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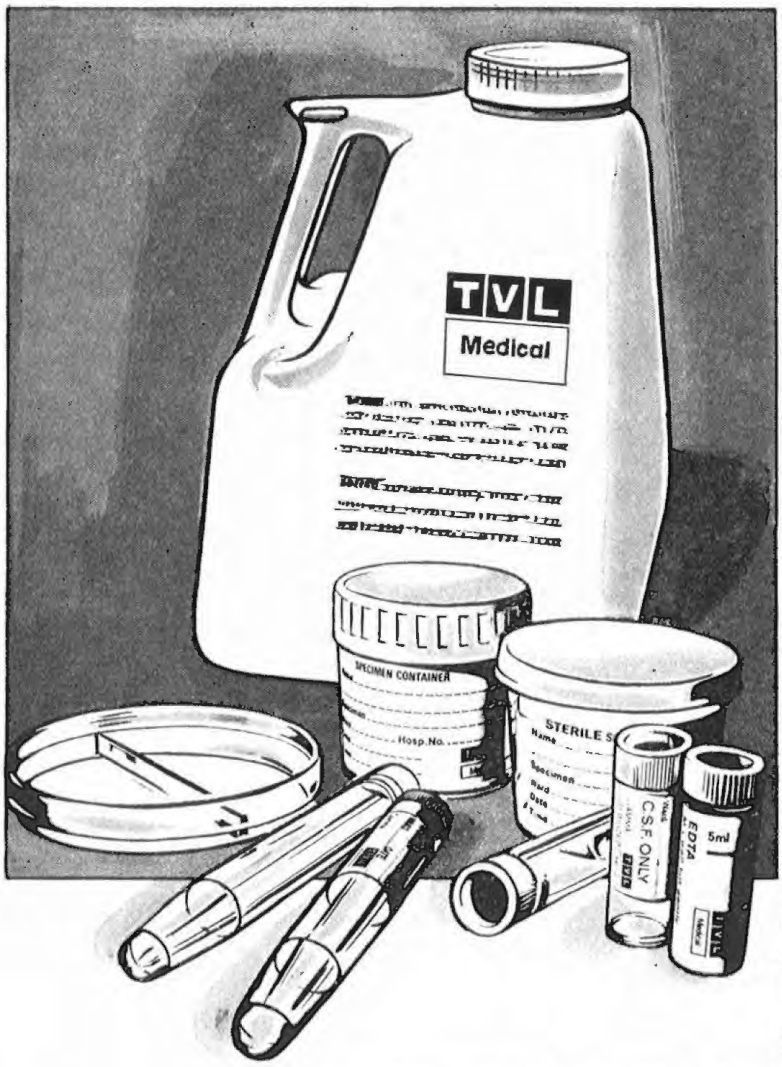

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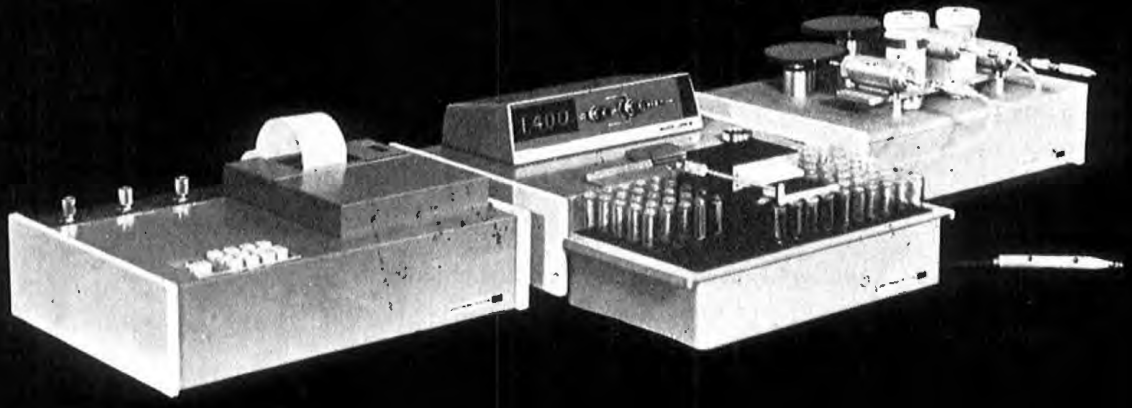

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T. H. PULLAR MEMORIAL ADDRESS, 1973

Clinical Pathology and its 'Body Politick'

Dr D. T. Stewart

Chairman, Pathology Services, Christchurch Hospital

Delivered at the 29th annual conference of the New Zealand Institute of Medical Laboratory Technology, Christchurch, August 1973

Tom Pullar, in whose memory this address is given each year, was known to pathologists for his great good sense and excellent judgment. I remember these qualities being very evident in his introduction of B.C.G. vaccination for the Department of Health.

To technologists he is best known, I imagine, for his interest in education, and for this reason it is appropriate that this year the address is being given in this five-star-plus temple of learning.

I do not know how you came into this building. As I approach it daily along the strictly functional hospital main corridor, I think of Alice in her long fall down the rabbit hole. After some intervening vicissitudes, she reaches the little door which leads into that glorious wonderland garden with its carpet-like lawns, peopled with strange creatures who, I hasten to add, bear no resemblance to the academic staff of the Clinical School.

The Minister, I am sure, appreciates that all this magnificence is a charge on the Department of Education. But you may think, with me, that it is strange that so much better quarters and facilities can be provided for those who are only learning the job than for those who are actually caring for patients. This ruler is symbolic of the difference—University issue— 15in, not 12in long—I would like the Minister to take it to brandish in the House when there is any argument between Health and Education for shares of the taxpayer's money.

The building too is symbolic. It is the first medical school building to be literally embedded in a New Zealand hospital. As such I hope that medical teaching here with its easy and close contact with patients and service departments, will cease to be academic and become clinically orientated. Pathology also should have an emphasis on clinical pathology in this ideal relationship.

Above us are two floors devoted to research: the activities soon to develop there should provide positions for technologists. I hope they will grasp them eagerly because they are well qualified to do so. Academic, clinical and research pathology should flourish here. Tom Pullar worked for a quarter of a century as a pathologist in Palmerston North, and this brings me to my title "*Clinical Pathology and its Body Politick*".

I have used the perhaps old-fashioned term "Clinical Pathology", to exclude Anatomical Pathology, which is one branch of the whole subject in which the dominant role of the pathologist is—as yet—unchallenged. (In parenthesis I say "as yet"—because if the national and world acute shortage of anatomical pathologists continues, some serious thought will have to be given to training technologists or science graduates in at least simpler or routine anatomical diagnosis.)

Clinical Pathology includes the other branches of: Clinical Biochemistry, Haematology, Immunohaematology and Microbiology, with Immunology soon to be added.

I must acknowledge the acquisition of "*Body Politick*" from an address given by the President of the Association of Clinical Pathologists in 1971. He quoted Shakespeare: "a body politick compact of all sorts and degrees of people". This body is worth anatomising in relation to the recent situation in Palmerston North and its possible implications.

Although I have quoted Shakespeare "all sorts and degrees of people", I must, for simplicity, divide the body into pathologists on the one hand and what I will call "technicians" on the other—lumping together technologists, science graduates and laboratory assistants. I know that "technician" is an offensive word to some, but I find it convenient here, as not singling out any one group of laboratory workers.

When I was a third-year medical student—in the mid-nineteen thirties—it was my job as a physiology demonstrator to show second-year students how to do a red cell count. I had

actually done two or three myself, and in retrospect my presumption astonishes me. It certainly did when I discovered later that one of the class was a trained technologist who had gone on to medicine. I still like to thank him for hiding his talent on that occasion.

Two years later I had "done" academic pathology and had some knowledge of drugs. I spent part of the holidays working in the Wanganui Hospital laboratory which was then under the charge of Mr Laurie Buxton. He was a technologist of considerable standing, but rather to my surprise, did not hesitate to advise clinicians and practitioners on how to treat the cases of pernicious anaemia and diabetes that he had diagnosed. I would point out, however, that 40 years ago the fields of laboratory medicine and of therapeutics were very much smaller and then a little medical knowledge was not necessarily a dangerous thing.

After my year as a house surgeon in Dunedin Hospital, I joined the Medical School Pathology Department. In order to continue to enjoy the advantages of living in the hospital I persuaded the authorities that a Resident Pathologist was most desirable. I did the night laboratory work which consisted of white cell counts, the typing of pneumococci in sputa and cerebro-spinal fluids, and the doing of blood sugars by Epstein's highly explosive method—almost invariably spattering the walls of the laboratory. Here was the budding pathologist turned technician: more or less coping with the night call work—but away back in the late nineteen thirties, when the range of laboratory tests was limited.

To learn some bench work I used to go to the diagnostic laboratory of the Microbiology Department in the Medical School. This was gloomily presided over by a kindly Scot, the late Andrew Logan. A considerable volume of diagnostic work came across the road from the hospital and he coped with this in a medical vacuum—no contact with clinicians and little or no advice from the academic microbiological staff. It appalled me as an example of "slot-machine" mechanical laboratory work.

When I came to Christchurch in 1939 I was, as you can imagine, glad that the main interest of my wonderful chief, the late Dr Arthur Pearson, was microbiology. I had, however, to do the Wassermanns and I am indebted to the late Tom Ross, another kindly Scot, for his tolerant instruction. My main interest was anatomical pathology, but I felt quite able to cope with the clinical pathology subjects of haematology and biochemistry—certainly as far as clinical consultation went. Clinical biochemistry was already beginning its astonishing expansion—and I was soon unable to cope. This was long before the days of chemical pathologists, but we were fortunate in having as biochemist the late John Murray, a man of astonishing skills, dedication and learning. He kept abreast of the biochemical literature—witness the two-foot pile of opened journals on his desk and adjacent benches—and many clinicians were deeply grateful for his advice, especially in the fields of diabetes and electrolyte imbalance.

The increased load and new knowledge in haematology and blood transfusion work led us to separation of disciplines by appointment of a haematologist in 1950, and an immuno-haematologist in 1970. Some 15 years ago it became evident to me that even the redoubtable John Murray could not cover both technical work and clinical consultation in the expanding field of biochemistry. It says much for him that the eventual appointment of a chemical pathologist worked out very well.

This history shows, I think, that there was a time when either technologist or pathologist coped with both the laboratory and clinical side of pathology—but it was a time of limited knowledge.

Today throughout the world there is thought being given to the roles of technologists and pathologists in laboratory medicine.

In the United States last December, Dr Daniel Weiss, speaking at a combined meeting of the American College of Pathology and the American Society of Clinical Pathology, said this:

"The role of the pathologist *was* that of a general consultant. Then technology overwhelmed him and drove him into the laboratory where for preference or force of circumstances he has stayed. Now the survival of pathologists may depend upon their resuming their original role and not becoming super-technologists. More sophisticated and mechanised testing methods and better trained laboratory personnel are freeing pathologists to concentrate once more on consultation."

The term "consultation" I interpret in a broad sense to include interpretation of results both in writing on a report and in bedside

discussion with the clinician, and advice on further appropriate laboratory tests, to establish diagnosis or control treatment. Haematologists are indeed entering the field of treatment of their own patients in hospital beds, and the extension of this principle to microbiology may be a solution to a staff shortage in that field.

The pathologist must indeed look to his future here, because since the war the organisation of hospital medicine has been changing. There has been the development, especially in medicine, of many units devoted to sub-specialties — nephrology, endocrinology, rheumatology, gastroenterology and the like. These are headed by young physicians who have come back to New Zealand with some laboratory knowledge and would prefer to run a laboratory doing tests appropriate to their sub-specialty—or at least have them done reliably in the general laboratory and interpret the results themselves.

It is becoming difficult for pathologists to have a full knowledge applicable to each highly specialised subdivision of medicine and this situation holds a challenge to their future.

A possibility to be considered is a medical laboratory staffed by technicians and geared as a factory to put out, as speedily and accurately as possible, the results of tests demanded by clinicans in all the specialties and sub-specialties in the whole field of medicine. The additional awful possibility arises that the staff of such factory laboratories could be trained either in technical institutes or in universities quite outside medical supervision.

What then are the advantages, apart from consultation in the broad sense, in having a pathologist supervising or running a medical laboratory?

In the field of *quality control* dedicated technicians could go to great and wasteful lengths in producing results the detail and accuracy of which would take no account of the biological state and variation of normal human beings. This is obvious in the fields of biochemistry and haematology, but in microbiology there are also many examples, such as the futile identification of commensal organisms in throat and vaginal swabs. Medical knowledge is important in medical laboratory quality control, and this must be applied throughout the training of technicians.

Morphological diagnosis is traditionally the field of the pathologist—not only in anatomical pathology, but also in haematology and cytology. Skill in this field is based on long association with microscopical work starting with biology and human histology in the first and second years of the medical course.

I know that cytology technicians can be trained in a few years to make a confident diagnosis of malignancy, and I have no doubt that similar technical training could be devised to cover interpretation of bone marrows and even the whole field of anatomical pathology. This would indeed be a major undertaking. I am perhaps old-fashioned enough to believe that morphological diagnosis should remain the province of the pathologist—and desirably—certainly when enough pathologists are available —the diagnosis of cancer should be made by a person medically trained.

Doctors are individualists and the shelves of theatre instrument rooms and the pharmacies in hospitals throughout New Zealand are littered with instruments and drugs got to satisfy a doctor's whim of the moment—often regrettably the result of uncritical reading of the literature.

So too are laboratories frequently subjected to demands for new tests. A laboratory with only a technical staff has no front line of defence against such medical requests. The pathologist therefore has an important function in assessing the clinical need for tests not routinely done. He has an equally important function in keeping the laboratory up to date both by his regular reading of the medical literature and by attendance at postgraduate courses—and not only courses arranged solely for pathologists. He should have his laboratory prepared to anticipate clinical demands for new tests of proven value. This is one protective and image-preserving function of a pathologist. Another similar function relates to out-of-hours service, which is widely considered to consume some 10 percent of a laboratory's maintenance funds. Considerable reduction in so-called urgent or essential out-of-hours work could be achieved if there were sufficient pathologists available to screen every such request. Without medical backing the technical staff are at the mercy of the sometimes unreasonable clinician and the work must be done without question. In the present state of pathologist staffing all that can be done is to remonstrate later with an occasional clinician and hope he will not offend in future.

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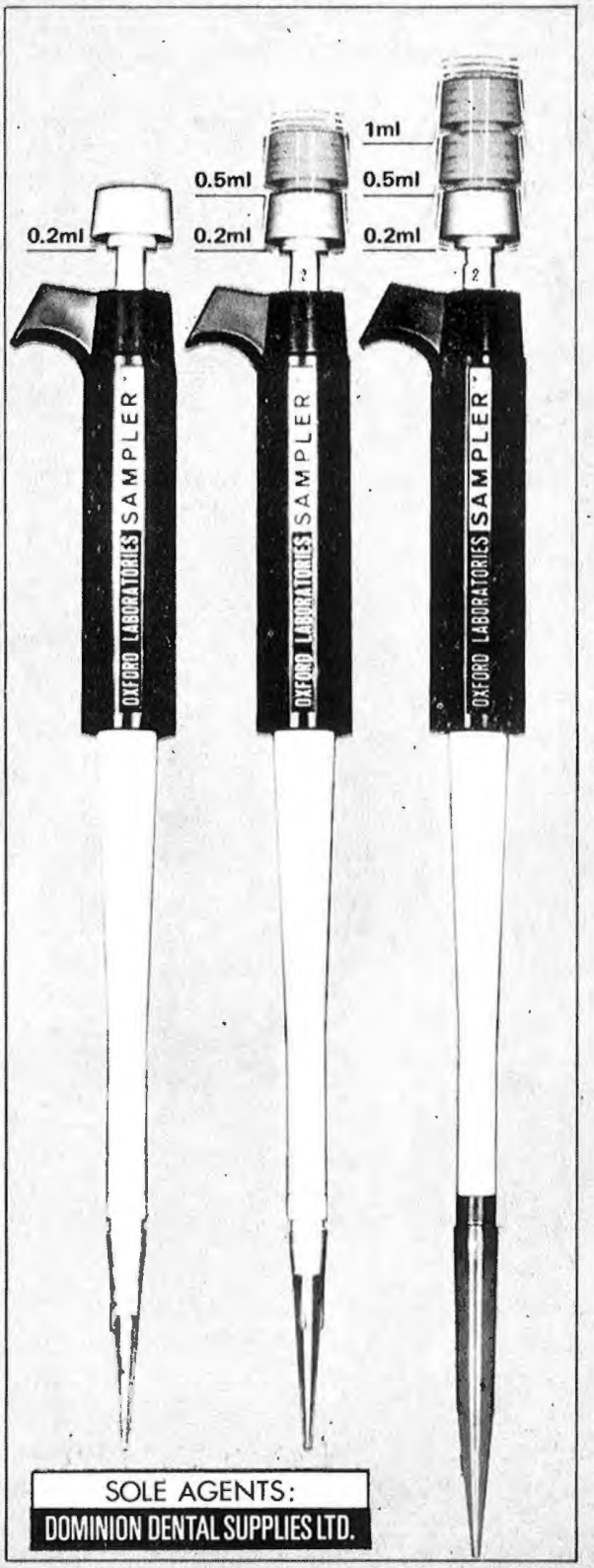

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Many of the audience will no doubt be thinking of smaller hospital laboratories without more than the tenuous medical supervision of the hospital medical superintendent or the district advisory pathologist.

In my view such laboratories should be under the charge of only senior technologists who have had a wide training with long and close contact with pathologists. They should be well recompensed for responsibility in isolation and have ample access to refresher courses.

There is justification for pathologists in more of these smaller centres. The solution to the probem, which is largely a financial one, may well be in some arrangement whereby the pathologist is based at the smaller district hospital and conducts a private practice from the hospital laboratory, or does the hospital work on a contractual basis.

Some pathologists like administration. I imagine that most of these have found their way into private practice where they are running large businesses. Hospital laboratories have also to be run, and many are also big business: the annual maintenance budget for the North Canterbury Hospital Board's Pathology Services now exceeds a million dollars. The administration of this empire—I must say that I have no sensation of being a millionaire—has grown upon me gradually since 1950. I have always been poor at maths, have no training in management, and it seems absurd that someone with my training and experience in anatomical pathology (in which branch there is a serious national shortage), and on my medical salary level, should have to devote a considerable time to administration.

