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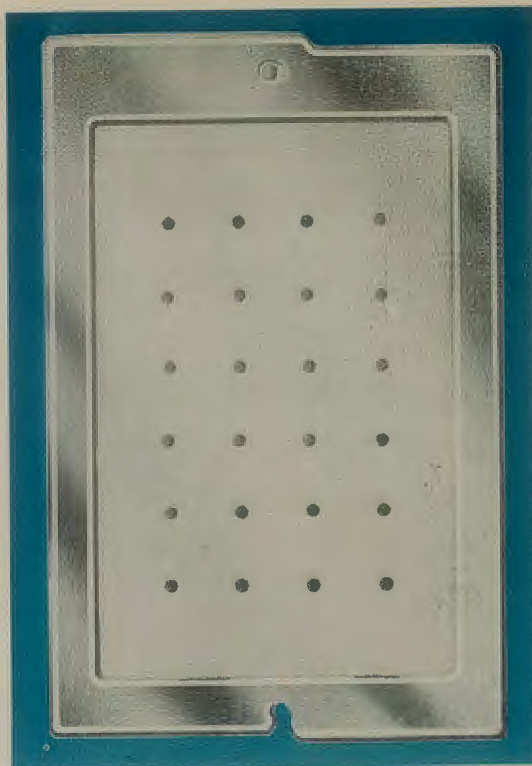

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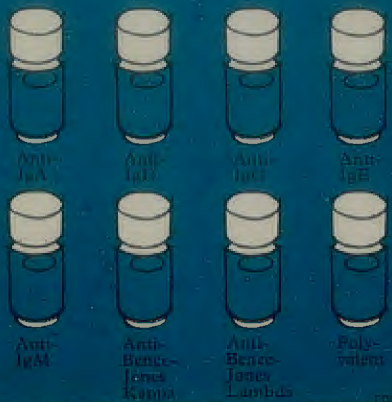

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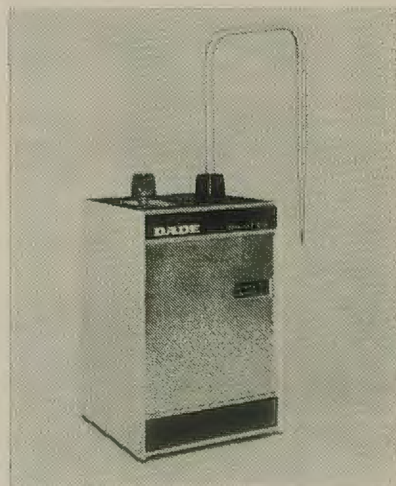

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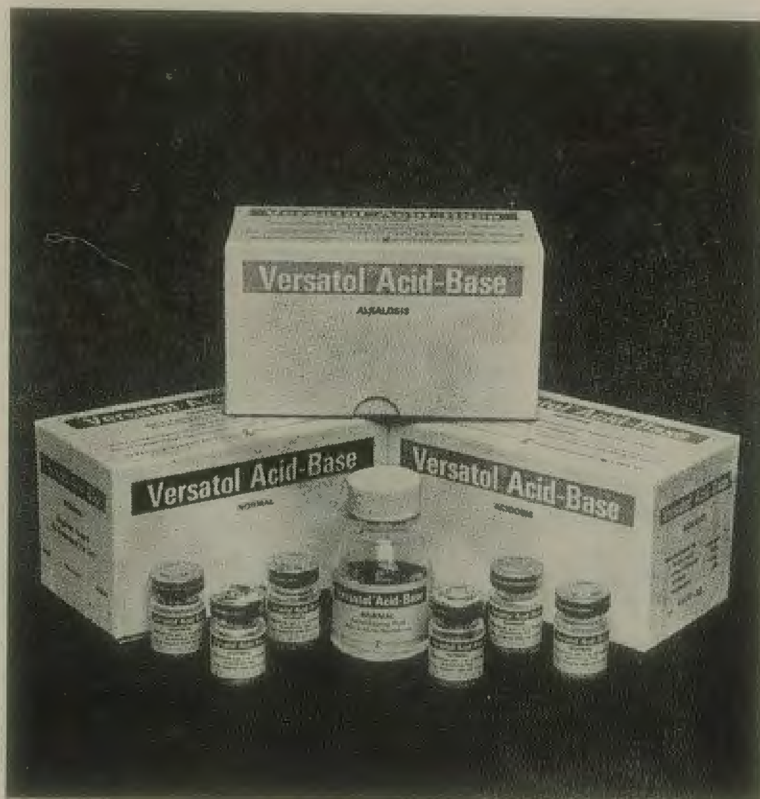

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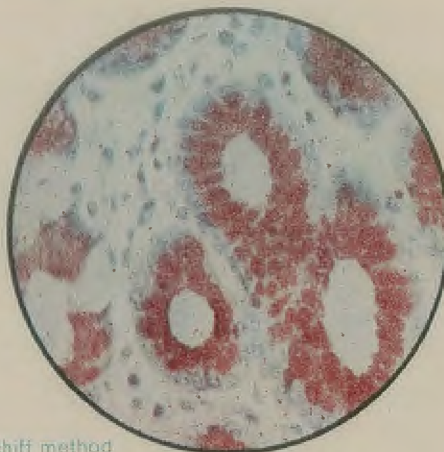