Many laboratories are experimenting in the management field. Here we have a Pathology Services Committee composed of the pathologists in charge of each department: when I retire it will have an elected chairman who, like myself at present, will deal with those day-to-day matters which cannot be dealt with by our lay administrative officer.

This officer is concerned mainly with staff matters and we have a second officer concerned with supply.

Though not essential, and depending upon the size of the organisation, a laboratory background can be of great help in these areas of management.

I hope an option will soon be provided in the C.O.P. course for proper management training to encourage those with talent to enter this field. It is at present a waste of medical manpower.

Earlier I discussed how the pathologist must take the laboratory to the wards in interpretation and consultation. An equally important medical function is to bring the wards to the laboratory. The pathologist should motivate the laboratory staff by clinical information, disseminated in informal discussions, seminars and staff clinicopathological conferences. Without this clinical correlation, laboratory work—like that done by Andrew Logan—becomes dull, routine and mechanical. Correlation is one of the features which make, or should make, laboratory work more attractive within hospitals: but it takes time and staff. This is an area in which the pathologist can make his medical knowledge and his place in the laboratory evident and needed. He should bring to the technicians (whose title derives from the Greek word technos—"skilful") the medical knowledge which is implied in his name—"path", indicating disease, suffering, patients.

My conclusion is that we must go along with the International Society of Clinical Pathology in holding that *the head of a clinical laboratory devoted to the diagnosis and treatment of human disease must be medically qualified.*

Technologists, science graduates, laboratory assistants and pathologists must continue to work closely together as a "body politick, compact of all sorts and degrees of people".

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Laboratory Standards

This title is used in a broad sense to indicate standards of performance and of comparison.

It is rather surprising to realise that it is over a quarter of a century since the first quality control survey was instigated in Pennsylvania. The gross discrepancies revealed at this time were found by subsequent surveys to be the rule, rather than the exception. The concept of quality control is now generally accepted but results are not always taken to their logical conclusion. The application of this knowledge to improve the standard of laboratory work has proceeded with varying degrees of speed. In the United States, the Medicare scheme requires from laboratories certain defined standards of staffing and equipment and some evidence of competence. Elsewhere, although the tools are available, progress is rather slow. In New Zealand most laboratories do employ quality control procedures but there is no statutory requirement to do so, nor machinery to follow up any deficiencies revealed by such a scheme.

There are many facets to this problem. Standardisation and documentation tend to impose rigidity on methodology and inhibit enterprise and experimentation. Different circumstances call for different approaches. A throughput of 100 specimens may require automation; two specimens call for different treatment. Certainly any technique used should demonstrably produce acceptable results. Poor methods should not be tolerated nor poor technique. Should the first step not be to examine the instruments and techniques used? Perhaps more to the point machinery should be set up to do this. The smaller isolated laboratory is vulnerable in this respect.

In the field of chemical pathology particularly, but also in the other specialities external evidence of competence can be obtained by the use of one of several quality control schemes. These can now be obtained at a reasonable frequency and with a wide range of estimations and methodologies. Results can provide the stimulus for correction and improvement.

What about comparability? At present laboratories may perform the same test by the same method and express it in different units. They may perform the same tests by different methods. They may be operating in the same district with interchange of patients. An added complication is the anticipated changeover to expressing concentrations in mol/litre. This is doubtless tidy and logical but will not directly improve treatment nor simplify understanding of results. There is a great deal to be said for co-ordination of such a change, perhaps in the nature of an "SI Day". As things stand any result which does not state the method used and the normal range related to it, is potentially dangerous.

It would appear that New Zealand because of its small population and relatively few laboratories, should stand a better chance of standardising and correlating laboratory results than most other countries. This Journal could make a contribution by publishing information comparing methodologies used in different laboratories throughout the country. It would be possible to tabulate the methods and normal ranges used in all such laboratories and this would be a safeguard for itinerant patients.

There have been some efforts to make comparisons in the sphere of haematology and of immunohaematology and I hope to have the opportunity to publish an account of these efforts in the future. There may have been other attempts to gain systematic knowledge of current methodologies which could be usefully reproduced.

An attempt to rationalise oestriol estimations is currently being undertaken by some members of the staff of the biochemical laboratory at Green Lane Hospital; namely, Miss S. M. Peace and Drs M. Lever and C. W. Small. This could be regarded as a very valuable pilot experiment in this field. A great deal of statistical and other information has been derived from it and this is to be published in appropriate journals. Praetical reports on the results achieved have been issued and I am indebted to them for permission to reproduce the substance of these reports. The foregoing remarks are in effect a preamble to this report.

The survey covers the period from November 1972 to July 1973 and 23 out of 25 laboratories performing pregnancy oestriol estimations participated. Six series consisting of three urines containing high, medium and low values were sent out.

To illustrate the results obtained, series six is shown here. An impression of the scatter of results obtained can be formed from this series. However, as was pointed out in the report

the precision of the method used in a particular hospital was of prime importance in following the trend of a series of results. One of the aims of the exercise was to develop a method of producing comparable results through the use of a common standard for all methods used. This is being investigated now. Table 2 shows the variety of methods in use but not all the modifications of these methods. They give different recoveries thus, Brown techniques give 50 percent, Oakey techniques 78 percent. The reference technique used produced absolute results.

Table I: Results Reported (mg/l) (Series 6).

Yellow	White	Red
74	25	4
71	20	5.3
60	19.5	6.2
60	16	9
59	19	8
56	24	4
55	22	5
51	28	7
50	24	4
47.0	18.3	4.0
47	18	3
46	26	5
46	20	5
42	21.6	4.5
38	16	5
33	13	4
30	10	2
29.6	12.6	3.5
28.6	14.5	3.3
28	10	2
27	35	6
26.4	13.4	8.1
22.6	16.5	7.5

Table 2: Means of Reported Results and Calibration Estimates

	Estriol μmole/litre (mg/l)		
	Yellow	White	Red
Direct Estimate	202 (58)	76 (22)	14 (4)
Brown (corrected)	213 (61)	76 (22)	12 (3)
Mean reported Oakey	162 (46.6)	74 (21.4)	19 (5.4)
Mean reported Brown	102 (29.4)	40 (11.6)	14 (4.0)

When a practical method is found, laboratories might agree to report absolute oestriol results and at the same time change over to μmol/litre or 24h specimen, in order to make a clean sweep. This could not be done without due notice and preparation and the physiological levels in the new unit clearly stated.

In the meantime, the specimens distributed have constituted a quality control system for oestriol determinations and must in some instances have indicated the need for investigating the method employed or the method of standardisation.

The nature of the estimation did not produce a normal distribution because of the underestimation in most cases and because of the different methods employed, so a simple Gaussian analysis could not be made. The specimens were not random samples and a conventional correlation analysis was not considered appropriate. The degree of consistency of the results was considered important. Two statistics were used to measure the comparability of performance.

1. **Coefficient of Non-determination.** This gives the proportion of the variance in each set of results which is not explained by the variance in the estimated oestriol content. Six laboratories had coefficients greater than 0.1. This represents more than 10 percent of such variation in the results and these are not consistent results. Those with results between 0.03 and 0.1 have room for improvement. By comparison the results obtained by GLC at Green Lane laboratory yielded a coefficient of 0.011 and the Brown results 0.006.

2. **Standard Error of Estimate of Laboratory Result.** This is a measure of the uncertainty with which the results reported can be predicted, using the regression equation from the reference results. Clearly the greater the uncertainty the less the consistency. Since the numerical value of the standard error depends on the range of oestriol results and as one laboratory might report results twice as high as another laboratory but with equal consistency, standard errors have been expressed as percentages of the mean result. Those laboratories with coefficients of non-determination greater than 0.1, have standard errors of estimates greater than 20 percent of their mean results. Those with coefficients between 0.03 and 0.1, between 16 percent and 19 percent. The other laboratories obtained results less than 15 percent.

Regression coefficients were also reported and this gave a measure of the average recovery of oestriol.

Although no statistical evidence could be adduced from the relatively small numbers of tests investigated it is likely that laboratories carrying out large numbers of tests will obtain

more consistent results. This is the usual finding. Laboratories which have not performed well should consider their quality control arrangements. Consistency can be checked by comparing results with the mean for the method similar to theirs and with the calibration results. Constant ratios should be obtained. The second ratio should be close to the regression coefficient.

If the number of specimens is very small it might be logical to send them to a larger unit. This is a sound general principle with tests which are not desperately urgent and by utilising special facilities parochial proliferation and duplication can be avoided and the best use made of limited resources.

It is to be hoped that financial support will be found to continue this scheme and initiate others like it. Their importance is obvious to those of us grappling with the problem of producing meaningful results.

—R.D.A.

The Comparison of Media for the Isolation of Salmonellae and Shigellae from Faeces

Jan V. Howe

Pathology Department, Wellington Hospital

Winning Entry, Student Essay Competition (Technical Section) 1973

Summary:

Three selective plating media and three enrichment media for the isolation of salmonellae and shigellae were evaluated. One hundred faeces specimens, from patients with diarrhoea in Wellington Hospital, were cultured onto these media. Results suggested that a variety of media should be used to obtain a high recovery rate of these enteric organisms. A larger number of specimens taken from a true cross-section of the New Zealand population should be tested. This would hopefully yield a wider range of salmonellae and shigellae strains and a higher percentage of isolations. Only then could conclusive results be obtained for the usefulness of these media in the isolation of these organisms.

Introduction:

There are several media which have recently been recommended as efficient for the isolation of enteric pathogens, specifically salmonella and shigella species, from faecal specimens.

Comparisons of three direct plating media, desoxycholate citrate agar (DCA), xylose lysine desoxycholate agar (XLD) and Hektoen agar (HEK) and three enrichment broths Gram-negative broth (GN), Selenite F broth and Rappaport broth have been made. A range of media from those losing popularity, those commonly in use and the newer media has been selected, with the aim of finding an efficient combination of media for the maximum number of isolations of the two genera.

Extensive evaluation of these media has been carried out overseas[1,2,4,5,6,7] but there is a need for evaluation in our environment, as the species of salmonella and shigella isolated in other countries are not necessarily species commonly found in New Zealand.

Materials and Methods:

During the period December 4, 1972, and April 30, 1973, 100 faecal specimens sent to the laboratory, Wellington Public Hospital, were collected from the routine Bacteriology Department and used in this study.

Three plating media, HEK, XLD and DCA and three enrichment broths, GN, Selenite F and Rappaport were compared.

All specimens were inoculated onto these media and incubated at 37°C for 24 hours in aerobic conditions. The enrichment broths were then plated out onto MacConkey agar.

The plating media, HEK (Difco), XLD (BBL) and DCA (BBL) were prepared from dehydrated media according to the commercial instructions. GN broth (BBL) and Selenite F. broth (Difco) were prepared from commercial dehydrated media and the Rappaport was prepared according to J. B. Iverson and N. Kovacs[3].

Biochemical and serological tests were carried out on all colonies suspected of being salmonella and shigella to confirm their identity.

Table I
Isolation of Salmonella and Shigella from Direct Plating Method

Organisms	XLD	XLD + HEK	XLD + DCA	HEK + XLD + DCA	HEK	HEK + DCA	DCA
Salmonella	1	0	0	5	0	0	0
Percent	16.6			83.4			
Shigella	1	3	0	5	0	0	0
Percent	11.2	33.3		55.5			

Table II
Isolation of Salmonella and Shigella from Combinations of Enrichment Broths

Organisms	RAPP	RAPP GN	Selenite	RAPP SEL GN	GN	GN SEL	Selenite
Salmonella	1	0	2	4	1	0	0
%	12.5		25	50	12.5		
Shigella	2	0	2	0	0	0	3
%	28.6		28.6				42.8

A qualitative study was also performed to further determine the effectiveness of the media. To compare the plating media for their ability to support growth from a small inoculum, six-hour broth cultures of *Salmonella anatum*, *Salmonella typhimurium* and *Shigella flexner* were used. Tenfold dilutions were prepared in saline from 10^{-5} to 10^{-8} for the salmonella species and 10^{-4} to 10^{-7} for the shigella species. These were tested by Miles + Misra counts on HEK, XLD and DCA. Nutrient agar (NA) was used as a control for this test.

To compare the enrichment broths 1g of faeces was emulsified in 10ml of saline. To three of these emulsifications two drops of *pure* six-hour broth cultures of *Salmonella anatum*, *Salmonella typhimurium* and *Shigella flexner* were added respectively. Tenfold dilutions of these suspensions were carried out from 10^{-1} to 10^{-6} in Rappaport, Selenite F and GN broths for each organism. The broths were then incubated at 37°C for 24 hours. A loopful of each broth was then plated out onto XLD. The 10^{-4}, 10^{-5} and 10^{-6} dilutions were done in triplicate.

Results:

Of the 100 specimens examined, *Salmonella typhimurium* was isolated from eight specimens, *Shigella sonne* isolated from five specimens and *Shigella flexner* isolated from five specimens.

DCA appeared to be the least efficient for the detection of shigellae, growing only five of the nine shigellae isolated. HEK and XLD appear to be almost equal in ability, HEK growing eight and XLD growing nine of the shigellae isolated.

When a salmonella species was isolated it was found on each selective medium in all cases except one, where a salmonella strain was isolated on XLD alone (Table I).

Fewer shigellae were isolated from the enrichment broths than from direct plating. GN broth failed to detect any shigellae, Selenite F and Rappaport broths detected five and four respectively, of the seven shigellae isolated.

Isolations of salmonellae from the enrichment broths, showed that Rappaport detected seven of the eight isolated, GN broth five and Selenite F broth six (Table II).

In no cases were salmonellae species isolated from the direct plates HEK, XLD and DCA and not from at least one of the enrichment broths but, in four cases salmonella species were isolated from the enrichment broths when there was no observable positive growth on the direct plates.

As there is a low incidence of cases of salmonellasis and shigellasis in the Wellington area qualitative tests were included in this study. Results of this testing are as follows:

MILES + MISRA COUNTS

Salmonella anatum: All selective media supported this organism to the dilution 10^{-8}, XLD compared more favourably with NA than HEK and DCA.

Salmonella typhimurium: All selective media supported growth of this organism to the dilu-

tion 10^{-8} and all compared favourably with NA.

Shigella flexner: All selective media supported growth of greater than 20 colonies of dilution 10^{-4} but there was no growth in the higher dilutions. These results compare well with those obtained on NA.

Resuts of the tests on the enrichment broths were graded according to the amount of growth.

Heavy — greater than 20 colonies
Moderate — 15 to 20 colonies
Light — 10 colonies
Scanty — five or fewer colonies.

Salmonella anatum:

Rappaport supported scanty growth at 10^{-6} dilution.
GN supported heavy growth at 10^{-6} dilution
Selenite F supported heavy growth at 10^{-6} dilution.

Salmonella typhimurium:

Rappaport supported a moderate growth at 10^{-6} dilution.
GN supported light growth at 10^{-6} dilution.
Selenite F supported a light growth at 10^{-6} dilution.

Shigella flexner:

No growth from Rappaport and Selenite F broths at any dilution.
GN supported a scanty growth at 10^{-5} dilution.

Discussion:

From the results of the direct plating method DCA appears to be the least efficient for the detection of shigella strains. XLD and HEK were almost equal in their ability to detect shigella strains. All three media performed equally in their ability to detect salmonella strains. In one case XLD detected a strain of salmonella when DCA and HEK gave negative results.

Throughout the study several features of the media were noticed. A relatively large number of false positive results were obtained from XLD agar. Colonies which after 18 hours incubation appeared as pink, were often found to have become yellow after 24 hours incubation. This could lead to false positive colonies being obtained in cases where routine faeces specimens have been cultured late in the afternoon and read the following morning, before 24 hours incubation have elapsed. Where there is a heavy growth of normal enteric flora on the XLD plate the yellow coloration of the colonies tends to diffuse, away from the colony, into the media. This is a possible cause of false negative results being obtained as this yellow coloration of the media tends to disguise any suspect pink colonies that may be present on the plate. A possible cause for these anomalous results may be that the XLD media used in this study was prepared from the dehydrated complete medium. Taylor *et al.* (1968)[5] states that to obtain satisfactory results from this medium it must be made from the base with the addition of heat labile substances after heating. Hektoen agar was a clearer medium to read as the orange coloration of normal flora colonies remained within the colony and did not diffuse into the media even when there was heavy growth of intestinal flora. This made the suspect green or green with a black centre colonies easy to see and consequently fewer false positives were obtained from this medium. DCA was difficult to read, it appeared to inhibit the normal intestinal flora to a greater extent than in HEK and XLD. Differentiation between the pink colonies of normal flora and colourless colonies of shigella and salmonella strains was difficult, due, possibly to the pink-brown colour of the media itself. Consequently, fairly large numbers of false positives were obtained. There were no distinguishing characteristics for the differentiation of salmonella and shigella strains such as the typical blackening of salmonella colonies due to the production of hydrogen sulphide on the XLD and HEK plates.

Rappaport and Selenite F enrichment broths inhibited the growth of normal enteric organisms quite efficiently and when salmonellae were isolated they appeared, in the majority of cases, in relatively large numbers. This showed that these two enrichment broths were performing well in their desired functions.

Both Rappaport and Selenite F appeared to inhibit shigellae.

Selenite F was inhibitory to a lesser degree as in three cases shigella strains were isolated from three specimens when GN and Rappaport gave no positive growth.

In one case Rappaport was the only medium to isolate a shigella strain from a specimen.

GN broth does not inhibit normal enteric organisms as it is designed to enrich all Gram-negative bacilli. For this reason, this broth performed badly in this study. It did not detect any shigella strains and only detected salmonella strains when the number of organisms present was high and both Rappaport and

Selenite F also gave a growth of salmonella. In one case alone the GN broth detected a salmonella strain when both Rappaport and Selenite F gave negative results.

When the GN broth was plated onto XLD and MacConkey agar after incubation, there was an overgrowth of normal flora therefore making it almost impossible to determine whether any shigella or salmonella strains were present.

Shigellae did not appear to thrive in the enrichment broth. This is probably due to the fact that shigellae tend to be inhibited by the reagents, in the broth, which are designed to inhibit normal intestinal flora.

From the qualitative study of the compared media it can be seen XLD, DCA and HEK detected *Salmonella anatum* and *Salmonella typhimurium* efficiently even when the number of organisms present is small. They only detected *Shigella flexner* when there were at least 20,000 organisms/ml saline.

The enrichment broths, Rappaport, GN and Selenite F supported and enriched the growth of *Salmonella anatum* and *Salmonella typhimurium* even when the number of organisms present in the faecal specimen were relatively small.

Rappaport and Selenite F did not support or enrich the *Shigella flexner* in the dilutions tested, showing perhaps that large numbers of organisms must be present before the inhibitory properties of these broths can be overcome and the shigella enabled to thrive.

GN broth gave a scanty growth of *Shigella flexner* from the 10^{-5} dilution. This may be due to the fact that by dilution of the number of other Gram-negative bacilli has been decreased to the point where the shigella is still present in small numbers but is not overwhelmed and is therefore able to thrive in the medium.

Ultimately only broad conclusions can be made from this study, due to the limited number of faeces specimens examined and the low isolation rate of the two genera, in this hospital. Tentatively, it could be suggested that XLD and HEK used for direct plating isolation are the most efficient of the media used in this comparison, especially for the isolation of shigella species.

Selenite F and Rappaport broth appear to be efficient in the enrichment of salmonella strains with the exception that Rappaport broth is known to be inhibitory to *Salmonella typhi*[3]. GN broth will only enrich this species when it is already present in relatively large numbers. It cannot from this study, claim to be sufficiently effective in the enrichment of shigella species. This result is contrary to overseas reports[4,6].

Rappaport and Selenite F broths appear to be almost equal in ability to enrich and support the growth of shigellae with Selenite F broth perhaps slightly superior. It is realised that the primary function of these enrichment media is to enrich the culture of salmonellae from faeces. The successful isolation of strains of shigellae from these enrichment broths is probably explained by the organism being present in large numbers which is often the case in bacillary dysentery.

From this study it may be suggested that a combination of XLD, HEK, Rappaport and Selenite F broth may lead to an increase in the numbers of isolations of salmonellae and shigellae from faeces specimens in the routine Bacteriology Department of Wellington Hospital.

As a result of this investigation it has been decided to use a combination of XLD and Hektoen agar as selective media and Selenite F and Rappaport broth as enrichment media for a further trial period. Hopefully this study will also encourage other laboratories in New Zealand to take a more searching look at the media they are using for the culture of faeces specimens and, perhaps after carrying out studies of their own will consider a change in media beneficial for the maximal isolation of salmonellae and shigellae.

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Assessment of an Automatic Integrator for Quantitation of Serum Protein Fractions

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Summary

Statistical comparison of serum albumin concentration established by means of Gilford 3023 Scanning and 3024 Integration accessories and by the Brom Cresol Green method.

Introduction

Of the various methods of estimating serum protein fractions, few are without some limitations. In recent years the technique using the albumin error of indicators, in particular brom cresol green (hereafter termed BCG) has gained considerable acceptance.

As protein electrophoresis is a routine procedure, the concept of quantitation of the electrophoretogram is attractive but also has well known limitations such as lack of uniformity of dye uptake by the various protein fractions and variations in technique resulting in non-uniform electrophoresis. Accessories available with the Gilford 300-N Spectrophotometer include a well-designed scanner adapting to the spectrophotometer by means of fibre optics and an integrator which in conjunction with a data lister will express protein fractions directly in concentration units on the basis of the total protein concentration.

The equipment produced by Gilford Instrument Laboratories Inc. Oberlin, Ohio, is part of the "300 series" system of modules applicable to the clinical chemistry laboratory. The spectrophotometer had been found to be a convenient, routine, automatic sampling, digital read-out instrument. The approximate prices of this instrument and the other modules are given in New Zealand dollars.

Spectrophotometer	$2,500
Strip scanner	$560
Integrator	$1,000
Data Lister	$1,600

The purpose of this paper is to present a valid comparison of albumin measurement by scanning with this equipment against estimation by the BCG method under routine conditions. Precision of the scanning is assessed and points of technique discussed.

Reagents

A. *Electrophoresis*:

1. Cellulose polyacetate—Titan II (Helena Laboratories, Beaumont, Texas, U.S.A. 77704).
2. Barbitone Buffer, pH 8.6
   Diethyl barbituric acid, sodium salt 12.77g
   Diethyl barbituric acid 2.08g
   EDTA, disodium salt ..... 4.0 g
   Dissolve in distilled water and make up to 1 litre.
3. Dye:
   Ponceau S 0.2g.
   Trichloracetic acid, 30ml of 10% solution
   Dilute to 100ml with distilled water.
4. Rinsing Solution:
   5% (v/v) Acetic acid in three containers.
5. Clearing Solution:
   Cyclohexanone 20ml.
   95% ethanol 80ml.

B. *Brom Cresol Green Method*:

1. BCG Buffered Reagent pH 3.8
   Dissolve each of the following separately in distilled water:
   Trisodium citrate dihydrate 5.1 g
   Citric acid trihydrate 6.86g
   Sodium azide 0.1 g
   Combine, mix and dilute to 800ml with distilled water. Dissolve 0.056g brom cresol green in 2ml of 0.1N NaOH and mix well to dissolve. Add this to buffered reagent, check pH and adjust if necessary to pH 3.8. Add 1.5ml 25% Brij-35 and dilute with distilled water to 1 L. Absorbance at 637nm with water as reference should be 0.16-0.18. Store in polythene bottle at 4°C.
2. Albumin Reference Standard
   0.5ml aliquots of pooled serum are stored

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- ☐ continuous linearity check within optional tolerances of 1, 2, 5 and 10%;
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- ☐ check on too high an enzyme activity which might prove unreliable;
- ☐ check on too low an enzyme activity if insufficient serum or substrate is added;
- ☐ choice of operating temperature at 25, 30, 35 and 37°C;
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*** During the 8th International Congress on Clinical Chemistry, held in Copenhagen in 1972, the Expert Panel on Enzymes recommended an overall temperature accuracy within 0.05°C for testing enzyme activities.**

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frozen as convenient secondary standards for this method. These are standardised against residual serum from a group quality control programme. As the mean values of albumin analyses on each of those samples performed by many laboratories are known it is worthwhile using them as albumin reference standards. There is some doubt as to the validity of using albumin standards prepared from crystallied human albumin as reference standards on account of the possible difference in available binding sites between this material and normal serum[5,6].

Techniques

1 in 200 dilution of serum in BCG reagent was made with a Griffen and George Diluspence dilutor. As the method has been shown to be linear to 5g albumin per 100ml, each batch of tests included a standard serum with which direct comparison was made. i.e.

$$\text{Albumin (g/100ml)} = \frac{T-B}{S-B} \times \text{Standard concentration}$$

Results

(1) Precision of scanning and integration system

A typical electrophoretogram was inserted, scanned and removed from the scanner 20 times to assess standard deviation of this quantitation procedure.

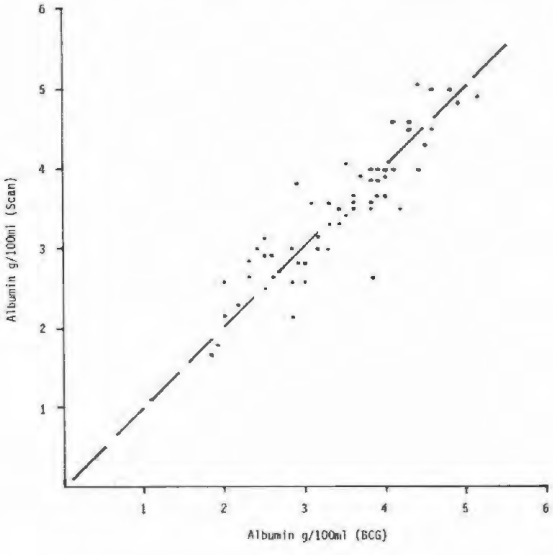
As the electrophoretogram is scanned in one direction the area under the hypothetical curve is integrated automatically and stored in a memory. When the end of the scan is reached a reverse scan automatically commences. At each valley (a rise in absorbance of 0.002 after a drop of 0.004) the data lister prints out the protein concentration indicated by the area under the previous peak. For the investigation of precision a total protein of 7.6 g/100ml was set on the integrator and the 20 independent scans yielded an albumin mean ± 2.S.D. of 4.87 ± 0.009g/100ml. Overall precision would be expected to be lower than this as variations in electrophoretic technique would be involved.

(2) Comparison with BCG Method

60 sera from hospital patients were compared in this survey:

	Number	Mean	Range
Albumin, BCG method:	60	3.40	1.8-4.9
Albumin, Scanning:	60	3.44	1.7-5.0

FIGURE 1.—Line of regression for the comparison of albumin values estimated by BCG and Scanning/Integration methods.



Statistical analysis of data using the t test of significance of difference between two means showed that on the basis of 95% probability tables for t, there is no significant difference between the albumin mean values obtained by the two methods.

Figure 1 illustrates the comparison of results by electrophoretic scanning and BCG methods.

Discussion

The results obtained indicate an instrument of good precision in the integrating and calculating facility. As usual in any scanning procedure, the quality of the electrophoretogram is of prime importance. The integrator calculates area by measuring the ordinates at 70 millisecond intervals and summing the results. Two different scans of the same strip may give slightly different totals because the positions of the measured ordinates will be different. For this reason it is desirable to use relatively long patterns. During forward scan the total area is automatically integrated and this sum is divided by the value pre-set for the total protein. As the last digit of the quotient is then discarded for the purposes of the final calculations during the reverse scan, it is desirable that the total integral be large so that

the discarded digit is of negligible effect. Thus ideally the protein bands should have relatively high absorbance.

On examining the duplicate results obtained by the two methods it was noted that 60% of the pairs showed differences in albumin concentration of 0.2g/100ml or less. On the other hand 15% of the pairs showed disturbing differences of 0.5g/100ml or greater. Although the corresponding electrophoresis results for this latter group were examined no common feature was identified which would account for these large variations.

It is intended to continue this study by the investigation of several features of the electrophoretogram which may be altered on the basis of buffer composition or protein stain used with a view to obtaining a more general correlation between these two convenient methods.

Acknowledgments

The author acknowledges the reagent details and validation provided by Mr B. Andrew and wishes to thank staff of Middlemore Hospital Laboratory for valued assistance in the analytical work involved in this survey.

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Estimation of Serum Inorganic Phosphorus; A Direct Method

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Summary

A method of estimating serum inorganic phosphorus, which avoids the removal of proteins, is presented. It involves the use of a non-ionic surfactant (Nonidet P40), a reducing agent (p-methyl ammoniumphenol sulphate) ammonium molybdate in sulphuric acid (which precipitates the proteins and combines with inorganic phosphorus and the reducing agent) and triethanolamine (which solubilises the proteins, makes the solution alkaline and thus releases the phosphomolybdate complex to produce a clear blue-coloured solution). A description of the optimum conditions of reagent concentration and other procedural parameters is given.

Introduction

Very few methods for the direct estimation of serum phosphorus have been reported. One method, that of Gindler and Ishizaki[1] used a surface active agent, Bion NE-9 (9 ethylene oxide unit adduct of p-nonylphenol: Pierce Chemical Co., Rockford, Ill.), and monoethanolamine to prevent precipitation of protein in the reaction mixture. Unfortunately, details of their method were not reported. Since Bion NE-9 was not readily available, a similar non-ionic detergent, Nonidet P40 (available from Shell Oil N.Z. Ltd) which was already in use, was substituted. Similarly, since monoethanolamine produced a dirty green/blue colour, triethanolamine was tested, and as it produced a clear pure blue colour, it was substituted for the original monoethanolamine. This paper establishes optimum assay conditions using these reagents. The resulting method has proved to be quick, accurate, sensitive and adaptable.

Materials

Reducing Reagent

Stock. Contains 1g of p-methyl ammoniumphenol sulphate and 3g of sodium metabisulphite in distilled water to a final volume of 100ml.

Working. Contains stock reducing reagent 75ml, Nonidet P40 3ml and distilled water to 500ml.

Refrigerate both solutions. Discard if any brown colour develops. The Stock solution is stable for years under refrigeration, and the Working solution for months at least.

Ammonium Molybdate Reagent

Dissolve 6g of ammonium molybdate in about 200ml of distilled water, add 80ml of 10 N sulphuric acid and make up to 500ml with distilled water.

Stable indefinitely at room temperature.

Triethanolamine Solution

Dilute 30ml of triethanolamine to 100ml with distilled water.

Stable at room temperature.

Standard Solution:

Stock. 50mg phosphorus per 100ml. Dissolve 0.2197g of potassium dihydrogen phosphate, anhydrous, in distilled water and make up to 100ml with distilled water. Add a few drops of chloroform to preserve. Stable at room temperature.

Working. 5mg phosphorus per 100ml. Dilute 10ml of Stock standard to 100ml with distilled water. Add a few drops of chloroform to preserve.

Stable at room temperature.

Method

1. To separately labelled tubes add 0.1ml of serum and standard solution.
2. To each tube, and another labelled Blank, add 3ml of Working reducing reagent. Mix.
3. To all tubes add 0.5ml of ammonium molybdate reagent. Mix well. This precipitates the proteins and may produce a faint blue colour.
4. To all tubes add 0.5ml of triethanolamine solution, and mix. This solubilises the proteins and a clear blue colour develops immediately.
5. Allow the tubes to stand at room temperature for at least two minutes to allow the fine hubbles to disperse.
6. At any time in the next 30min read the Absorbances of the Test and Standard solutions against the Blank set at zero on the spectrophotometer, at a wavelength of 650nm.

Calculation.

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 5 = \text{mg inorganic phosphorus per 100ml}$$

Discussion

Preliminary experiments were conducted, using the reagents already in use for the estimation of inorganic phosphorus by Gomorri's method[2]. By varying the contents and proportions of the reagents, it was soon apparent that the new method was considerably more sensitive than Gomorri's method. Similarly, the relative proportions of the ammonium molybdate and triethanolamine reagents required to produce adequate colour and a clear solution, were found without much trouble. Eventually, the method as proposed was decided on, and it remained to prove that the conditions as described, were optimal. It was found that these original conditions were, in fact optimal, but that considerable variations were possible, without sacrificing anything in the way of accuracy, sensitivity or precision. Results by both methods are compared in Table I. Experiments were conducted, therefore, using the method as described, but varying in turn, one or other of the reagents, and noting the effects.

Surfactant

The non-ionic surfactant mentioned in the abstract was not readily available, so Nonidet P40 was used. This is a Shell product, octyl phenol ethylene oxide. The amount added to the reducing reagent was varied, with the results as shown in Figure 1.

Reducing Reagent

A series of Working Reducing Reagents was prepared, in which only the amount of p-methyl ammoniumphenol sulphate (Metol-BDH) was varied, and these were used to estimate the inorganic phosphorus in a series of samples with and without protein, and with varying concentrations of phosphorus.

Colour development was not maximal with 20ml of Stock reducing reagent used to prepare 500ml of Working reagent, and only just when 40ml was used. The use of 75ml allowed a margin of safety. Note that the shape of the curves was the same within a range of phosphorus concentration from 4mg to nearly 20mg per 100ml, whether protein was present or not

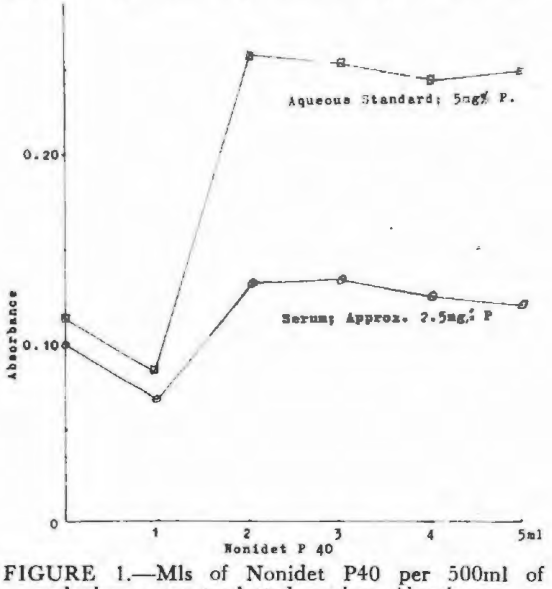


FIGURE 1.—Mls of Nonidet P40 per 500ml of reducing reagent plotted against Absorbance.

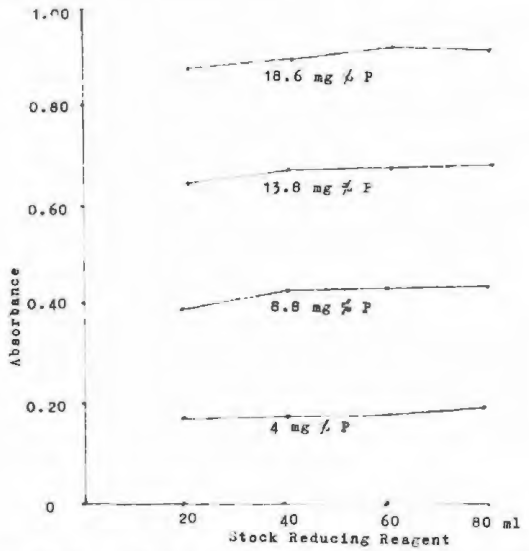


FIGURE 2.—Mls of Stock reducing reagent per 500ml of Working reducing reagent plotted against Absorbance, at four levels of phosphorus concentration, in protein containing solutions.

(the latter curves although not shown, were identical in shape up to at least 20mg per 100ml). See Figure 2.

These tests also showed that there was sufficient reducing agent present to cope with all likely concentrations of inorganic phosphorus that might be encountered clinically, and that Beer's Law held throughout this range. It was also evident that moderate variations in the amount of reducing agent made little difference to the amount of colour produced.

Ammonium Molybdate Reagent

The amount of this that is needed, is related to both the amount required to react with all likely levels of phosphorus concentration, and the amount of triethanolamine needed to alkalinise the mixture and solubilise the proteins. A series of reagents was prepared, containing 1, 2, 4, 5, 8, and 10g of ammonium molybdate in 500ml of reagent, with the proportionate amount of 10 N sulphuric acid in each case. The results obtained when these reagents were used are shown in Figure 3. Note that considerable variation from the recommended amount of 6.0g is possible without affecting the results.