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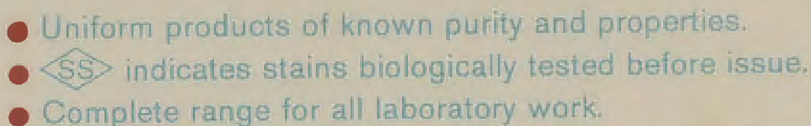

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Volume 26, No. 1 March, 1972

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

Anomalies

One of the literary devices used in science fiction is the assumption of the existence of plural probability; thus any possibility not only can. but does happen in an endless series of parallel worlds. Slight erosions in the fabric of these parallel worlds could produce co-existent states and explain a lot of contradictions and anomalies. (Close that space-time continuum, I can feel a draught!) This could explain enthusiastic attempts to overpopulate the globe and equally enthusiastic attempts to make life on the planet impossible. In another sphere it could explain how claims of a healthy economy and satisfactory balance of payments can be correlated with a million unemployed.

At a parish pump level we might thus reconcile the much heralded increase in leisure with reductions in our annual leave!

We also have the issue of Registration. It too presents anomalies. In the nature of living things there is growth and development and this applies to organisations as well as individuals. We have reached a stage in our corporate development where we are aware of our contribution in maintaining the laboratory service and as an emerging profession are striving to define our legitimate status and rightful place in the scheme of things. There is certainly a gap between our conception of the situation and that propounded by the Society of Pathologists and a further difficulty in reconciling this official view with individual opinions which many of us have diligently sought. In these days of change, re-examination and reappraisal, when even secondary schoolchildren are campaigning to exert an influence on their curricula, a more flexible and conciliatory attitude would have been appropriate. As it is the issues are clouded. There are problems in multiple staffing of laboratories in regard to relative responsibility, job content evaluation and interaction. In a profession as universal as medicine, problems and their solutions reflect universal practices. Various groups have their own recognised education, organisation and status. Graduates engaged in clinical chemistry and physics may have qualifications gained elsewhere and usually own allegiance to professional bodies at home and abroad. Technical assistants and similar groups should have some form of training, certification and job evaluation as in the United States. The Institute has on its own initiative gone as far as it can in remedying this situation.

Medical Technologists who form the largest group are now urged to safeguard the public interest by becoming a registered body. This is implicit in the establishment and maintenance of standards of education and training. This is a straightforward and separate issue and need not be complicated at this stage with related problems.

It is timely to consider our position in regard to other para-medical groups. Most of those groups possess Registration Boards the constitution of which is naturally of interest. It is to these groups that we must look for community of interest and mutual support. Events in any case have taken such a turn as to make this course desirable, perhaps inevitable. For one thing, we learn that a common set of conditions of employment regulations is being drafted and that these will have to be negotiated on a group basis. The vexed question of curtailed leave usually founders because of lack of unanimity. For another, the presentation of a case for negotiating committees or tribunals calls for more skill and resource than one group can muster or afford since professional assistance is required. The issue of membership of the Combined Hospital Employees Organisation will come up again at our annual conference. Sober thought should be given to the problem now.

R.D.A.

Quality Control and Fault Finding in an Automated Laboratory

A. G. Wilson, A.I.M.L.T., A.N.Z.I.M.L.T.
Chemical Pathology Laboratory, Dunedin Hospital.

Received for publication April 1971.

Introduction

One of the most important aspects of a clinical laboratory is the accuracy and precision of its work.

In recent years, surveys have shown that the standard of work in many laboratories is unsatisfactory. Tonks[3] suggested that if results are to be clinically significant, errors should not exceed one quarter of the normal range, and we have provisionally adopted this as a workable hypothesis.

With the advent of automated analysis, where large numbers of tests are processed, control of precision and accuracy becomes increasingly important.

Continuous flow analysis, such as the Autoanalyser system, has a number of special problems peculiar to its mode of action and methods of quality control need to detect errors which can occur randomly throughout a run.

In this laboratory, conventional methods of quality control were found to be inadequate for detecting some of these errors. Over the past year a more comprehensive scheme has been gradually introduced and as a result of this improvements have occurred.

Apparatus and Methods

The laboratory is largely automated, processing approximately 150 specimens a day, of which about 80 to 100 are for electrolytes, urea and creatinine, the remainder for liver function and enzyme tests.

The 14 Autoanalyser channels in use are Generation 1 and peaks are read manually.

The control system now in use, consists of a number of methods to aid fault finding.

1. Known Controls:

With every batch of tests, commercial known control sera are included as the operator's check of accuracy. If these controls are outside prescribed limits, i.e. 2 Standard Deviations (S.D.) of the best monthly performance to date, the run is stopped and an attempt made to find the fault.

The known controls are processed early in the run as an initial check of accuracy and plotted on charts which are displayed in the laboratory.

2. Unknown Controls:

These are included in every batch of tests and the results over a monthly period give an indication of the precision of the method.

The mean and standard deviation of the known and unknown controls are logged daily over a period of a calendar month. For comparative purposes, the coefficient of variation (C.V.) is then calculated, i.e. $\frac{\text{S.D}}{\text{mean}} \times 100 =$ C.V. % and these are displayed on a notice board.

When a significant improvement has occurred, the working limits for the known control are narrowed.

3. Blind Controls:

This method is not often used because it is time consuming and difficult to operate. To be successful, no one should know of its existence. A pool of sera is dispensed in vials sufficient for a day's use. Each sample is given a fictitious name, a laboratory number and processed as a patient's test.

The report forms are intercepted at the dispatch desk.