Triethanolamine Solution

Gindler and Ishizaki used monoethanolamine, but it was found that this produced a blue/green colour, whereas triethanolamine produced

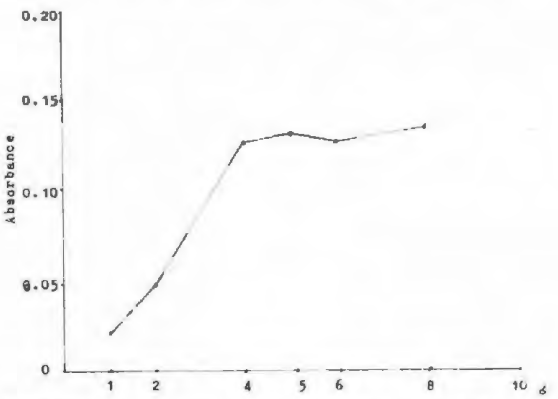


FIGURE 3.—Grams of ammonium molybdate per 500ml of reagent, plotted against Absorbance.

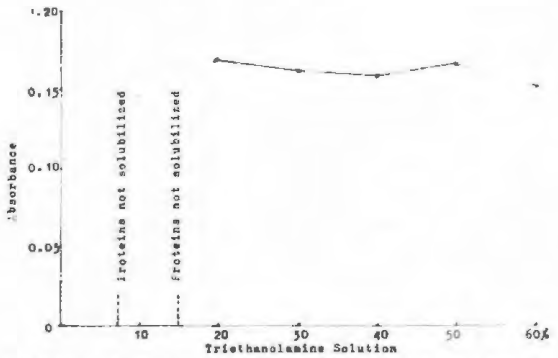
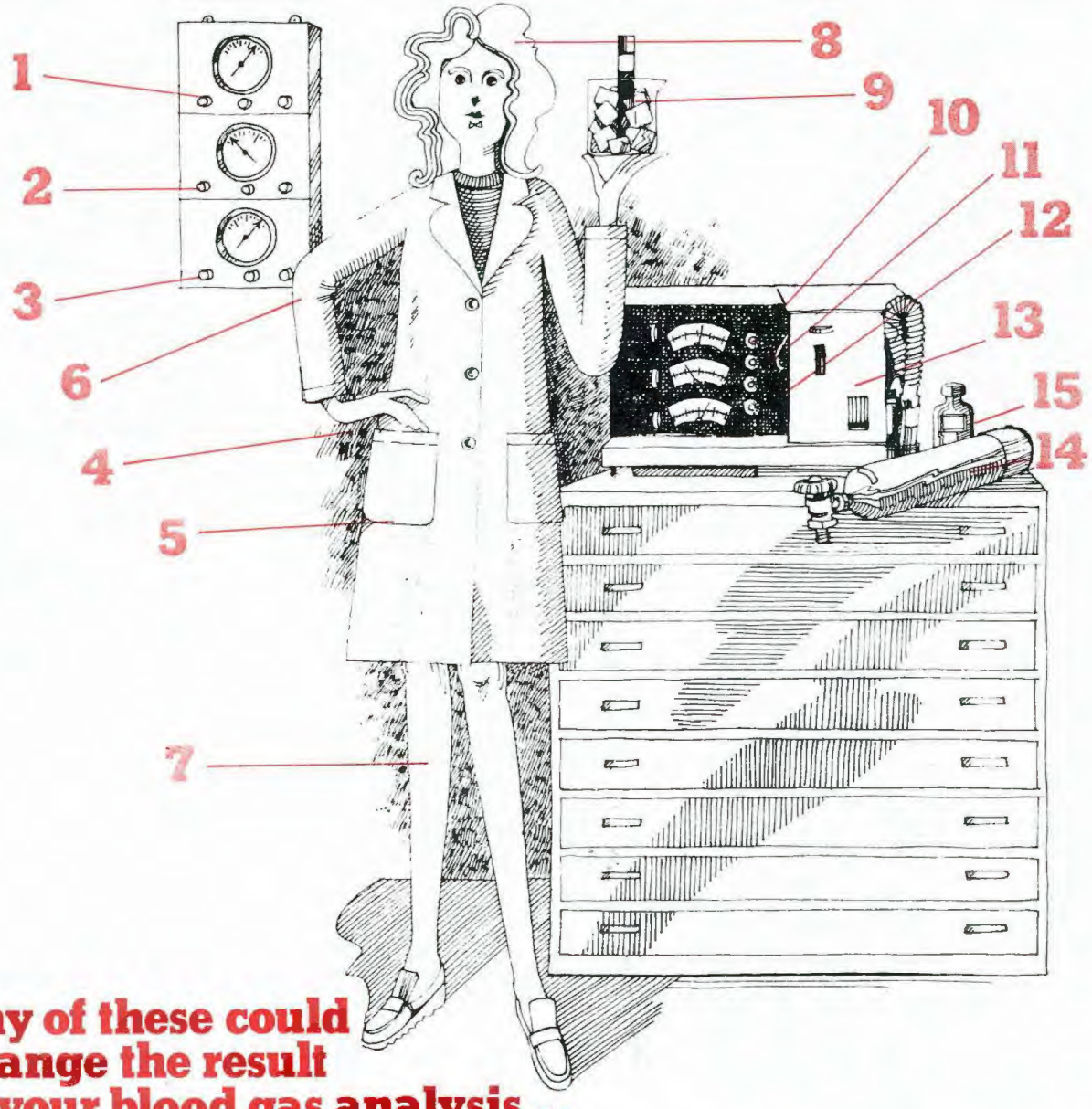


FIGURE 4.—Differing strengths of triethanolamine solution plotted against Absorbance.

a clear blue solution. This latter was preferred because any possible haziness would be more readily seen (although none has been seen to date), and because of its greater spectral purity.

Varying percentages of triethanolamine solutions were used and the results are shown in Figure 4. Below 20%, the proteins were not solubilised, and 60% tended to depress the amount of colour produced. A 30% solution seemed to offer a wide margin of safety.

It is plain from the above experiments, that reagent concentration may be varied within quite wide limits, without significant influence on the final result.



Any of these could change the result of your blood gas analysis...

1. pressure
2. temperature
3. humidity
4. technic
5. "miracle fabric" uniforms
6. jostling
7. nylons
8. hair spray
9. sample handling
10. protein build-up
11. damaged electrodes
12. broken membranes
13. unsaturated KCL
14. improper calibrating gases
15. contaminated buffers

..and you might never know it unless you controlled your system with Versatol Acid-Base, controls with known pH and Pco_2 values which simulate blood samples.

A practical approach to quality control: Calibrating at the beginning of each shift, no matter how carefully done, will not guarantee that everything is working properly. The answer: quality control of pH and Pco_2 in all three physiological ranges with Versatol Acid-Base. Because it is based on human serum, Versatol Acid-Base reacts in your instrument the same way as a patient sample. If you don't recover the Versatol Acid-Base value (within acceptable limits) you should anticipate that your patient values may be incorrect and therefore not reliable as a guide in diagnosis or therapy.

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serum calibration reference and control for pH and Pco_2 assays

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TELEPHONE 573-109

Blood Gas Instrument Troubleshooting Guide

Versatol®
Acid-Base System

pH Problem	Possible Cause	Corrective Action
Versatol **Alkalosis**..... **Lo** **Normal**..... **Lo** **Acidosis**..... **Lo**	1. Calibration Buffers TOO COLD	1. Allow sufficient time for calibration buffers to equilibrate to 37°C before calibration.
	2. Saline used as pH electrolyte	2. Replace saline with correct electrolyte, saturated KCl.
	3. Damaged electrode	3. Replace electrode.
	4. Instrument TOO WARM	4. Adjust instrument to 37°C.
	5. Controls TOO WARM	5. Allow sufficient time for control samples to equilibrate to 37°C before analysis.
	6. Air bubble entrapped in salt bridge (I.L.)	6. Tilt salt bridge repeatedly to dislodge and remove air bubble.
	7. High calibration buffer contaminated with blood	7. Obtain fresh buffer solution and recalibrate instrument.
	8. High calibration buffer exposed to air excessively	8. Obtain fresh buffer solution and recalibrate instrument.
Versatol **Alkalosis**..... **Hi** **Normal**..... **Hi** **Acidosis**..... **Hi**	1. Instrument TOO COLD	1. Allow sufficient time for instrument to fully equilibrate to 37°C before use.
	2. Controls TOO COLD	2. Allow sufficient time for control samples to equilibrate to 37°C before analysis.
	3. Calibration buffers TOO WARM	3. Allow sufficient time for buffers to equilibrate to 37°C before calibration.
	4. Controls exposed to air excessively	4. Obtain fresh control samples and revalidate instrument.
	5. Controls stored TOO WARM (unrefrigerated) for excessive period	5. Obtain fresh control samples and revalidate instrument. Store refrigerated.

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Blood Gas Instrument Troubleshooting Guide

Versatol® Acid-Base System

pH Problem	Possible Cause	Corrective Action
Versatol **Alkalosis**..... Lo **Normal**..... Lo **Acidosis**..... Normal	1. Damaged electrode	1. Replace electrode.
Alkalosis..... Lo **Normal**..... Normal **Acidosis**..... Lo or Normal	1. Protein contaminated electrode	1. Flush measuring electrode with cleaning solution provided by manufacturer; or with 0.1N HCl.
Alkalosis..... Hi **Normal**..... Normal **Acidosis**..... Lo or Normal	1. Low calibration buffer contaminated with blood	1. Obtain fresh buffer solution and recalibrate instrument.
	2. Low calibration buffer exposed to air excessively	2. Obtain fresh buffer solution and recalibrate instrument.
Versatol **Alkalosis**..... **Normal**..... **Acidosis**..... } No response	1. Air bubble entrapment in capillary of measuring electrode	1. Introduce new sample carefully, avoiding air entrapment in electrode capillary.
	2. Measuring electrode has become dehydrated	2. Rehydrate by soaking for several hours with calibration buffer.
	3. Open electrical circuit or poorly grounded equipment	3. Insure that all cables are properly connected, and that the instrument is properly grounded.
	4. Reference electrode junction clogged with KCl crystals	4. Carefully warm reference electrode tip in warm water until KCl solution flows freely.
	5. Damaged electrode	5. Replace electrode.
Versatol **Alkalosis**..... Lo **Normal**..... Normal **Acidosis**..... Hi	1. Controls diluted with saline	1. Introduce new control samples using proper handling sequence: SALINE-AIR-SAMPLE.

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Blood Gas Instrument Troubleshooting Guide

Versatol® Acid-Base System

Pco_2 Problem	Possible Cause	Corrective Action
Versatol **Alkalosis**..... **Lo** **Normal**..... **Lo** **Acidosis**..... **Normal**	1. High calibration gas TOO COLD, low gas temperature satisfactory	1. Readjust High gas flow to 2-3 bubbles/sec.
Alkalosis..... **Lo** **Normal**..... **Normal** **Acidosis**..... **Lo or Normal**	1. Protein contaminated membrane	1. Flush sample cuvette and membrane with 1% detergent solution. Replace membrane if problem persists.
	2. Spacer material at measuring tip not completely wetted with electrolyte solution (Corning).	2. Slide membrane back and forth to force fresh electrolyte solution in front of sensitive glass tip (Corning).
Versatol **Alkalosis**..... **Lo** **Normal**..... **Normal** **Acidosis**..... **Normal**	1. Low calibration gas TOO COLD, High gas temperature satisfactory	1. Readjust low gas glow to 2-3 bubbles/sec.
Versatol **Alkalosis**..... **Normal**..... **Acidosis**..... } **No response**	1. Air bubble entrapped in measuring cuvette	1. Introduce new control sample carefully, avoiding air entrapment in sample.
	2. Air bubbles entrapped beneath membrane tip	2. Remove air bubbles by gently tapping or shaking the electrode.
	3. No electrolyte at measuring tip	3. Slide membrane back and forth to force fresh electrolyte solution in front of sensitive glass tip.
	4. Open electrical circuit or poorly grounded equipment	4. Insure that all cables are properly connected, and that the instrument is properly grounded.
	5. Damaged electrode	5. Replace electrode.

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Blood Gas Instrument Troubleshooting Guide

Versatol® Acid-Base System

Pco_2 Problem	Possible Cause	Corrective Action
Versatol **Alkalosis**..... **Lo** **Normal**..... **Lo** **Acidosis**..... **Lo**	1. Calibration gases TOO COLD	1. Reduce gas flow rate to 2-3 bubbles/sec., to allow sufficient time to equilibrate to 37°C.
	2. Instrument TOO COLD	2. Allow sufficient time for instrument to fully equilibrate to 37°C before use.
	3. Controls TOO COLD	3. Allow sufficient time for control samples to equilibrate to 37°C before analysis.
	4. Inadequate gas line flushing	4. Allow sufficient time for adequate flushing; i.e., 5 minutes at fast flow rate before calibration.
	5. New Pco_2 electrolyte TOO COLD	5. Allow sufficient time for reassembled electrode to reach 37°C.
	6. Membrane leak	6. Replace membrane.
	7. Controls exposed to air excessively	7. Obtain fresh control samples and revalidate instrument.
	8. Controls stored TOO WARM (unrefrigerated) for excessive period.	8. Obtain fresh control samples and revalidate instrument. Store refrigerated.
	9. Controls diluted with saline	9. Introduce new control samples using proper handling sequence: SALINE-AIR-SAMPLE.
Versatol **Alkalosis**..... **Hi** **Normal**..... **Hi** **Acidosis**..... **Hi**	1. Calibration gases TOO WARM	1. Reduce gas flow rate to 2-3 bubbles/sec., to allow sufficient time to equilibrate to 37°C.
	2. Instrument TOO WARM	2. Allow sufficient time for instrument to fully equilibrate to 37°C before use.
	3. Controls TOO WARM	3. Allow sufficient time for control samples to equilibrate to 37°C before analysis.
	4. Membrane leak	4. Replace membrane.
	5. New Pco_2 electrolyte TOO WARM	5. Allow sufficient time for reassembled electrode to reach 37°C.

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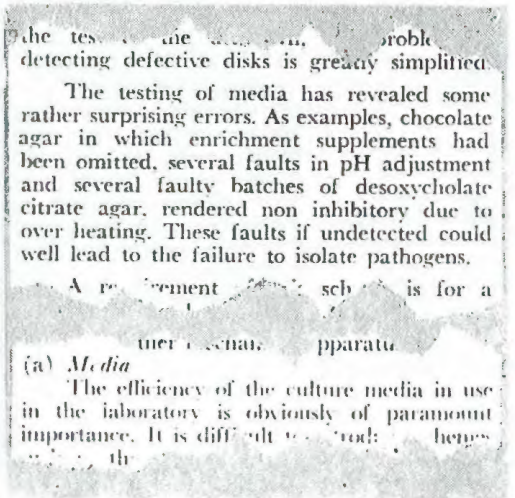

the tes[illegible] detecting defective disks is greatly simplified.

The testing of media has revealed some rather surprising errors. As examples, chocolate agar in which enrichment supplements had been omitted, several faults in pH adjustment and several faulty batches of desoxycholate citrate agar, rendered non inhibitory due to over heating. These faults if undetected could well lead to the failure to isolate pathogens.

A re[illegible] sch[illegible] is for a

ther[illegible] pparatu[illegible]

(a) *Media*

The efficiency of the culture media in use in the laboratory is obviously of paramount importance. It is diffi[illegible] hanges th[illegible]

Extract from November NZIMLT Journal by D. G. Bolitho, Pathology Dept., Cook Hospital, Gisborne.

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Wavelength

Here a compromise was struck between sensitivity and scale readability. If greater sensitivity is required, as when only small samples of serum are available or very low levels of phosphorus are being estimated, a wavelength of 680nm or 700nm may be used, and in the former case, 0.05ml of serum may be used without loss of accuracy. However, a standard solution containing 5mg phosphorus 100ml gives an absorbance of 0.250 at a wavelength of 650nm with a 1cm light path, and this seems very satisfactory. Variations in the volumes of the reagents used, are also possible, provided the concentrations are altered appropriately, and in this way also, changes in the overall sensitivity of the method are possible.

Beer's Law

Using the method as recommended, Beer's Law held up to a concentration of at least 20mgP/100ml with aqueous solutions, and to at least 18.6mgP/100ml with protein-containing solutions (these being the upper limits tested). For these experiments, 0.1ml amounts of aqueous phosphorus standard solutions containing 5, 10 and 15mg of phosphorus/100ml were added to 0.1ml amounts of serum of known phosphorus concentration, and the mixtures treated as in the proposed method using 2.9ml working reducing reagent to maintain correct volume. The results are shown in Figure 5. The expected absorbances were obtained also, indicating that recovery of the added phosphorus was complete.

Colour Stability

The colour develops immediately the triethanolamine is added and the tube contents mixed, and is stable for at least 45min. It is however, advisable to wait for about 2min after mixing, to allow the numerous small bubbles to disperse. Since the surface tension of the final mixture is very different from that of water, any water remaining in a flow-through cuvette (if one is used) will cause fluctuating readings for a short time after pouring in the Blank solution at the commencement of a series of readings. After stability is attained, there are no further delays in obtaining steady readings.

Accuracy and Precision

Using two batches of Versatol quality control sera of differing phosphorus concentrations, no significant differences from the stated values were obtained, repeated estimations giving results that in all cases came within 3% of those values.

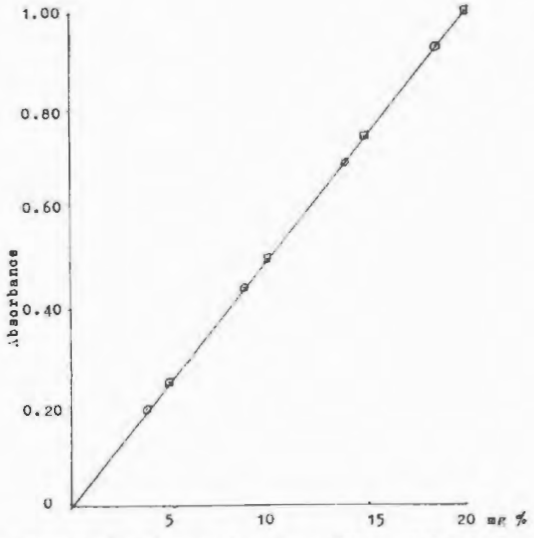

FIGURE 5.—Illustrating the validity of Beer's Law. Squares: Aqueous standards. Circles: Protein containing solutions.

Standard Deviations were calculated at two levels of phosphorus concentration, based on 25 estimations in each case, and were as follows:
At 3.4mg Phosphorus/100ml, S.D. = 0.13mg.
At 6.7mg Phosphorus/100ml, S.D. = 0.15mg.

Interferences

Lipaemia.

Only severe degrees of lipaemia produced significant opacity in the final solution, and a serum blank was then required. For this purpose it is best to replace the ammonium molybdate reagent with 0.5ml of distilled water, all other reagents being present in the usual amounts.

Hyperbilirubinaemia

Serum bilirubin levels of up to 20mg/100ml produced no significant absorption at the wavelength used, although the colour of a serum blank was a deep yellow.

In any instance where there is any doubt about possible interference, a serum blank should be set up as a check.

(See Page 114)

Table 1: Comparison of results by Gomorri's method and the proposed method, in mg Phosphorus per 100ml.

REFERENCES

1. Gindler, E. M. and Ishizaki, R. T. (1969), *Clin. Chem.* 15, 807.
2. Varley, H. (1964), *Practical Clinical Chemistry*, 4th Edit., p. 448.

Technical Communications

Assay for Muscle Phosphorylase Activity

The estimation of the enzyme phosphorylase in biopsy specimens of muscle is of use in the investigation of suspected anomalies of muscle glycogen metabolism. Phosphorylase is an enzyme involved in the degradation of glycogen catalysing the transfer of glucosyl residues from the glycogen chain to inorganic phosphate as an acceptor forming glucose-1-phosphate. This reaction is readily reversible in vitro and it is this reversed reaction that is used in the procedure of Hers and van Hoof[1]. This method as originally described is unsuitable for use in the majority of clinical chemistry laboratories. A modified method using standard equipment is described below.

Reagents:

Buffer: Citrate (0.2 M) pH 6.5 containing mercaptoethanol (0.04 M).

Substrate: Glycogen (2% W/V) glucose-1-phosphate (0.1 M) and sodium fluoride (0.2 M) dissolved in above buffer.
Sodium molybdate (2.5% W/V)
Aminonaphthol sulphonic acid (0.04%)
Sulphuric acid (1.3 M)
Trichloroacetic acid (10% W/V)
Phosphate standard (0.4 mg P/100 ml) in trichloroacetic acid (6.7%). Prepare from potassium dihydrogen phosphate and 10% trichloroacetic acid solution.

Preparation of tissue extract:

It is best to perform the following steps in a cold room (4°C) although a beaker of ice will suffice. Use 4 ml buffer (pre-cooled) per gram of muscle tissue. Homogenise finely cut tissue in a Potter homogeniser surrounded by an ice bath. Centrifuge the resultant homogenate at 3,000 r.p.m. for 10 min at 4°C. A small bench centrifuge is suitable provided it is pre-cooled in a refrigerator prior to use.

Enzymic Reaction.

Dilute the supernatant 1 in 5 with buffer. To the diluted supernatant (0.5 ml) add buffered substrate (0.5 ml) and incubate at 37°C for 10 minutes. Stop the reaction by addition of 2.0 ml trichloroacetic acid (10% W/V). Mix well then centrifuge at 3,000 r.p.m. for 5 minutes.

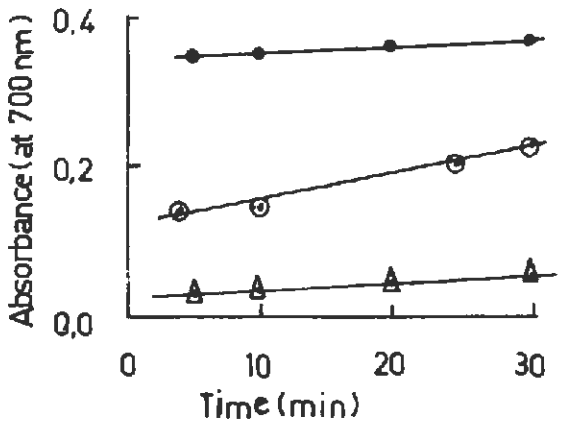

Figure 1.—Absorbance at 700 nm given by released phosphate with respect to incubation time.

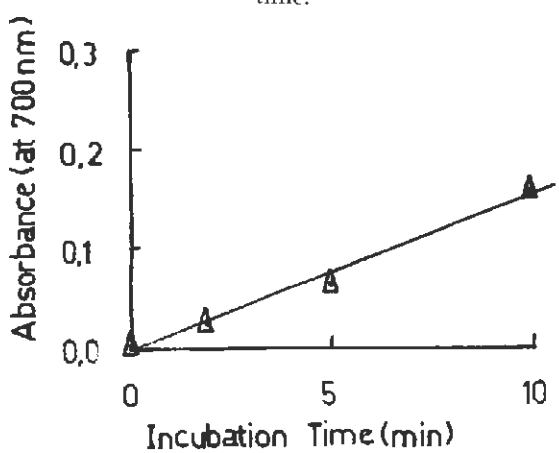

Figure 2.—Stability of colour reaction with time.

Measurement of released Phosphorus

Take 2.0 ml portions of supernatant, standard and blank. For the blank use a mixture of 1.33 ml trichloroacetic acid and 0.67 ml buffer. Add to each tube 0.5 ml H_2SO_4 (1.3 M), 0.5 ml sodium molybdate (2.5 %) and 1.0 ml aminonaphthol sulphonic acid (0.04%). Allow to stand 10 minutes then read at 700 nm.

The enzyme reaction is linear for the first ten minutes (fig. 1). It was found that the colour development increases steadily with time (fig. 2), but contributions from non-enzyme sources are small if a ten minute colour development is used. Blank values for the reaction can

TABLE I.—Phosphorylase levels as founds in fresh and post-mortem muscle specimens.

Time after death (hours)	μmol phosphorus min/gram
12–16	0.43
24	1.08
8–12	0.47
Fresh tissue	0.42

be kept low if acid-washed glassware is used throughout.

Table I gives the results of a number of phosphorylase determinations performed on both post-mortem and fresh muscle tissue.

Diana M. A. Johnson
D. M. Wilson
C. W. Small

Pathology Department, Green Lane Hospital, Auckland.

August, 1973

Reference

1. Hers, H. G. and van Hoof, F. (1966). *Methods in Enzymology* 8, 525.

Who's Who In The Institute

This is the fourth item in our series which spans a number of years and once more one of our prominent members has been prevailed upon to satisfy our curiosity by telling us something about himself. I must get more people to do this because it is interesting. To paraphrase the conventional opening remark of extra-terrestrial visitors, "let me take you to our leader".

"It's like undressing in public," he complained and I found myself idly wondering if he had a bow tie tattooed on his neck; however, he was persuaded to disgorge a few sparse details which are reproduced in the accompanying data sheet. All that one can do is to flesh them out (oh! Sorry Des!) with the aid of the imagination. I never knew he was an organist and a chorister. Is he a bass, a baritone, a tenor or what? Perhaps even a counter-tenor! Does he take part in those obstacle courses they organise at youth camps swinging over muddy creeks on ropes like Tarzan? The mind boggles.

Let's try to be serious. He obviously plays his part in the community and must organise his time well to keep up with his diverse pursuits for he keeps a large and varied stock of hats.

Des Philip, the discoverer of the metaphysical enzyme, "Institutase" which controls the growth and development of Institutes is plainly a reliable source of this vital principle, for as far as I can recollect, he has always been there. I remember when he was Treasurer. He was much younger then—in fact we all were. He kept an untidy desk and lost the Conference cheque from Dunedin. Ah well, we all make mistakes. He has continued in the service of the Institute ever since and grown in stature from year to year. I mean this seriously and

sincerely and with no intention of perpetrating a double entente. It just so happens that Des is pun-prone.

What else do we know of him? I recall a fellow member on the negotiating committee remarking that he was the first to grasp a point but apt to ruminate before giving voice to his conclusions. Certainly he shows a most impressive control of the business in Council and his habit of reflection fits him admirably for the position of Chairman. We have been fortunate of late with our choice of Presidents and par-

ticularly so at the present time.

We are grateful to Des Philip for overcoming his natural reticence and allowing us a brief glimpse into his modus vivendi.

DESMOND JAMES PHILIP

Born in Auckland 27 November, 1928.

Educated at Otahuhu and Dominion Road primary schools and Mt Albert Grammar school (1942-46). Played soccer at Mt Albert for two years, saw the light and turned to rugby which he has supported as player and coach ever since. Represented the school at athletics and also played softball and tennis. School prefect.

Commenced training as a "cadet" at the Auckland Hospital at the end of 1946 rotating through the major Auckland hospitals.

Qualified in 1952 and on qualification was placed in charge of Middlemore Hospital Laboratory (staff of four) and has remained there ever since (now a staff of 65).

1959-1965 Treasurer NZIMLT, 1961-1971 Vice-president, and has been President since then. Has been convener of the Education and Negotiating Sub-committees of the Institute and was on the committee that negotiated the major changes in the Hospital Employment Regulations (Laboratory Workers) 1969.

Has been a member of the Medical Laboratory Technologists Board since 1967 and is currently a member of the new Registration Board.

Married, five children (two boys, three girls).

Interests

Actively involved with youth work—organising camps, raising funds, etc.

Keenly interested in local church as Treasurer and Young People's Leader.

Fond of music—organist at local church and was member of Choral Society and Dorian Singers.

Keeping up with varied activities of a growing family occupies any spare time.

A spasmodic gardener who has a good spring and summer garden and a wonderful winter showing of the largest collection of weeds in the Southern Hemisphere.

Chairman of the local Hospital Canteen Committee and was leader of a committee that raised funds to build a magnificent Hospital Swimming Pool.

Chairman of the Auckland Hospital Board Canteen Association.

Note:

Has worn bow ties since the day in 1952 when he dragged a conventional long tie through a rack of slides covered with Crystal Violet. The rumour that he started wearing them after people called the other ones "Rainbow ties" because there was a pot at the end is definitely untrue.

Laboratory Reminiscences

E. L. F. Buxton

Received for publication May 1973

The Council at its Conference Meeting in August of this year, resolved to make an effort to record the details of the Institute's development and of the people involved in this from the early days.

Mr Buxton who has been retired for a number of years, is a life member of the Institute and a founder member of the Associations which preceded it.

We are greatly indebted to him for making this valuable contribution to our records.—Editor.

My earliest knowledge of bacteriological laboratories was in 1917 when I was appointed to the Veterinary Laboratory at Wallaceville to fill a vacancy caused by call-up of Mr C. S. M. Hopkirk for service overseas in the Army Veterinary Corps. At that time the whole of the original staff were on active service. Those known to me were Colonel Reid, O-I-C, Veterinary Laboratory. Mr Fred Smith (later a Senior Assistant at the Public Health Lab-

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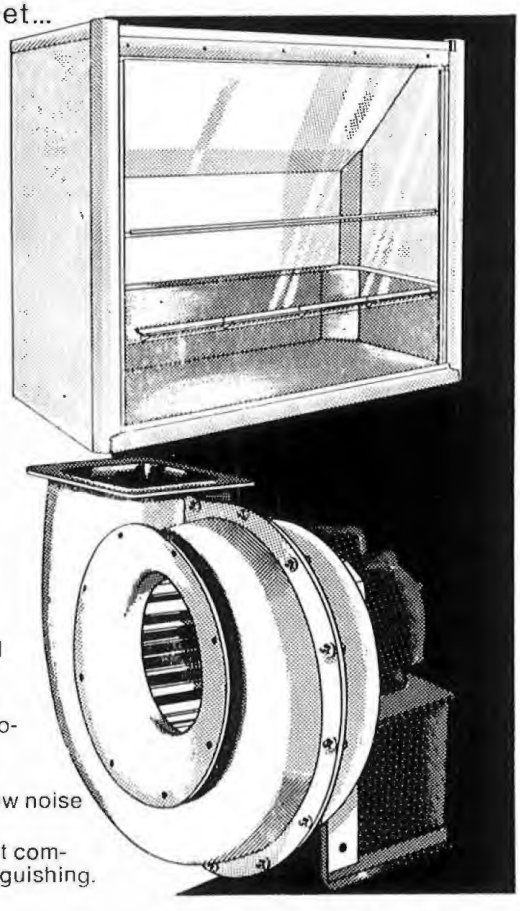

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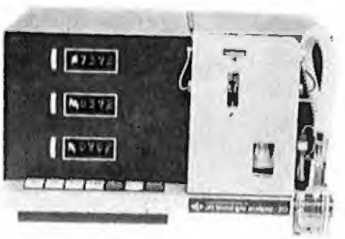

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oratory, Auckland) and Mr Cyril Hopkirk, who later graduated as Doctor of Veterinary Science and became O-I-C of Wallaceville Laboratory.

When the staff returned from the First World War, I was transferred to the Chemical Laboratory of the Department of Agriculture in Wellington. It was about this time (early 1920) that the Department of Health brought in its Bacteriological Trainee Scheme. First appointments were given to returned servicemen who had had experience in army laboratories overseas. In the latter half of 1920 I was appointed as a trainee at Wellington Hospital.

At that time Mr J. A. Hurley was Bacteriologist-in-Charge, Messrs A. B. Dore and Athol Pierard were assistant bacteriologists, and Messrs Jack Pierard, Len Cooper, William Nuttall and myself were trainees. Dr C. M. Hector was part-time pathologist. Soon after I was appointed, Jack Pierard and Len Cooper qualified as assistant bacteriologists and in 1923 Bill Nuttall and I also obtained our C.O.P. in bacteriology and clinical pathology.

For years we old-timers were very jealous of the title of bacteriologist. It was the title conferred on us by the Department of Health and when we formed the Association in 1945 we were recognised as belonging to the New Zealand Association of Bacteriologists. But, of course, the scope of hospital laboratory work has broadened considerably since those early days, and it was natural for the narrow term of "Bacteriologist" to give way to the wider designation of "Medical Laboratory Technologist".

As far as I can remember, by 1923 there were hospital laboratories in most of the main centres.

Dr Walter Gilmour was pathologist to the Auckland Hospital with George Haylock and Thelma Walker (now Mrs Dotchin) as assistants. Mr F. L. Armitage was Bacteriologist-in-Charge of the Health Department's laboratory at Auckland with Horace Holt and Fred Smith and Reginald Brooks as assistants. Then Len Haden was located at Whangarei and Jim Smith at Hamilton. Mr Phil Hicks was in charge at Napier and Mr Wilberfoss at Palmerston North. Besides the staff already at Wellington Stan Hodson seemed to have a roving commission. He used to bring in specimens from the army camps nearby and at one time he was sent to help cope with an epidemic, (? diphtheria), at Invercargill.

Dr A. B. Pearson was Pathologist at Christchurch Hospital and he brought his senior assistant, Tom Ross, with him from England.

Mr Andy Logan was bacteriologist at Invercargill and Ivor Saunders was his trainee.

Otago Medical School ran the laboratory for the Dunedin Hospital. Dr Murray Drennan was Professor of Pathology and Dr Champtaloup was Professor of Bacteriology. Bevan Brown was a member of the laboratory and managed to gain his M.D. while on the staff and later became assistant pathologist at Auckland Hospital and later still, medical superintendent at Oamaru Hospital. Other members of the staff at that time I am not sure of but Mr S. O. Jarratt and Alf Samuels were on the staff at a fairly early period, also Vince Hawke, at Apia to be later followed by Jack Peddie who later took charge of the hospital laboratory and still later by Mr Rasmussen who was still in charge when I visited Samoa in 1961.

I should have mentioned earlier that Bill Carruthers was in charge at Gisborne Hospital laboratory at this period. He was later followed by Mr M. O. Ekdahl, ex New Plymouth.

With the opening of laboratories in the provincial towns the idea of an Association for the betterment of laboratory workers was discussed by the members of the staff of the Wellington Hospital laboratory, some of whom at the time were on the pay-roll of the Hospital Board and the rest were paid by the Health Department.

Len Cooper and I worked side by side on a bench about six feet long and often got our heads together on all sorts of questions. So in the early twenties, it may have been 1923, members of the staff met one evening at the home of Dr C. M. Hector, the part-time pathologist, and discussed the formation of an Association, which became known as the "Bacteriological and Pathological Association of New Zealand (Incorporated)."

Objects of the Association, Membership, etc., were carefully considered and the "Constitution and Rules" were printed by O'Kane and McKenzie, Wellington, in 1925. The Hon. Secretary and Treasurer to the Association was Leonard F. Cooper, Bacteriological Laboratory, Hospital, Wellington.

I have a printed copy of the "Constitution and Rules" amongst correspondence dating from 1937 when a second attempt was made to form an Association as the first attempt fizzled out, as I was transferred to Auckland in 1924

and later Len Cooper was appointed to Palmerston North after Stan Hodson (who had succeeded Mr Wilberfoss) was appointed to the newly formed laboratory at the New Plymouth Hospital.

Len Cooper when writing to me in 1937 said he had plenty more copies of the Constitution and Rules of 1925 and all the other books and correspondence. Where they are now I would not know. I understand that Mrs Cooper is still living in Palmerston North.

I had a hand in the formation of what became known as the New Zealand Association of Bacteriologists in 1945.

When Stan Hodson was appointed bacteriologist to the Rangitaiki Dairy Company his place at New Plymouth was taken by Vince Hawke who had been in Samoa. Vince and I communicated several times and tried to stir up enthusiasm amongst those we met, but it was not until Vince was appointed to the new laboratory opened at the Nelson Hospital that serious negotiations took place. I have a letter dated December 29, 1936, from Vince Hawke in which mention is made of circularising members of laboratory staffs and calling a meeting of those interested. It was February 1937 before a circular was drawn up, and then the next move was to contact Mr Len Cooper for his advice. What happened after that I am not sure, but nothing definite resulted until May 11, 1945, on which date several of us met in the board room of the Wellington Hospital as a Provisional Committee to go into the question of forming an Association of Bacteriologists. There should be minutes of that meeting in the early files of the Association. I was appointed Chairman of the Provisional Executive, Mr Norm Ellison brought forward a proposed constitution and it was decided to send out a letter and questionnaire to bacteriologists—Mr Hawke was made responsible for contacting the laboratories in the South Island, and I accepted responsibility for getting in touch with those in the North Island. By June 25, 1945, we were assured of support by most of the laboratory staffs.