The information given by this series of control samples is the unbiased precision of the laboratory and can be affected by errors not usually detected by other control sera, for example clerical errors.

4. Independent Laboratory Controls:

This laboratory has utilised freeze dried sera submitted for analysis by the American College of Pathologists survey scheme. This provides an additional check of accuracy from an independent source.

5. Pooled Sera for Drift Control:

In continuous flow analysis, a phenomenon called drift occurs. This may be sporadic or gradual and can be caused by a variety of reasons. To check for this, a pool of sera is placed after the standards and in every fifth sample cup and compensation can sometimes be made when reading results. If a serious drift is noticed early in a run, the analysis is stopped.

6. Daily Means:

Daily mean data is calculated for all automated tests where 20 or more are processed. This method of control, suggested by Hoffman and Waid[2], is based on the hypothesis that 90% of the tests done are normal and have a Gaussian distribution.

When calculating daily means, truncation limits are used to exclude grossly abnormal values. Some truncation limits are shown in Table I and are those suggested by Whitehead and Morris[5].

Daily means for most tests, especially electrolytes, fall within remarkably narrow limits with correct operating conditions. The data is calculated as soon as possible after a run has been completed. The running mean is calculated from the previous month's daily means and results outside of 2 S.D. are used as an indication for action.

7. Logging of Absorbancies:

For some methods where control is sometimes difficult, e.g. enzymes, it was found useful to log the absorbancies of the standards to compare day to day variations in controls and standards. This often resulted in detection of anomalies.

8. Control Officer:

The control officer is a key figure in ensuring the success of a control programme. Initially it is necessary to explain the aims of the operations of the programme to the staff and gain their confidence and co-operation. The information should be gathered and evaluated before reports are issued. Any problems associated with quality control are referred to him and it may be necessary to analyse the autoanalyser charts critically or make exhaustive enquiries to trace a fault. This is often a very difficult and unpleasant task as there is a tendency for operators to regard the investigation as a reflection on their work.

Sources of Errors

1. Standards:

On a number of occasions, standards have been found to be wrong, especially when a number of pipettings are required. We have found that in order to reduce the frequency of errors in preparation, one person should be responsible for their preparation.

Occasionally some standards have been found to deteriorate on keeping.

Cholesterol standards in one instance, resulted in low controls and daily mean values. Being made up in isopropanol, they had probably evaporated as fresh standards rectified the error.

Commercial control sera used for enzyme standards have caused considerable trouble, mainly as a result of incorrect reconstitution or dilution.

Bilirubin standards deteriorate rapidly if exposed to light. Improvements in the precision of enzymes and bilirubin tests has occurred since separate vials have been reconstituted daily and those for bilirubin stored in a dark box.

As mentioned before, the logging of absorbancies of standards has helped to control their preparation.

2. Reagents:

Reagent preparation is a common fault. For some Autoanalyser tests, the strength of a reagent is critical. With careless preparation, a colour reagent may result in reduced span and therefore sensitivity. Faults in reagents are not always made known to a control officer, but can sometimes be traced when precision deteriorates, particularly when a less competent operator takes over a series of tests.

Other reagent faults can occur when reagent bottles are not frequently washed out. An example of this has occurred with the urea method. The thiosemicarbizide in the colour reagent tends to settle out in the stock bottle and can be carried along the transmission tubing and become deposited in the flow cell, causing 'noisy' peaks.

Noise can also occur when reagents have not been filtered. Filters are now available for insertion in reagent lines and are to be recommended.

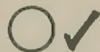
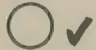
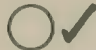
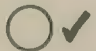
3. Apparatus:

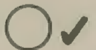
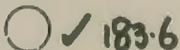
The dialyser is the most common cause of trouble, especially when aqueous standards are used to compare with sera. The use of pooled sera at regular intervals in a run is often the only indication of a dialyser fault. Membranes 'conditioned' by use in one method may not be satisfactory for another. Fig. 1 shows an autoanalyser chart for serum iron. Although dialysis is satisfactory for the aqueous standards, the sera has not been satisfactorily dialysed.

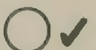
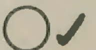
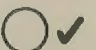
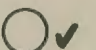
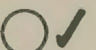
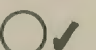
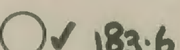
Dialysis trouble for the tandem calcium phosphate method has been largely eliminated by changing the membranes before a run, and in the iron method pre-treatment with diluted HCl seems to help.

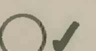
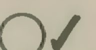
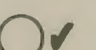
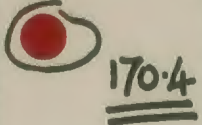
Occasionally the heating baths fail to maintain the correct temperature. When the 95°C bath overheats in the glucose method, the peaks are diminished. Overheating baths in the enzyme tests can cause havoc when plasma coagulates and causes blockages.

In the alkaline phosphatase method, low absorbancies were found for the standards, but not the controls when the 37°C heating bath heated to 45°C.

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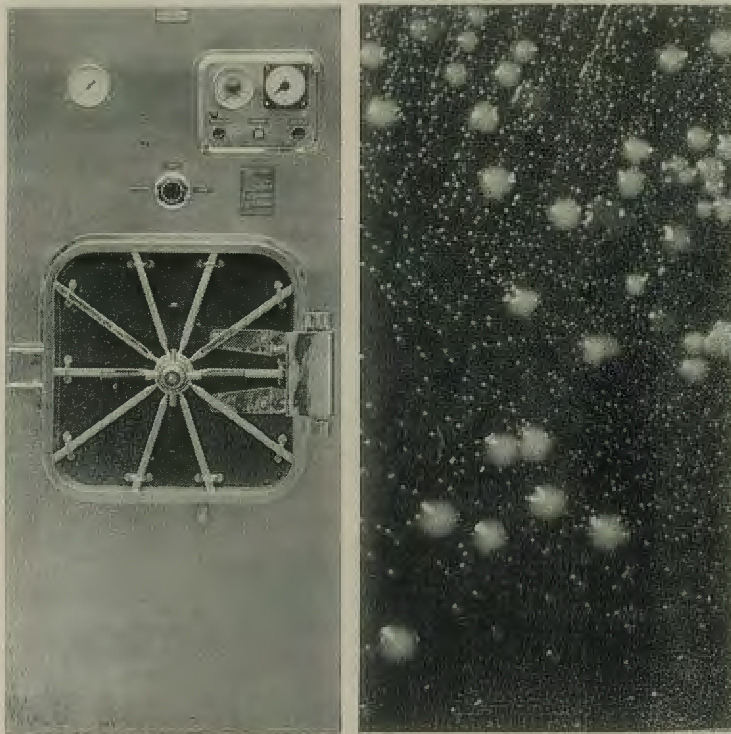

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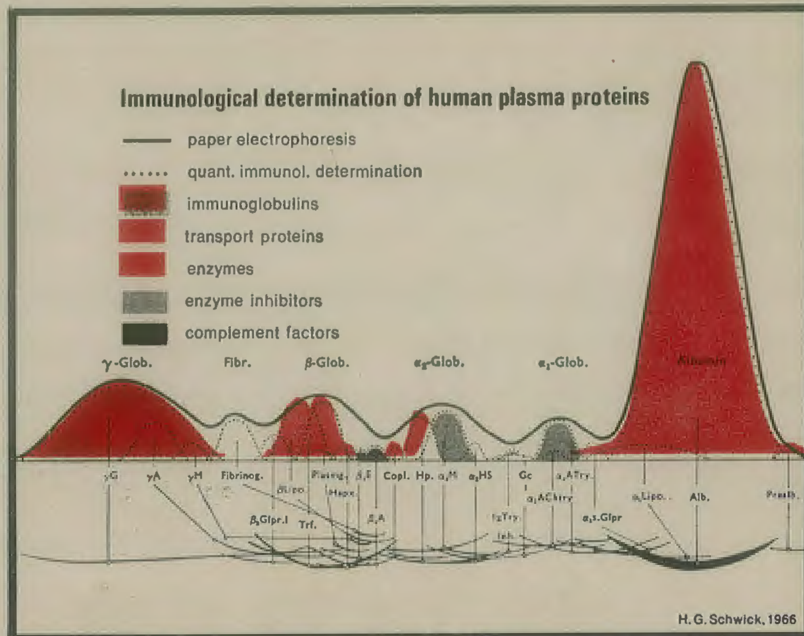

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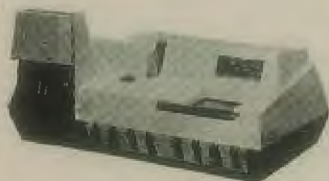

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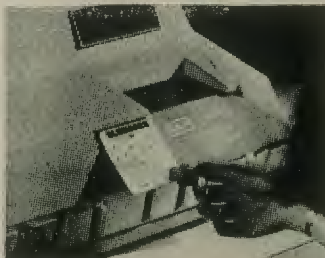

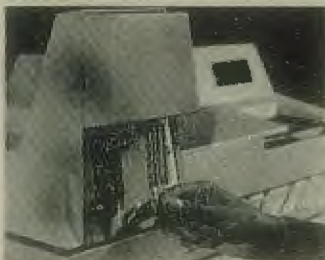

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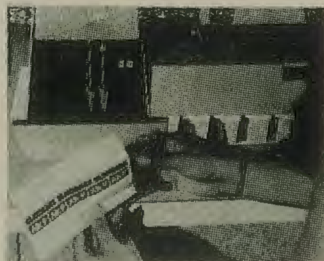

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Table I

	LOWER LIMIT	UPPER LIMIT
Na	100	200
K	1.5	10
Cl	50	140
Urea	5	80
Creatinine	0	2.0
Albumin	1.5	6.0
Protein	2.0	10.0
Bilirubin	0	2.0
Alk. Phos.	5	100
Transaminase	5	100
Calcium	4	18
Phosphate	1	10
P.B.I.	3	15
Cholesterol	100	500

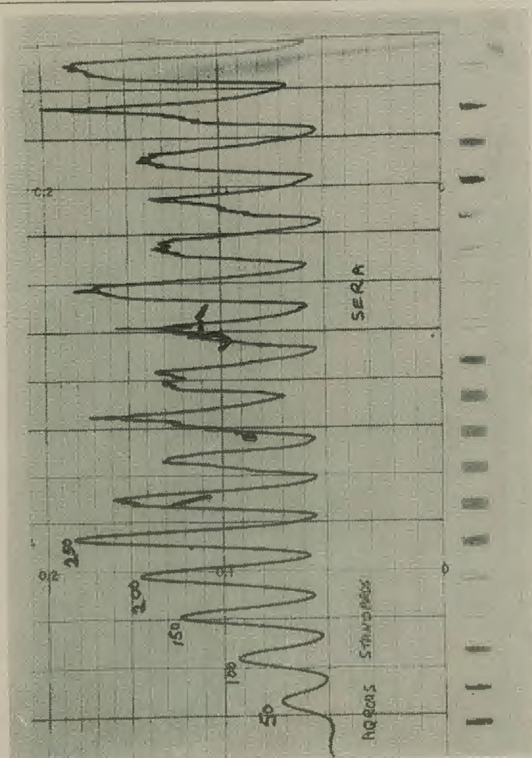

Figure I. Serum iron chart with aqueous standards and sera showing effect of a faulty dialyser membrane.