A meeting of Miss Winstone, Mr Hawke and myself was arranged for early in July and a decision made to call the first conference, to be held at the Wellington Hospital on August 7, 1945.

We had many teething troubles to get the Association off the ground. By the end of November, over three months later, we had only 24 financial members. Some of the larger laboratories showed very little response.

By November 1945, Mr Sutherland who had been chosen as Editor of the Journal resigned as he had been appointed to the Council of the New Zealand Institute of Chemistry. His place was taken by Mr Doug Whillans of Auckland who made an excellent Editor and proved a tower of strength to the Association over many years.

In May 1946, Miss Ernestine Winstone, our first Secretary, resigned prior to leaving for Japan to become bacteriologist to the 6th General Hospital. Fortunately, Mr S. O. Jarratt of Palmerston North was willing to take over the position, and was also prepared to organise the second annual conference to take place at Palmerston North.

Numerically, the second conference was a great improvement, and testified to the fact that the Association, at last, had become a functioning organisation.

Although I was appointed to Wellington as a bacteriological trainee under the Department of Health, there was little evidence of training, and there seemed to be an atmosphere of opposition to the scheme on the part of those staff employed by the Hospital Board. Trainees were encouraged by the Health Department to take university lectures in the appropriate sciences, but obstacles were put in the way of those desiring time off to attend those lectures, even though hours off had to be made up. At first I was the only trainee keen to do the B.Sc. degree. Work to be carried out was allotted by the senior assistant bacteriologist and often I would hang around with specimens awaiting to be done, but not given to me until a few minutes before 4 p.m. when I had to dash away to get to a lecture a few minutes later. Then because I wondered why the delay I was reported to the boss as unsatisfactory, and when a relief was required urgently to help out at Auckland, I was the one chosen for the job. That was a godsend as far as my future in the work was concerned, as it gave me an opportunity to discover other methods and gain experience which I found difficult to obtain in Wellington, and which eventually led to my being chosen to commence the laboratory at the Wanganui Hospital.

Although I was 21 when appointed to Wellington, and had had three years at Wallaceville, as I was the newest arrival I was delegated to the media making and preparation of outfits for specimens, making up of staining solutions, and going to the abattoirs up Ngahauranga Gorge to get serum for making Loeffler's media. Examination for K.L.B. was an important job in those days.

Next year I spent most of the year examining sputa for T.B. We were supposed to be able to sit for the Diploma at the end of two years, but although our syllabus was much simpler than present requirements, I had been able to make myself far from proficient in sitting the examination. The third year I did urinalysis, the occasional blood count, and some of the more senior work, but the senior members of the staff jealously kept the senior work for themselves, and if it had not been for Len Cooper I would not have been ready to sit for the Diploma even after three years. He gave me as much time as he could spare and we spent the last evening before the test going through the syllabus to see what final preparation was needed. We first sat a three-hour paper set by the Professor of Bacteriology at Dunedin. Then we had a week to do the practical examination. Fortunately in those days we could get help from text-books. Then after I had my several sheets of answers to the practical exam nicely written and underlined here and there with red ink, the oral examiner arrived in the person of Dr Maurice Watt, later Director-General of the Health Department. He looked through my practical examination results, asked me a few relevant questions and thus the agony was over, but it was six months before Bill Nuttall and I heard whether we had passed. Fortunately our increase in salary was back-dated, as we had been on £160 to £180 over the three years, and passing the examination gave us a good lift for those days. Two years later when I became married I was earning the princely sum of £260 p.a.

Three years later, at the beginning of 1928, I was appointed to Wanganui at £350 p.a. for which I had to drop £5 p.a. but I considered the ultimate salary would rise higher as the grading at Auckland had a maximum of £420 p.a. But there was no grading of hospital board bacteriologists in those days, and one had to impress the board with comparisons of salaries made by neighbouring boards—Palmerston North and Taranaki—before any increase was given. Eventually the Pathologists' Association recommended a maximum for such positions at £450, and that was where we stuck until Dr P. P. Lynch who had established a private laboratory in Wellington invited me to join his staff, promising an immediate increase of £50 with another £50 in 12 months, and a further £50 a year later. I must have satisfied the Wanganui Board by this time with my ability, because they offered me the same terms to stay. This I was pleased to do as I considered the cost of living in Wellington was equal to another £100 p.a.

So you can see the great need there was to establish an Association through which salary scales could be established, such questions as annual leave and sick leave, standardising of methods, the training of staffs in bacteriologist-controlled laboratories, refresher courses, and the general improvement of standards of work considered. The pioneers of the Association put in a lot of hard work, and present-day members of the Institute have a lot to be thankful for, and should do their best to carry on the good work for improving their own lot and those who will follow them.

Book Reviews

Ultracentrifugation. J. S. McCall, B.Sc., M.Sc., A.R.I.C., and B. J. Potter, B.S.c., M.Sc., A.R.I.C., 126 pages, illustrated. Bailliere Tindall, 798 Henrietta Street, London WC2 8QE. Price: U.K. £2.50.

The authors who describe their work as "a concise introduction to ultracentrifugation" are to be congratulated on having succeeded so ably in their purpose.

The advent of immuno-technology and its development into a relatively low cost means of characterising and estimating, sometimes with very considerable accuracy, a wide variety of macromolecules using only simple apparatus has reduced the number of occasions on which the diagnostic laboratory would arrange a sedimentation analysis. Even so the ultracentrifuge still provides the most convenient means of isolating a variety of molecules and particles in the fields of virology, immunology and general protein chemistry, and of assessing their molecular weights and so on.

The technologist or chemist who has to make casual use of ultracentrifuge invariably finds this a rather intimidating apparatus with the calculation of results a daunting procedure. In "Ultracentrifugation" the authors have produced a small, clear, logical work, wherein theory is adequate; its mathematical expression concise and easily followed. It is not cluttered with methodolgy, or descriptions of apparatus and its operation, concerning which manufacturers' literature is excellent.

Individual chapters deal with: techniques in common use; practical considerations, optical systems, normal rate separation, density gradient separation. sedimentation equilibrium, rotors and cells for special purposes, collection problems, and complicating factors. These are propounded with unusual clarity and simplicity being shorn of unnecessary verbiage whilst the mathematics necessarily involved are well within the compass of the high school graduate. A few key references are appended to each chapter.

This then is a small book advantageously limited by its size which the reviewer regards as having made a major contribution to the literature of ultracentrifugation and recommends for the laboratory library and for the bookshelves of those technologists and chemists concerned with ultracentrifugation.

—J.V.D.

Microbiology Procedures by Rose M. Morgan M.S., M.T.(A.S.C.P.), and Dorothy S. Good M.S., M.T.(A.S.C.P.), 1972. Charles C. Thomas, Publisher, Springfield, Illinois. 235 pages. Price $(U.S.)8.95.

"The Selection and Interpretation of Current Tests for Physicians, Nurses, and Paramedical Personnel"—this is the sub-heading on the title page. On first reading through the book one gets an impression of an odd mixture of facts and there seems to be little cohesion in the book as a whole.

The authors have divided the book into sections on 1. Clinical Bacteriology; 2. Mycology; 3. Virology; 4. Rickettsiology and 5. Clinical Parasitology.

Each section in turn is devoted to a description of a test procedure, identification of a micro-organism or the culture of specimens from certain sites. As an example the Indole Test is described with the following sub-headings.

Synonyms, Nurse's Responsibility, Principle of the Test, Specimen required, Preservative, Length of Time required to Perform Test, and Positive and Negative Results.

This rigid treatment of all sections seems to make the information given appear disjointed, however, under the headings of Abscess culture, Eye culture, Faeces culture and other similar sites the directions for collection of the specimen and preparation of the patient are most useful.

Under the heading of Nurse's Responsibility a list is given of materials and instruments required and then a series of instructions describes in detail how the specimens are to be obtained.

Considering the book from the medical technologists' point of view, information given regarding identification of bacteria, fungi, viruses and parasites would be better obtained from the texts devoted to these disciplines. The four pages of coloured illustrations are poor and could just as well be left out of the publication.

For the medical technologist much useful information is included on the collection of specimens and at the end of each section is a good list of references.

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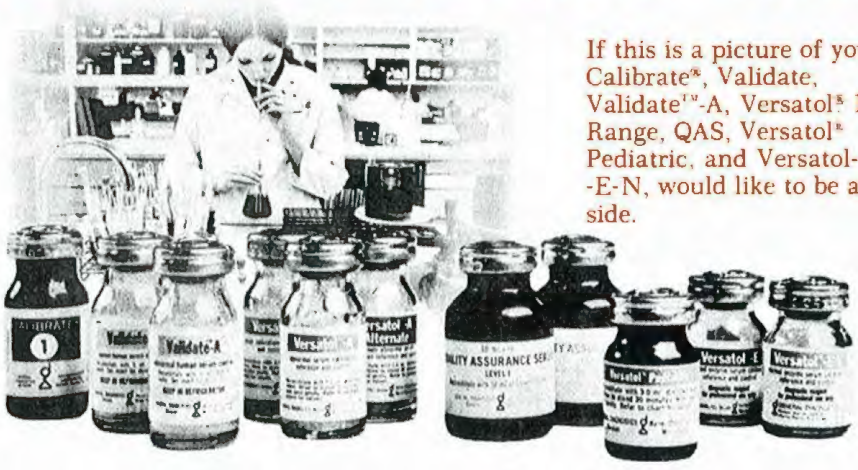

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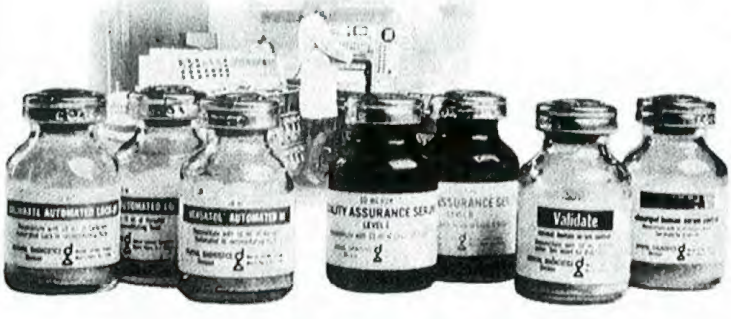

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For the physician, the nurse and other paramedical people the information regarding microbiological tests will be useful by making them aware of the limitations of the techniques and tests used.

This book can be recommended to the medical technologist for the instruction given in collection of specimens.

—G.R.R.

Servicing Electronic Laboratory Equipment. L. W. Price, M.A., C.Eng., M.I.E.R.E. (1973). 197 pages, illustrated. Bailliere Tindall, London. Price (U.K.) £2.80.

One of the problems resulting from the mechanisation of clinical laboratories is that of maintaining and servicing complex laboratory equipment. This compact little textbook is one in the series of Laboratory Monographs published by Bailliere Tindall and is devoted to helping with this problem.

Probably the ideal situation is to have professional staff available to service such equipment and this is frequently arranged, and indeed in hospital service laboratories where it is essential to keep equipment constantly in working order this is certainly desirable but perhaps a council of perfection. Many of us do find ourselves in the position of having to effect repairs, the extent and complexity of the faults tackled are only limited by the knowledge and experience of the operator.

This book will be a boon to those who have a leaning towards electronic gadgetry, those who of necessity must make repairs and also electronic engineers required to deal with laboratory equipment. It is not a simple book and requires some background knowledge of electricity and terminology but the items selected for study are very thoroughly discussed.

Initially there is a dissertation on electronic principles and fault finding. This covers resistors, valves, semiconductors, electrical contacts and insulation and the testing equipment includes the principles and use of the transistor tester, multirange testmeter and oscilloscope. Semiconductors are clearly explained with the aid of diagrams and the "tricks" of replacing and testing are revealed. The rest of the book is devoted to practical instructions for servicing specific items of equipment with the aid of detailed fault tables.

General laboratory equipment includes temperature measurement, electrical motors, vacuum systems and other electronic apparatus. The shattered remains of a high speed ultracentrifuge is illustrated. This disintegrated under working conditions due to the action of corrosive fluids which had not been washed off. The construction of a Beckman L261 ultracentrifuge is shown.

Electrochemical measuring and analytical equipment covers pH meters and other electrodes and gas chromatographs. Flame ionisation and electron capture detectors are illustrated. The method described for testing pCO_2 electrodes by current leakage, is as a matter of interest, now built into the current blood gas models.

Electro-optical measuring instruments cover a variety of lamps; tungsten, deuterium discharge, and infra-red sources. The simple EEL colorimeter, Unicam 500, 600 and 800, the Beckman recording spectrophotometer and Perkin Elmer 257 are all exemplified and illustrated. Atomic Absorption is represented by a Unicam SP 90, rather briefly. As in other cases it is noted that certain work should only be attempted by a service engineer or other qualified person.

Recording and Indicating Devices include moving-coil meters, galvanometers, moving-coil recorders and potentiometric recorders. Honeywell Recorders and XY recorders are described. Other specific equipment and information include the principle and calibration of the oscilloscope, the Geiger-Muller counter, scaling units, ratemeter, planchet counters and scintillation counters. There is an appendix of electrical and electronic data and an excellent bibliography.

—R.D.A.

Laboratory Planning. Marion J. Purvis, A.I.S.T., Grad.I.S.M. (1973). 97 pages, 8 illustrations. Bailliere Tindall, London. Price (U.K.) £2.00.

"Laboratory Planning", is the last of the six titles in the Laboratory Monograph series which deals with selected aspects of medical laboratory work.

The arrangement of the book is into three sections, initial planning, detailed planning and communications with additional chapters on safety and staff relations.

Having been involved on several occasions in this type of exercise I was curious as to how the various problems were tackled. Anyone so involved soon learns the important features and those who have not will find a

good preview of them in this book. It is necessary to consider what is ideal in the way of space and equipment and to be prepared to compromise in terms of what is possible financially or spatially. The tendency to transfer piecemeal the all too familiar design of a previous laboratory to a new building is a pitfall which must be guarded against. Design should be freshly considered in regard to the actual work requirements, work flow, equipment and staff. Special situations arise when heavy equipment needs supporting or is too bulky to move in by the usual means of access; balances require vibration-free mountings; toxic and radioactive elements call for special facilities often defined by statute or local regulations.

The author states in the preface that it was not her intention to recommend this or that material for a specific purpose but to point out the advantages and disadvantages of a number of systems.

I was rather disappointed to find that the information was too scanty and not sufficiently discursive to achieve this aim.

Details of different finishes on floors and benches would have been useful. What about a good lino on a bench for example? It is comfortable to work on, slightly yielding for glassware, deadens noise and can be easily replaced when worn. Definite advice on specific matters would have been welcome too. How high should a bench be for sitting at and for standing beside? What about knee room for sitting? What actual space is required between bays and in corridors? What is the best basic arrangement of benches as a spatial configuration? What is a sensible number of power points? The possibility of movable benches with plug-in services for redeployment is another interesting possibility. All sorts of questions come to mind but are not, alas, answered.

Most things receive a brief mention but in this small book only about half the pages are concerned with laboratory planning.

There are many books dealing with safety precautions and staff relations and here again the brief dissertations on these topics really do not do them justice.

What is said, is however, sensible enough and could prove helpful to an inexperienced person confronted with the complexities of laboratory planning. —R.D.A.

Microbiology. A Programmed Presentation. Second Edition. W. J. Payne, Ph.D. and D. R. Brown, Ed.D. 283 pages (illustrated). C. V. Mosby Co., St. Louis (1972). Price $6.75(NZ), N. M. Peryer Ltd., Christchurch.

This is a programme of self-instruction designed to introduce the student to the fundamentals of microbiology. It takes the form of a series of exercises in which the student is required to fill in the missing word or words and so complete the sentence.

The text covers the growth and physiology of micro-organisms, antimicrobial methods, pathogenicity and resistance, microbiology of foods and milk, water and air and the useful chemical activities of micro-organisms.

The reader is reminded by the authors that to reap maximum benefits one must work at a comfortable speed, writing in the answers before revealing to oneself those that have been provided. It is stressed that after completion of an exercise the reader makes use of the references provided and increases his knowledge by further reading.

I had the feeling that the authors had difficulty in choosing alternative answers to some questions. Here are a few examples:—

" a hypersensitive skin test similar to the tabernacle/tuberculin (circle one) tests is very useful."

" If a soluble antigen is added to test cereal/serum (circle one) containing specific antibodies . . . "

" It may also be useful to note a gradual rise in titer in a series of agricultural/agglutination (circle one) tests that occure as the patient recovers ".

In these examples I think the reader would have little difficulty choosing the correct answers whether he was conversant with the subject or not.

I think that this book is of limited value to the medical laboratory technologist who is connected to a training programme although it may be useful for a first-year student or as a revision course for examination candidates. Only 43 pages are devoted to the description of specific organisms including spirochaetes, acid-fast organisms, fungi and Gram-positive and negative organisms. Due to the practical nature of our work perhaps greater emphasis should be placed on this aspect.

The book's aim is to inspire further reading and study and if this is achieved its purpose is fulfilled.

—M.J.

Histopathology: Selected Topics. Edited by H. C. Cook. Published by Bailliere Tindall. 296 pages. Price $5.25 (N.Z.)

This is one of the first of the new era books describing histological techniques, and will no doubt set the pattern for future publications. It consists of nine chapters, written by 10 technologists, each an "accepted authority" in his field, and covers a range of topics including techniques for amyloid, chromosome studies, neuroglia and microglia, marrow aspirations, urine cytology, lipids, fluorescent microscopy and a chapter on the history, preparation and types of waxes.