A faulty pump affected the precision of bilirubin, C.P.K., calcium and phosphate, for a time. It was found that the rollers were defective, and had ground away the side rails on one side of the pressure plate.

A sampler gave trouble, when odd shaped peaks and gaps were found to be caused by a fault in a sampling cam.

Automatic diluting apparatus has been the source of some errors. Worn parts were found to be responsible for a diluting error in the glucose method, and a 'Zipette' fault resulted in a higher daily means and controls for cholesterol. It was found that the barrel of the 'Zipette', delivering isopropanol, would not travel the full distance if not thoroughly lubricated with isopropanol before use.

4. Manifold Defects:

The kinetic parameters of continuous flow analysis, have been considered by others[4] and as generation 1 and 11 Autoanalyser equipment will be in use for some time, they require consideration.

A published manifold diagram may require modification to give satisfactory performance. The calculations of the parameters, lag phase factor and exponential factor, give information which can lead to the correction of a manifold such as the ideal sample to wash ratio, the elimination of long sections of unsegmented stream, and selection of correct joins and tubing. In most instances, the per cent interaction will be small with a properly designed manifold and can usually be ignored.

By running standard profiles, and calculating these parameters, we have been able to modify some of our methods and improve precision.

The electrolyte manifold was redesigned to give optimum flow rates to the dialyser and considerable improvement was made in the calcium manifold from the original design by removing the tygon mixing coils and replacing them with a reduced number of glass coils.

Faults with manifolds have occurred when remade without reference to the flow diagram or when regular maintenance has been neglected.

An apparent blockage in the transaminase manifold was found to be the result of a pump tube being placed on the wrong connection of a D1, glass joint, causing the larger volume to go through the capillary and the smaller volume through the 'straightaway'. This resulted in insufficient pressure to pump through the 40 feet heating coil and colorimeter. For some time the fault was thought to be a blocked heating bath, a common cause of trouble in this method if the cleaning regime with alcoholic potash is neglected.

Glass joins where plasma is introduced, require frequent cleaning as small fibrin clots can partially block a tube and may be carried along the stream and block dialysers or heating baths.

In the urea method, the strong acid erodes the glass coils in time, resulting in a larger manifold volume.

The increased incubation time in the eroded 95°C bath, results in bigger peaks.

An example of poor peak profile is shown in Fig. II, a CO_2 graph. This was caused by a faulty join at the debubbler where the gas stream is sampled. The liquid stream was not flowing efficiently to waste.

To maintain the optimum function of a manifold, it is necessary to renew the pump tubes and joins frequently and carry out a regular programme of manifold maintenance. This entails re-analysis of the standard profile, and such procedures as checking the volumes of heating bath coils which are subject to acid erosion.

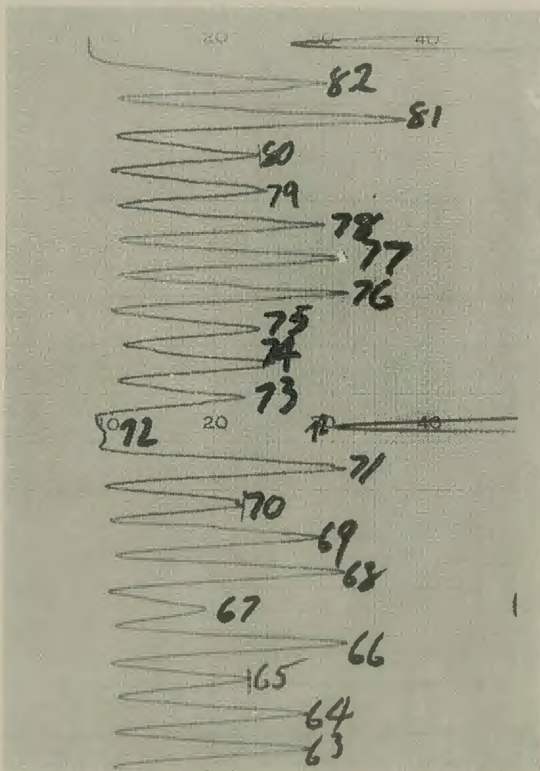

Figure II. CO_2 chart showing the effect of a manifold malfunction.

5. Operators:

Some operators seem to get better results than others and it is obvious that some are more competent. There was a general deterioration of results in one period which could be traced to operator changes and improved under a new operator.

From the fault finding point of view, an operator who withholds information, is worse than a less competent one, who will discuss difficulties. In the latter case, gradual improvement can be seen as confidence is gained.

Operator errors can sometimes occur, such as failure to check that adequate reagents are available. The absence of wash water for the Sampler causes alteration in peak profile. Failure to adjust the baseline sometimes occurs which results in loss of sensitivity and in some instances wrong filters or sampling cam have been found.

Drift is a serious problem for an operator who must carefully peruse the chart before some sort of compensation is made. The intermittent pool used for drift control, is used to draw a compensating curve, and as a logarithm relationship exists between concentration and absorbance, this is difficult.

A line drawn parallel to the original calibration line would be incorrect in these cases.

6. Identification Errors:

These have occurred at every stage from collection of blood to reporting of results. Some errors have occurred when the preprinted form for another patient from that bled, has been signed by a doctor, the specimen label attached and sent to the laboratory.

In the laboratory, errors have occurred when the sera is transferred to the wrong autoanalyser cup, peaks have been misread, results recorded wrongly, or clerical errors when the results are reported on the wrong cumulative report.

Many errors occur in instances where the original request form gives only scant details on the patient's identification and the hospital number is omitted.

7. Environmental Causes:

Temperature variations have been found to cause drift and improvement obtained by keeping the temperature constant.

Light can affect some tests, and a smaller S.D. for creatinine was achieved by protecting the 40ft delay coil from light. Bilirubin tests, which are markedly affected by light, are protected by keeping the sampling module in a box.

One of the greatest difficulties in estimating protein bound iodine is atmospheric iodine contamination. In an effort to eliminate contamination, iodine is excluded from the laboratory where the test is done and from the area where distilled water and reagents are prepared.

It is significant that bad days sometimes occur when a number of small operator faults are found and could be due to working conditions. This has happened for example on days when it has been oppressively hot and the central heating system has not been turned off.

Results and Discussion

The known controls provide only a random check on accuracy and precision and

are sometimes of little use in fault finding. Because the operators know of their existence and value, they tend to get preferential treatment, especially when being read. Thus the result may be biased towards the expected result or the graph adjusted to fit the known result.

The unknown control also provides only a random check of precision. Initially the results are unbiased but the approximate value becomes apparent in time. However, these are changed monthly.

Control sera does not detect reading or clerical errors. Any unexpected results ought to be checked immediately by the operator.

Table II shows the coefficient of variation of the unknown controls during 1970. The figures in the column headed 'Acceptable Values' are based on Tonk's criteria that errors should not exceed one quarter of the normal range and have been calculated from our normal ranges derived from blood donors.

The blind control is a better check of laboratories precision because operators are unaware of its existence.

The results of a blind control are shown (Table III). A total of 40 samples were analysed. When discrepancies were found, the worksheet and autoanalyser charts were critically examined. Misreading of peaks (a common fault when a large number of peaks are read manually) was detected on three occasions in this series. As a result of this finding a double checking technique is now used when reading results.

Table III shows the coefficient of variation for the known, unknown and blind control for January, 1971. It can be seen that there is a definite bias towards the known control.

The figures in the column marked 'O.S.U.' are those published for a blind control at Ohio State University.

Drift Control:

In continuous flow analysis, when a succession of samples are pumped through a manifold and various modules before the concentration is monitored, gradual or sporadic changes in the function of a module, or in reagents can occur.

Dialysis is the most common cause of drift. The use of pooled sera placed at close intervals is often the only indication of a dialyser fault. This is illustrated in Figure III, the pool being indicated in every fifth position. This proved to be the result of a faulty membrane. Numbers 14 and 16 are blind controls and although the operator attempted compensation for the drift, the two identical sera were reported as:—

	Na^+	K^+
Number 14	140	4.5
Number 16	137	5.3

This serves to demonstrate the sporadic action of a faulty membrane for which compensation cannot be made.

Temperature changes have been found to be a major contribution to drift, when reagents stored at 4°C gradually warm to room temperature throughout a run. Drift in the albumin method has been largely eliminated by placing the dye on ice throughout a run. Other methods using cold reagents, e.g. transaminase also require temperature control.

Drift was found to be caused by a variety of other reasons, such as layering of reagents due to topping up a reagent bottle during a run, or to the failure of a pump to maintain consistent pumping.

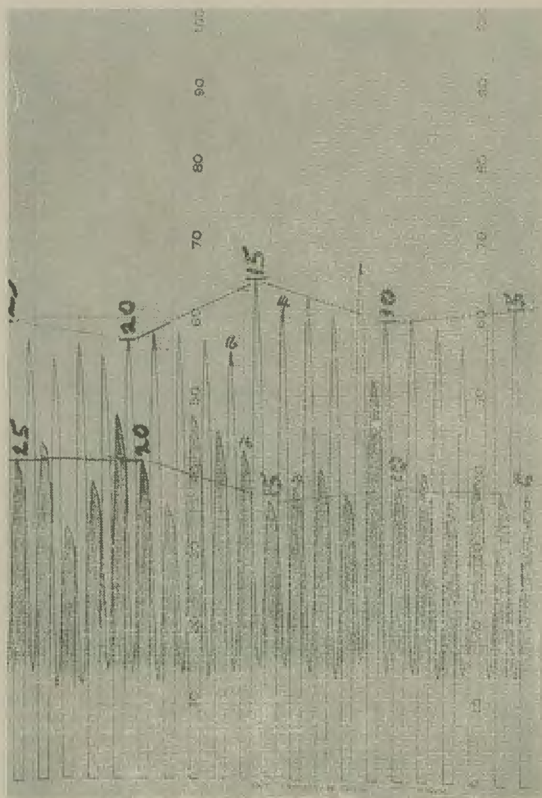

Figure III. Sodium and potassium chart showing excessive drift caused by a faulty dialyser.