In general the pattern is to present a review of historical and current views on the subjects presented, followed by the techniques being used by the authors. The chapter on amyloid covers the classification of amyloid diseases, structure and ultrastructure of amyloid, chemical composition, staining reaction and fluorescent techniques and polarisation methods. The techniques for chromosome studies commences with the classification of human chromosomes according to the Denver Study Group and Chicago System of Nomenclature followed by tissue culture reagents and lymphocyte, marrow and fibroblast culture methods. Sex chromatin methods are also discussed.

An interesting chapter on "Organ transplantation and the Histology Laboratory" is presented by Brenda D. Disbrey which is concerned mainly with renal transplants. The range of expected findings in these patients, includes fungi and viral inclusions. The usual basement membrane and routine staining techniques are discussed.

Lipid diseases and lipid identification techniques are discussed in detail. Marrow aspirate and bone biopsy techniques include lytic and non-lytic methods of fixation and a methacrylate embedding technique. A history of urine cytology is included and the results of a comparison of three fixatives for preserving urine prior to Millipore filter preparation is given and the chapter is well illustrated with both cytology and histology photomicrographs of normal and abnormal cells. The chapter on fluorescence covers auto-fluorescence of tissues and pigments, formaldehyde-induced fluorescence and several fluorescent staining techniques.

All chapters have a list of references and an adequate index is provided. It is a book which can be picked up and read for interest or can be used for consultation, or as a bench book.

—D.T.

Laboratory Microbiology. 2nd Edition. L. Jack Bradshaw, Ph.D. 311 pages, W. B. Saunders Company, Philadelphia 1973. $5.25 (NZ), N. M. Peryer Ltd., Christchurch.

Irrespective of the place of employment the reader of this book will have a timely message regarding the value of setting out basic techniques in relation to microbiological experiments. Although the author would agree that his work is no substitute for Bergey's Manual of Determinative Bacteriology it is clear from the outset that the ultimate aim is a better understanding of such a manual.

Perhaps a major disadvantage may be experienced outside of the author's campus, namely that the whole layout of the book was intended to correlate with a set course. Nevertheless, the publication may well be adapted to a basic laboratory practice in the Polytechnic or similar teaching situation. Supervision of the work supporting the layout would be essential otherwise the active student mind might well be tempted towards guesswork.

Greater emphasis should have been placed on the role of gene determinants and should a further edition be forthcoming this would find a place alongside the relatively short third chapter.

In this day and age of ecology action the comprehensive experiments in chapter five are worthwhile. Although not covering the questions of destruction and inhibitions of micro-organisms at any real depth the fundamental principles of viability and surival are clearly related.

The fact that the author has made a proper reference to "Bergey" adequately covers the chapters devoted to the taxonomy of micro-organisms in general.

The filing puncholes in the bound copy make it clear that so many pages are included in readiness for practical benchwork (no doubt increasing the cost of the book), in the local situation, a good idea.

If one supports the idea that fundamentals are still the basis of good teaching then the little book is worthwile in the beginner's hands.

—H.C.W.S.

Selected Abstracts

Contributors: D. G. Bolitho, M. Jeannette Grey, J. Hannan, A. G. Wilson

CHEMICAL PATHOLOGY

Serum Lipids and Lipoproteins in Children from Families with Early Coronary Heart Disease. Tamir, I., Bojanower, Y., Leutow, O., Heldenberg, D., Dickerman, Z. and Werbin, B. (1972). *Archs Dis. Childh.* **47**, 808.

It is now well established that individuals with certain forms of primary hyperlipoproteinaemia have an increased risk of developing coronary heart disease at an early age, and that the risk is greatest for those with familial hyper-β-lipoproteinaemia (Type II). If therapy designed to lower the serum β-lipoprotein concentration is to be effective in preventing or delaying coronary heart disease in these patients, it is probably important to start treatment in childhood.

Serum lipids and lipoproteins of 64 men who survided for more than 6 months after a myocardial infarction, and of their children were investigated. Twenty-six fathers and all their 55 children had normal serum lipids and lipoprotein patterns. Thirty-eight fathers had normal serum lipid concentrations and abnormal lipoprotein patterns, of these, 23 had hyper-β-lipoproteinaemia, 11 had increases in both β and pre-β-lipoprotein, and four had increases in pre-β-lipoprotein only. Thirty of the 85 children whose fathers had abnormal lipoproteins were found to have Type II hyperlipoproteinaemia regardless of the type found in the father.

—J.H.

Quantitative Determination of the Alphafoetoprotein (AFP). Hirsch-Marie, H. and Huguet, C. (1972). *Digestion* **7**, 156.

Quantitation of AFP by Laurell's unidimensional electroimmunodiffusion method was carried out on 81 patients, 71 of whom had a hepatoma.

This technique allowed the detection of as little as 1mg/litre of AFP. There was a great diversity in serum levels: 1-1,880mg/litre. The lowest levels (1-100mg/litre) were the most frequent. After hepatectomy, the serum AFP drops rapidly; sometimes it may even disappear. The rate and duration of this decrease depend on the remaining AFP-secreting tumoural hepatocytes.

Other authors have detected, in 11 normal subjects, levels of serum AFP from 4.0 to 10.5 μg/litre by radioimmunoassay using free and antibody-bound ^{125}I-labelled AFP. Thus, it would seem that the suppression of the foetal gene responsible for AFP production is not complete in the adult.

—J.H.

The Influence of Quality Control Programs on Evaluation of Laboratory Data: Some Statistical Observations on Reference Sera. Riddick, J. H., Jr. (1973). *Human Path.* **4**, 31.

Several investigators have recently questioned the accuracy of manufacturers' assay values for pooled human serum preparations as calibrating standards and quality control materials. Although the printed values accompanying each lot of this material are said to represent the pooled results of a number of reference laboratory assays, the accuracy of these printed results cannot be assessed because of turbidity, lack of primary standards for enzymes, and the impossibility of the use of water or other simple solvent standards because of viscosity and dialysis problems inherent in multichannel analysers.

In this study, significant differences in the performance of the SMA 12/60 concurrent with a change in lots of calibrating serum indicate that, at least in the case of two manufacturers, printed values provided by the manufacturer cannot be relied upon for accuracy in calibrating the SMA 12/60.

—J.H.

Possible Error Resulting from Use of "Nitrogen Filled" Vacutainers for Blood Gas Determinations. Long, G. E., Mueller, R. G. and Hunt, P. K. (1973). Letter to Editor. *Clin Chem.* **19**, 559.

This letter and its reply makes interesting reading. It is concluded that nitrogen-filled vacutainers are not suitable for PO_2 estimation.

—A.G.W.

A Proposal for a More Uniform Output in Laboratory Data. Lo, J. S. and Keller, J. A. (1972). *Clin. Chim. Acta* **41**, 239.

The authors suggest a simple mathematical conversion which gives one single set of numbers for all laboratory tests with fixed values for upper and lower "normal limits". This "clinical unit" system would eliminate the multiple "normal values" now existing between hospitals and might allow clinicians to compare laboratory results more easily.

—A.G.W.

Determination of Amylase Activity in Serum and Urine Using Blue Starch Substrate. Irie, A., Hunaki, M., Bando, K., and Kawai, K. (1972). *Clin. Chim. Acta* **42**, 63.

The authors investigated the Phadebas Amylase test and showed that it is simple and reproducible. They found the colour produced is stable and not affected by haemoglobin, bilirubin or sugars. The addition of albumin to protein-positive urines enabled reliable amylase determination.

—A.G.W.

Disadvantages in the Use of Animal Serum for Quality Control of Serum Iron Binding Capacity Estimation. Butler, V. (1973). *Clin. Chim. Acta* **43**, 451.

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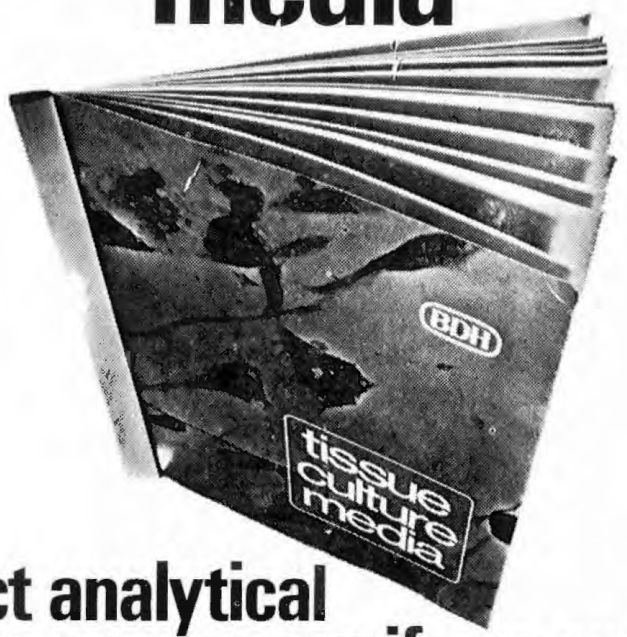

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Discrepancies in the results of serum total iron binding capacity estimation were obtained when using freeze dried control sera of animal origin. (Well-comtrol-Burroughs Wellcome). The author's investigation showed that increasing amounts of magnesium carbonate lowered the iron binding capacity of fresh and freeze dried bovine sera but not human sera.

-A.G.W.

The Attainment of Increased Sampling Rates in Continuous Flow Analysis. Pennock, C. A., Moore, S. R., Collier, F. M. and Barnes, I. C. (1973). *Med Lab. Technol.* 30, 145.

This article shows in simple terms how to analyse a standard profile and effect improvements to auto-analyser manifolds. The authors suggest that regular analysis of the standard profile may be a useful quality control guide as worn pump tubes or unauthorised manifold changes are detected before results deteriorate.

—A.G.W.

Trial of a New Flowcell for Continuous Flow Analysis. Tweedie, A. K. (1973). *Med. Lab. Technol.* 30, 183.

An improved flowcell for use in continuous flow systems made by Fisons Scientific Apparatus has been evaluated. It is a solid one-piece unit with a de-bubbler immediately adjacent to the light path so that the length of unsegmented stream is reduced to a minimum.

—A.G.W.

Anomalous Response of Macroamylase to Assay Temperature. Henderson, A. R., King, J. and Imne, C. W. (1973). *Clin. Chem.* 19, 123.

An anomalous response to increased reaction temperature is described for two samples of macroamylase, as contrasted with normal serum and pancreatic amylase. A possible method of differentiating macroamylase by assays of activation at 45°C and 25°C is suggested. The ratios of activities found at these two temperatures for a normoamylasemic population are given.

—A.G.W.

Radioimmunoassay Review. Skelley, D. S., Brown, L. P. and Besch, P. K. (1973). *Clin. Chem.* 19, 146.

In this review the principles of radioimmunoassay are described. Methods are outlined for preparing radiolabelled antigenic compounds and antibodies. Commercial suppliers of various components needed for radioimmunoassay are listed. A list of normal values for hormonal and nonhormonal values for humans is given.

-A.G.W.

Australia Antigen Content of Commercial Quality Control Sera. Simon, R. G., Langhofer, L. A. and Hendrik, E. J. (1973). *Clin. Chem.* 19, 221.

The Australia antigen content of 36 sera was determined for radioimmunoassay. Twenty-three of the 36 sera were strongly positive for Australia antigen. Most of the control sera tested are available in New Zealand.

A.G.W.

Method for Determination of Cyclic A.M.P. in Plasma. Rabinowitz, B. and Katz, J. (1973). *Clin. Chem.* 19, 312.

Cyclic adenosine 3' 5' monophosphate (cAMP) plays a key role in numerous physiological and pathological processes. Its assay is likely to become of considerable clinical interest. A simple radio-immunoassay procedure is described for measuring cAMP based on competitive binding of cAMP by a protein in crude adrenal extracts, and precipitation of the cAMP protein complex by ammonium sulphate.

—A.G.W.

HAEMATOLOGY

HEMPAS: Congenital Dyserythropoietic Anaemia (Type II). Verwilghen, R. L., Lewis, S. M., Dacie, J. V., Crookston, J. H. and Crookston, M.C. (1973). *Quart. J. Med.* 42, 257.

This paper presents 39 cases of hereditary erythroblastic multi-nuclearity associated with Positive Acidified Serum test (HEMPAS) or congenital dyserythropoietic anaemia Type II. The haematological findings are described in detail.

The absolute reticulocyte count and the leucocyte and platelet counts are generally normal. Red cell morphology included marked anisocytosis with some irregularly contracted cells, tear-drop poikilocytes and stippling as well as an occasional erythroblast (exceptionally binucleated).

The red cell osmotic fragility was slightly increased in 10 out of 20 patients. Bone marrow showed marked hyperplasia of erythroblasts which formed 50 to 90 percent of all nucleated cells. The Ham test was positive in all 39 patients, but not with patients' serum and only with about one-third of normal sera.

-M.J.G.

Erythrocyte Deformation as a Sign or Renal Homograft Rejection. Guevarra, A. K., Morita, Y. and Reyman, T. A. (1972). *Transplantation* 14, 683.

In 1968, Lichtman *et al.*, described a haemolytic anaemia characterised by the presence of many "schistocytes" or fragmented and deformed RBCs in the peripheral blood of patients undergoing renal homograft rejection. In the present report, data from eight patients who rejected renal homografts and seven patients with functioning kidneys provide further evidence for this entity.

Using Wright's stain, schistocytes were counted in 20 oil immersion fields along with total RBC counts in the same fields. These values were averaged to arrive at the percentage score for schistocytes. Areas with rouleaux, poor cell distribution, and scratches were avoided.

In two control groups, the schistocyte count did not exceed 1 percent, with a few exceptions (in which the percentage was 1-2). In patients rejecting renal homografts the percentage of schistocytes rose to as high as 9.1. A significant number was considered to be 2 percent. These cells appeared as small, rounded or irregular fragments, helmet- or triangular-shaped, blister cells or very pale "exploded" forms.

—J.H.

A Macromolecular Serum Component Acting on Platelets in the Presence of EDTA—"Platelet Stain Preventing Factor". Stevem, P. and Berg, K. (1973). *Scand. J. Haem.* **10**, 202.

The EDTA blood of an apparently healthy person was found to cause weak, pale staining of platelets with May-Grunwald Giemsa, PAS and Sudan Black stain. This occurred after the EDTA blood was kept at room temperature for more than 30 minutes before blood films were made. Twenty control bloods in EDTA for two hours at room temperature showed a variable degree of impaired staining of platelets but an impairment of staining of a degree comparable to that of the original patient's platelets was never observed. The "platelet stain preventing factor" did not agglutinate platelets. After extensive investigations relating to this plasma factor, the nature of this macromolecular serum component remained unknown.

M.J.G.

Lysozyme Activity and Nitroblue-Tetrazolium Reduction in Leukaemic Cells. Catovsky, D. and Galton, D. A. G. (1973). *J. clin. Path.* **26**, 60.

The cytochemical methods for lysozyme and nitroblue-tetrazolium reduction have been used to study the blast cells of acute myeloid leukaemia. Both proved useful in characterising the cases with predominant monocytic differentiation.

The demonstration of lysozyme activity helped to define two main groups: (a) with predominantly lysozyme-negative cells (myeloblastic-promyelocytic), and (b) with considerable numbers of positive cells (monoblastic-monocytic). In addition this test was also of value in the differentiation of other leukaemic disorders. Reduction of nitroblue-tetrazolium was also a feature of monocytic differentiation. The combination of these two methods with those for myeloperoxidase and non-specific esterase activity contributes to the cytological characterisation of acute myeloid leukaemia.

—M.J.G.

The Observer Error in Peripheral Blood. Bacus, J. W. (1973). *Amer. J. clin. Path.* **59**, 223.

Coloured photomicrograph (Kodachrome) transparencies of the six types of leucocytes commonly classified in a differential were shown to 11 senior haematology technicians in the United States of America for independent assessment. The testing involved identification of 1,041 individual cells images. When the segmented and band neutrophils were lumped together as one class, the resulting average observer error per type of leucocyte was approximately 1 percent. The error in classifying segmented versus band neutrophils was estimated at about 7 percent per cell type for those observers with the least systematic bias or attitude toward naming one of these cell types preferentially. In fact, in this particular series of tests, all of the 11 observers had similar basic discriminatory ability, and all came from the same laboratory.

—M.J.G.

Studies on the Diagnostic Significance of Haemoglobin F Levels. Newman, D. R., Pierre, R. V. and Lineman, J. W. (1973). *Mayo Clin. Proc.* **48**, 199.

Alkali denaturation tests for foetal haemoglobin in peripheral blood were done on 193 adults having bone marrow examinations. Of these, 87 were found to have HbF values greater than 3.1 percent. Highest levels were 25.7 percent in a patient with polycythaemia vera and busulfan-induced marrow hypoplasia; and 19.1 percent in a case of myelomonocytic leukaemia (MML). Three other cases of MML had HbF levels between 7.5 percent and 11.5 percent. Most of the cases tested were myeloproliferative disorders. Three cases of blastic transformation of chronic granulocytic leukaemia showed values between 6.9 percent and 13.7 percent. Patients with benign haematological disorders (e.g., deficiency states) did not show the raised levels of HbF. The mechanism responsible for this erythrocytic response in serious marrow disorders is unknown.

—M.J.G.

Paroxysmal Nocturnal Haemoglobinuria (PNH): Clinical Manifestations, Haematology and Nature of Disease. Dacie, J. V. and Lewis, S. M. (1972). *Ser. Haemat.* **5**, 3.

Diagnostic Tests for P.N.H. Jenkins, D. E. (1972). *Ser. Haemat.* **5**, 24.

These two papers form part of a collection of nine papers on various aspects of P.N.H. The first paper is a review of present knowledge of this disease and deals with such fields as factors which precipitate attacks of haemolysis, the haematology of P.N.H., the association of P.N.H. with other blood diseases, and evidence for P.N.H. being a stem cell disorder.

The second paper is an excellent review of the Ham acid haemolysis test, the thrombin modification of that test and the sucrose haemolysis test. Methods, interpretation of results, technical sources of error and mechanisms underlying these tests are all well reviewed.

—M.J.G.

A Comparison of Serum Folate Estimations Using Two Different Methods. O'Broin, J. D., Scott, J. M. and Temperley, I. J. (1973). *J. clin. Path.* **26**, 80.