Daily Means:

Daily means seem to be extremely valuable in detecting faults, because they are quite independent of the random controls, which may be 'protected'. In some cases they revealed processing difficulties which the operators were reluctant to reveal.

Table II

	MEAN VALUE	ACCEPTABLE C.V.	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEP	OCT	NOV	DEC
Na	145	1.3	1.5	1.25	1.1	1.2	1.3	1.0	1.0	1.4	0.4	0.5	1.2	0.8
K	4.2	5.5	3.7	4.3	1.9	2.3	2.3	3.3	2.9	3 3	2.2	1.5	2.8	1.8
Cl	107	1.8	4.2	2.3	2.5	0.9	1.5	1.6	1.4	0.8	0.6	0.8	1.2	1.2
Urea	28	11	20.0	11.4	6.6	5.3	2.6	4.2	5.7	4.6	1.4	1.6	4.4	5.0
Creatinine	0.8	7.8	20.0	25.0	12.0	12.0	5.0	6.8	7.7	7.1	3.7	2.8	4.4	6.6
Glucose	100	8.3	5.3	5.1	2.9	5.0	4.5	2.6	3.7	5.6	2.3	3.3	5.3	2.2
Albumin	3.4	6	4.2	5.7	6.1	4.9	4.7	4.7	3.9	3.4	4.6	3.6	2.5	4.2
Protein	6.3	3.5	3 3	3.1	3.9	2.4	1.7	1.9	2.3	5.4	2.1	1.9	1.3	2.4
Bilirubin	1.0	15					4.4	5.9		17.0	14.0	8.0	9.8	14 0
Alk. Phos.	20	8	16	16	16	8.0	6.6	10.4	7.1	4.5	4.7	6.0	7.5	7.0
S.G.O.T.	18	13	17	15	25	12	5.7	6.2	11.1	7.2	4.9	5.9	5.8	9.5
C.P.K.	4	16				16		6.5	8.9	4.8	8.0	11.8	16.8	11.1
Uric Acid	4.3	5.7	6.6	4.3	4.4	7.0	2 2	3.6	3.3	2.9	2.6	2.8	1.7	4.4
Calcium	9	4.7		2.0	2.2	2.9	1.3	2.9	2.9	1.8	1.7	1.3	3.3	1.8
Phosphate	3.4	7.0		2.2	4.3	3.2	3.3	4.0	2.9	2.1	2.9	2.9	2.7	4 6
Cholesterol	180	7.5	6.5	3.1	5.1	5.7	3.0	5.9	3.6	10.6	7.1	1.3	7.6	7.3
Iron	100	11.0				21.0		5.8	7.5	8.8	10.3	10.5	14.0	8.4
P.B.I.	5.2	10								13.4	6.7	9.1	10.5	5.8

Table III

	KNOWN CONTROL		JNKNOWN		BLIND		O.SU. BLIND	
	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
Na	125	0.9	139	1.2	140	1.5	140	1.7
K	7.2	1.4	4.4	2.5	4.4	5.4	5.0	3.0
Cl	92	1.0	101	1.4	100	1.4	102	1.6
Urea	66	1.7	54	2.9	52	6.1*	26	6.6
Creatinine	4.0	2.2	1.7	5.7	1.5	4.7	1.7	6.5
Albumin	4.4	2.9	3.5	5.7	3.6	3 8	5.4	2.9
Protein	7.2	1.7	6.5	2.2	6.6	2.3	7.6	2.5
Bilirubin	2.5	3.1	0.5	15	0 5	13.4		
Alk. Phos.	99	6.4	44	6.0	50	8.2	6.9	6.2
Transaminase	64	5.3	46	7.0	36	15*	133	9.3
Calcium	6.8	1.1	9.3	1.7	9.6	2.9	4.8	4.0
Phosphate	8.1	0.8	3.6	4.6	3.8	4.4	4 5	6.7

* affected by misreading of peaks.

Table IV

DAILY MEANS — DECEMBER 1970

	Action Limits										
Na	138 — 142	139	141	139	138	141	140	138	139	139	139
K	4.1 — 4.5	4.3	4.1	4.1	4.4	4.3	4.3	4.4	4.2	4.3	4.3
Cl	97 — 102	98	100	99	98	99	101	100	99	100	99
Urea	33 — 42	42	41	40	39	38	37	33	36	36	42
CO_2	23 — 27	25	25	26	26	26	24	25	26	24	26
Creatinine	0.9 — 1.1	1.0	1.2	1.0	1.0	1.1	0.9	1.0	1.1	1.2	1.1
Albumin	3.1 — 3.9	3.3	3.5	3.8	3.4	3.4	3.4	3.4	3.9	3.5	3.2
Protein	6.1 — 7.1	7.0	6.4	6.9	6.7	6.6	6.9	6.9	7.0	6.7	6.4
Bilirubin	0.4 — 0.7	0.6	0.6	0.6	0.7	0.5	0.5	0.6	0.5	0.5	0.3*
Alk. Phos.	35 — 51	39	53	43	38	48	46	42	37	39	48
S.G.O.T.	20 — 43	23	43	35	31	31	26	34	27	31	31
Ca	9.2 — 9.6				9.4			9.4		9.2	
PO_4	3.9 — 4.5				3.7			3.8		4.0	
Cholesterol	192 — 242		197		197		217		233		209
PBI	5.6 — 7.6	6.4		6.3			5.5				6.5

* Controls low. Standards had been left exposed to light.

On a number of occasions they have been the only means of indicating a faulty run.