The two methods referred to are, the routine autoclaving and *L. casei* method (Waters and Mollin) and the method with no autoclaving but using a

chloramphenicol-resistant strain of *L. casei*. These authors obtained results from the "chloramphenicol technique" that gave a 20 percent higher mean result than the values from the other method mentioned above. It is suggested that autoclaving may destroy some folate despite the presence of ascorbate, or some folate may be bound and precipitated with protein.

—M.J.G.

Thrombocytosis and Thrombocythemia: the Laboratory and Clinical Significance of an Elevated Platelet Count. Davis, W. M. and Ross, Alicia O. (1973). *Amer. J. clin. Path.* 59, 243.

One hundred consecutive patients with platelet counts greater than 500,000 per cu.mm. in a 16 month period were studied. The counts were performed by phase contrast microscopy although the initial clue to the presence of thrombocytosis often came from judgments of increased platelets in the blood film. The largest group of thrombocytosis (36) came from malignancies. The second largest group (15) were recent splenectomy cases of varying types. Eight cases of well-documented iron deficiency anaemia showed increased platelet counts.

—M.J.G.

MICROBIOLOGY

Isolation of Aeromonas Species from Clinical Sources. McCraken, A. W. and Barkley, R. (1972), *J. clin. Path.* 25, 970.

During one year in one hospital 14 isolates of *Aeromonas hydrophila* were made. This is a reflection of: either increased awareness of the possibility of this organism being a significant pathogen or a reflection of the fact that it is becoming increasingly pathogenic in itself. This paper is in accordance with the findings of several other workers. The paper gives clinical descriptions of the 14 cases and a resume of the biochemical testing and identifying features of *Aeromonas hydrophila*.

This study serves a useful purpose in stressing the value of carrying out the oxidase reaction on fermentative as well as oxidative organisms isolated in routine clinical laboratory.

—D.G.B.

The Localisation of Viable Bacteria in Tissue. Messrs P. D. and Hunt, A. C. (1972), *J. clin Path.* 25, 1000.

This technical note describes a very simple technique for the localisation of bacteria in cryostat sections. With the exception of a cryostat no special equipment is needed.

—D.G.B.

Stimulation of Growth on a Deficient Medium of a *Klebsiella aerogenes* species by Sulphonamide, Para-aminobenzoic acid, Nitrofurantoin and Some Other Substances. McGhie, D., Hutchinson, J. G. P. and Finch, R. G. (1972), *J. clin. Path.* 25, 976.

A strain of *Klebsiella aerogenes* is described which was unable to grow on direct sensitivity test (Oxoid) with 5 percent lysed horse blood. Growth on a deficient medium was promoted by sulphonamides and nitrofurantoin as well as by para-aminobenzoic acid, para-aminosalycilic acid and some other substances.

The organism is interesting for those interested in studying transferable drug resistance and also in pointing out the limitations of all disk diffusion sensitivity testing in that its sensitivity to various antibiotics could be varied at will by varying the content of thiamine of the medium. It is of even more importance to those laboratories who still carry out relatively uncontrolled disk sensitivity test techniques by either dropping large numbers of disks on a single plate or using rings containing disks at intervals. A perusal of this paper by any laboratories using such a method should encourage them to adopt the methods of either Kirby-Bauer or Stokes, as one illustration to this paper shows sulphamethoxazole/trimethoprim stimulating the growth of this organism in the centre of a mast-ring, thus giving the appearance of sensitivity to the eight antibiotics at the periphery of the ring.

—D.G.B.

Identification and Characterisation of *Moraxella phenylpyruvica*. Snell, J. J. S., Hill, L. R. and Lapage, S. P. (1972). *J. clin. Path.* 25, 959.

This paper describes the isolation of the organism *Moraxella phenylpyruvica* from human clinical material derived from a variety of sites. A description of the identifying characteristics of the organism and tests used in identification are given. The organism appears to he a low grade pathogen.

The authors also discuss the taxonomic position of this organism.

—D.G.B.

A Further Study of Strontium Selenite and Selenite F Broths for the Isolation of *Salmonella typhi*. Chau, P. Y. and Forrest, C. R. (1973). *J. clin. Path.* 25, 966.

This is a report on a comparative trial of the efficiency of Strontium selenite and Selenite F. Strontium selenite was introduced by Ivsen and Shelley (1969) and was reported to be superior for the isolation of Salmonellae to Selenite F. In this study 625 stool specimens were examined and the earlier reports of the greater effectiveness of strontium selenite were confirmed. An additional benefit was that fewer non-lactose fermenting nonpathogenic organisms were isolated from the strontium selenite medium. It appears that strontium selenite media may well have a place in the isolation of Salmonellae and it should come into more wide-spread use when commercial preparations become available.

—D.G.B.

Factors Contributing to the Microbial Contamination of Cold Water Humidifiers. Airoldi, T. and Litsky, W. (1972). *Amer. J. med. Technol.* 38, 491.

This paper stresses the dangers of contamination of the water supply in cold water humidifiers. This danger has been extensively documented in the last two or three years. The paper considers only one model of cold water humidifier which may or may not be widely used in New Zealand. The authors suggest that besides the use of sterile distilled water in these units and thorough cleaning; that ethylene oxide sterilisation should be resorted to. If it is in

fact the case that this is the only way which the problems of this particular type of humidifier can be overcome it seems to this reviewer that the time has come to radically review the design of these units.

—D.G.B.

Hydrolysis of Casein—A Differential Aid for the Identification of *Serratia marcescens*. Salisbury, W. A. and Likos, J. J. (1972). *J. clin. Path.* 25, 1083.

The authors suggest the use of a skim milk medium as an additional identifying test for *Serratia marcescens*. They have discovered that 97 percent of strains of *Serratia marcescens* hydrolyse casein. The only other member of the Enterobacteriaceae to hydrolyse casein is *Proteus mirabilis* and confusion between these two organisms is unlikely to arise. The medium is simple to prepare and this may well be a useful additional identfying characteristic for nonpigmented Serratia strains.

Experiences with a screening test for Bacteria. Brupacher, R. and Dominque, G. (1973). *Amer. J. clin. Path.* 59, 552.

An elaborate comparison of the testuria miniculture method with conventional quantitative cultural techniques for determining bacteriuria is presented. The authors use sophisticated statistical analyses to demonstrate that the testuria screening test is suitable for use in screening patients attending a urological outpatient clinic but that in a mass screening programme its reliability was insufficient.

—D.G.B.

A Comparison of Three Anaerobic Systems for the Isolation of Anaerobic Bacteria from Clinical Specimens. Kilgore, G. E., Starr, S. E., Del-Bene, V. E., Whaley, D. N. and Dowell, V. R. (1973). *Amer. J. clin. Path.* 59, 552.

A comparison of three anaerobic systems for the isolation of anaerobic bacteria from clinical specimens. These were an anaerobic glove box including materials for handling and incubating specimens in an inert atmosphere of nitrogen, hydrogen and CO_2 plus the necessary media held in a prereduced form and an integral air lock which could be used to facilitate an anaerobic introduction of the specimens. This system was compared with a prereduced roll tube technique using commercially prepared medium and the BBL Gas-pak system. The gas-pak system compared very favourably with the much more expensive and time-consuming anaerobic glove box. The advantages and disadvantages of each system are fully discussed.

—D.G.B.

Selective Media in the Isolation of Tubercle Bacilli from Tissues. Mitcheson, B. A., Allen, B. W. and Lambert, R. A. (1973). *J. clin. Path.* 26, 250.

A comparison of the results of direct culture on Lowenstein-Jensen slopes and on three media made selective for tubercle bacilli by the addition of antibacterial agents and of guinea-pig inoculation is presented. The media were Lowenstein-Jensen slopes without potato starch, selective 7H11 oleic acid albumin agar with Malachite green. Elective liquid Kirschner medium with Phenyl red but without penicillin and a selective media containing polymixin B, Carbenicillin, Trimethoprim and Amphotericin B. Only 15 of the 490 tissue specimens proved positive and from this fairly limited number the authors conclude that the most efficient culture media was the selective 7H11 medium which is not only superior to the other culture media but to guinea-pig inoculation.

—D.G.B.

Identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by Carbohydrate Disk Technique. Stacey, P. and Warner G. (1973). *J. clin. Path.* 26, 303.

This technical note describes a simple method of testing the carbohydrate fermentation reaction of these organisms on Thayer-Martin medium by using disks saturated in the appropriate carbohydrates.

D.G.B.

A Controlled Trial of the use of Dip Slides in General Practice for the Diagnosis of Urinary Infections. Maskel, R. (1973). *J. clin. Path.* 26, 181.

A comparison of the results of duplicate dip slides, one being incubated in a bacteriological laboratory and the other being incubated in a general practitioner's surgery and interpreted by him. It was found that 87 percent of those found to be positive by the laboratory were also found to be positive by the general practitioners. It is suggested that the cost of providing small incubators to general practitioners would be outweighed by the speed and saving of laboratory time which could be gained if they carried out these procedures for themselves.

—D.G.B.

Selectagerm—A New Approach to Clinical Bacteriology. Greaves, P. W. (1973). *J. clin. Path.* 26.

The author uses a novel approach to the isolation and identification of bacteria by using Repli dishes. These square dishes are divided into 25 small squares. The small containers are each filled with 2ml of various selective culture media—10 media in all are used. Clinical material, usually swabs is inoculated into nutrient broth which is then used to inoculate the squares of the individual culture media. The technique is an interesting variation but with the high cost of Repli dishes in New Zealand and the high cost of media preparation would appear to be prohibitively expensive.

D.G.B.

Simple Economical Anaerobiosis. Barton, A. P. and Winzar, J. A. (1973). *J. clin. Path.* 26, 238.

A simple economical method of anaerobiosis is described using plastic bags and iron wool with acetic acid and a wetting agent to maintain an anaerobiosis. The authors claim that using this method anaerobic conditions sufficient to enable the growth of *Clostriduim tetani* can be maintained.

—D.G.B.

MISCELLANEOUS

A Review of the Male Factor in 231 Infertile Couples. Van Zyl, J. A. (1972), *S. Afr. J. Obstet. Gynaec.* 10, 17.

This article is a presentation of findings based on a study of 23 infertile couples with a 56.3 percent conception rate arising out of 126 apparently normal women. A new technique for the sperm count is described, and a modified classification for the evaluation of semen is explained. Abnormal sperm morphology was found to be the most important parameter of semen and in most cases abnormal sperm morphology was coupled with abnormal motility. The importance of repeated semen analyses and chromosomal analyses is stressed. When the count was below 10 million/ml there was a reduction in the conception rate.

—J.H.

What's New?

STABLE-ISOTOPE "TRACERS"

With increased availability and better detection methods, compounds labelled with C-13, N-15 and O-18 are finding many new applications in biochemistry, medical research and clinical diagnosis, as safe alternatives to radio-isotopes.

More than 100 standard chemical compounds labelled with atoms of the naturally occurring non-radioactive isotopes C-13, N-15 and O-18 are now available from the British company *Prochem BOC*, London SW19 3UF. Many other labelled compounds can be synthesised to order. Isotope percentages up to 90 percent for C-13, 99.9 percent for N-15 and 20 percent for O-18 can be achieved.

It is only in recent years that the problems involved in the very-high-efficiency distillation of tiny quantities of liquefied gases have been overcome, thus enabling these stable isotopes to be produced commercially. Equally important are sensitive new instrumental techniques for detecting and estimating the isotopes after they have passed through one or more reactions. Methods now available include mass spectrometry for absolute determination of concentrations; pulsed NMR (nuclear magnetic resonance) spectroscopy for obtaining structural information; and most recently—a highly sensitive technique based on the optical emmission spectrum of nitrogen gas, for assessing N-14/N-15 ratios.

Medical Science

In medical research and human biochemistry, C-13 can be detected almost as readily as the familiar radio-isotope C-14, and is completely safe. There are no radiation hazards, so there is no objection from the patients' angle. It is hoped, for example, to develop routine diagnostic tests for metabolic disorders using C-13 labelling. A possible mass screening test for diabetes involves feeding labelled glucose, then analysing CO_2 in breath samples.

N-15 is even more important, for the radio-isotopes of nitorgen are all short-lived. Current studies using N-15 include protein and amino acid metabolism, the longevity of red blood cells, the action of certain drugs, and the part played by uric acid in gout.

Further information from: PROCHEM/BOC Ltd., Deer Park Road, London SW19 3UF, England.

Varian Associates today introduced the first low-cost nuclear magnetic resonance spectrometer designed specifically for 13C analysis. Called the CFT-20, the instrument is smaller, less expensive and easier to operate than any previous 13C spectrometer. It includes an integral computer that supervises NMR experiments, analyses the data and presents the results on an X-Y plotter. The CFT-20 costs $44,500—less than a third the price of the machines now being used for 13C work. Deliveries will begin in midsummer.

Nuclear magnetic resonance spectroscopy is a technique for determining molecular structures. A sample of the molecule under study is placed in a very strong magnetic field and irradiated with electrical energy at radio frequencies. Under these conditions, certain kinds of atomic nuclei in the molecule will "flip"—that is, they will change their orientation with repect to the magnetic field. The frequency at which a nucleus flips indicates the immediate chemical environment of the atom: a nucleus that is part of a methyl group will flip at a particular frequency, a nucleus in a carbonyl group at a different frequency, a nucleus in a cyanogen group at still another frequency, and so forth.

Only certain types of nuclei will flip in a magnetic field, and NMR spectroscopy can be used only when those atoms are present in the unknown molecule. Fortunately, both carbon and hydrogen—the two most important constituents of organic compounds—have isotopes that flip. In the case of hydrogen, it's the common isotope 1H, which makes up more than 99 percent of the hydrogen in nature. But for carbon, the flipping isotope is the relatively rare 13C, which accounts for only about 1 percent of natural carbon.

Scientists have known for two decades that NMR spectroscopy could be used to analyse both the hydrogen and the carbon structure of organic molecules. But most of the NMR work done to date has been on hydrogen. Because the required carbon isotope is relatively rare, and because it flips under much lower energy inputs, a spectrometer for carbon NMR must be 5,700 times more sensitive than one for hydrogen. As a result, carbon NMR has been practical only for highly skilled scientists, working with instrument arrays costing as much as $150,000.

Varian's new system incorporates a number of important advances to overcome this 5,700-to-1 handicap, yet it costs only $44,500—less than one-third the price of previous systems. And its sensitivity is unprecedented in 13C instruments.

"This means", says Varian's Dr Shoolery, "that many more scientists will be able to work directly with the skeletal carbon structure of organic molecules instead of just the outer covering of hydrogen.

"We expect the CFT-20 to make some important contribution to the science of NMR spectroscopy itself, as well as to our knowledge of large organic molecules -eventually even the natural polymers such as enzymes, sugars and starches and nucleic acids."

For further information contact: Mr Richard Galetti, Varian Associates, 611 Hansen Way, Palo Alto CA 94303.

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(Addendum Page 97)

Sample No.	Gomorri's Method	Proposed Method	Sample No.	Gomorri's Method	Proposed Method
1	3.2	3.4	11	2.9	2.8
2	3.6	3.6	12	2.7	2.6
3	3.7	3.7	13	3.1	3.0
4	3.8	3.7	14	3.8	3.7
5	3.4	3.5	15	3.8	3.6
6	5.1	5.0	16	7.0	6.8
7	3.7	3.6	17	3.3	3.0
8	3.1	3.2	18	2.8	2.7
9	3.3	3.3	19	4.1	4.1
10	3.0	2.9	20	8.1	8.3

Table 1: Comparison of results by Gomorri's method and the proposed method, in mg Phosphorus per 100ml.

INDEX TO VOLUME 27

Directions for Contributors

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

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

Illustrations

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

Nomenclature and Units

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

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

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

N.B.:

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

2. No space should be left between the symbol for a prefix and the unit. A space is left between the symbols in derived units,
e.g., ms = millisecond
m s = metre x second
Where ambiguity could arise abbreviations should be written in full.

3. **Numbers.** The decimal is indicated by a full stop. Commas are not used to divide large numbers but a space is left after every third digit.
A zero should precede numbers less than unity. Units which give a number between 0.1 and 1000 should be chosen when possible.

References

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

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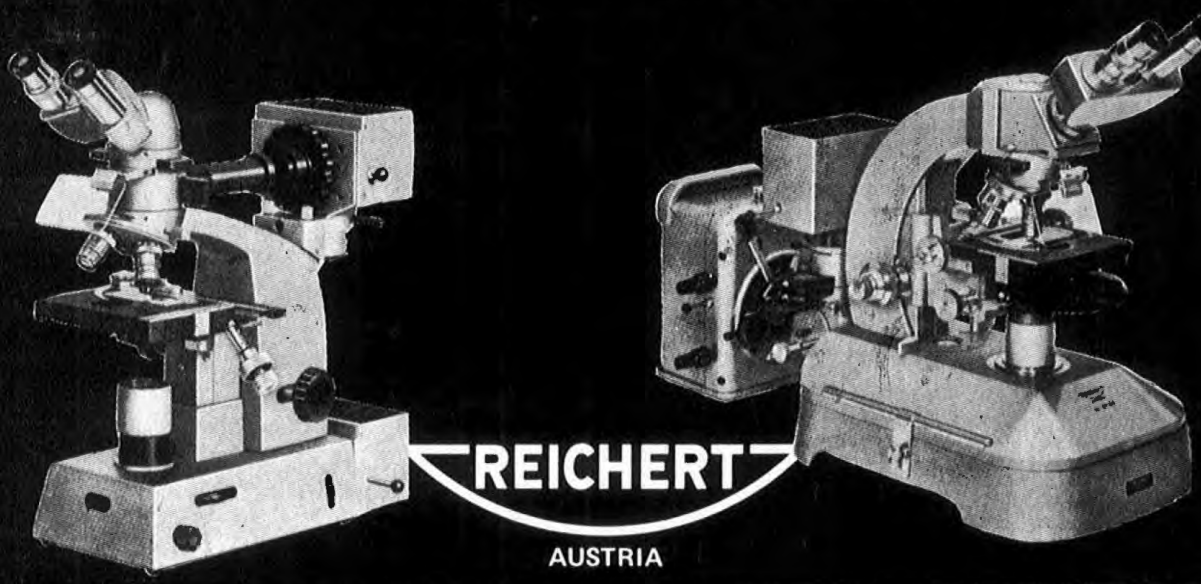

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