When the known controls have revealed faulty standards or reagents, the daily mean has been outside limits as expected, but daily means have been especially useful when the other controls are within acceptable limits but an error has occurred. This has been found when electrolytes standards which require a number of accurate pipettings, have been prepared carelessly or when drift has not been compensated.

Daily mean data has on a number of occasions, shown faults due to incorrect standards, incorrectly adjusted flame, omission of the intermittent pool used for drift compensation and dialysis faults.

On one occasion, total protein standards had been prepared wrongly and the known controls 'protected'. The daily mean in this instance was the only indication of an error.

A rising albumin mean was found to be due to noisy peaks, making them difficult to read as in the method, an absorbance of 0.1 approximates 1.0g of albumin. It was discovered that the wetting agent was being used in the wrong concentration. Table IV shows some daily means from December, 1970.

Logging of Absorbancies:

We have found that logging of absorbancies of enzymes, bilirubin, protein and albumin standards, to be a useful additional method of control. These standards are prepared from commercial control sera and accurate dilutions from small volumes are required. With consistent preparation of these standards and stable reagents, there is little day to day variation, in any case the linear or logarithmic relationship that should exist between different concentrations of the standards, should be the same. Some laboratories use computer programmes to check this relationship.

Summary:

For the detection of faults, one method alone is often insufficient and in most cases it is a combination of methods which permits the fault to be detected. However, the calculation of the truncated daily mean is of paramount importance since it is objective and statistically valid in that it applies to all tests performed. By contrast the control sera have only a random sampling justification. The daily mean can of course be affected by the influx of abnormal results from a select group of patients such as clinics and this is a further factor to be considered when assessing results. Human fallibility is still a large factor particularly in regard to misidentification and checks should be instigated where ever possible.

The role of the control officer is very important in maintaining the success of the programme.

Acknowledgment:

The author is indebted to Mr R. D. Allan for his advice and criticism.

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Experience with a Micro Sodium Electrode for the Detection of Cystic Fibrosis.

G. G. Broad, A.N.Z.I.M.L.T.
Medical Laboratory, Hamilton.

A paper read at the 27th Annual Conference of the N.Z.I.M.L.T. August 1971.

Introduction

Since the first reported observance by Darling et al (1953)[1] of the increase in sweat electrolytes of patients with cystic fibrosis, there have been a number of tests evolved for the determination of increased concentrations of sweat electrolytes.

The purpose of this paper is the introduction of a new and relatively inexpensive method for the detection of cystic fibrosis first described by Lawson et al (1967)[4].

Cystic fibrosis is now more generally referred to as mucoviscidosis due to the easily detected increase in viscosity of the mucus. Condition of the disease is centred round the exocrine aspects of the pancreas, and in particular the secretion of the fat splitting pancreatic enzymes.

Characteristic symptoms that lead to the investigation for cystic fibrosis are (1) increased viscosity of mucus; (2) fat intolerance (malabsorption) and (3) respiratory problems — (a) persistent cough or wheeze; (b) *Staph.aureus* infection of the sputum; (c) shadows on the lungs shown radiologically.

Techniques

Swachmann and Gahn (1955)[7] were the first to introduce a technique for the qualita-

tive estimation of the chloride ion in sweat.

The patient's hand was induced to sweat and then brought into contact with silver nitrate and potassium chromate, both of which were suspended in an agar base. Chloride ion was precipitated as a fine white silver chloride precipitate, the intensity of which was observed against the reddish brown colour of the indicator silver nitrate.

Disadvantages of this method were: (1) difficulty in retaining permanent results, (2) agar plates dried out and (3) difficulty in obtaining sweat.

The latter was the most troublesome factor, in that it often entailed encapsulating the whole body in a plastic bag to induce sweating. Some patients tried forcibly to leave the bag, while for some adults up to two hours were needed to obtain sufficient sweat.

There is now commercialy available an impregnated fibrous pad which has proved unsatisfactory in that it is hard to read the test results and give reliable diagnostic information.

For these methods chloride concentrations greater than 60mEq/L were considered indicative of cystic fibrosis.

Gibson and Cooke (1959)[2] introduced the now standard procedure of sweat induction using pilocarpine followed by iontophoresis.

For this method two electrodes supplying 0 to 20 volts across the electrodes are prepared. Pilocarpine impregnated filter paper is placed on the inside of the forearm and the positive electrode is secured to it. On the outer part of the forearm some sodium bicarbonate is put on the gauze-covered negative electrode. Once securely positioned the electrodes are connected to a power source and the current is increased slowly to give 2 ma.

Iontophoresis is continued for five minutes, after which the electrodes are removed and the skin is cleaned and dried. Preweighed filter pads are then placed over the pilocarpine site and all sweat produced over a 30 minute period is collected. After elution of the sweat, sodium is estimated using flame photometer, and the chloride using the technique of Schales and Schales[6].

Diagnostic values are, sodium greater than 80mEq/L (normal 10-80mEq/L); chlorides greater than 60mEq/L (normal 4-60m Eq/L).

Disadvantages of this method are: (1) patient discomfort, (2) relatively time consuming, (3) skill needed in using Iontophoresis, (4) estimation of the electrolyte content of the filter pad required.

McGrady and Bessman (1956)[5], and Johnson (1956)[3] first reported findings of an increase in the parotid sodium levels in patients with cystic fibrosis.

Lawson (1967)[4], published an article concerning the use of a micro-sodium electrode in the diagnosis of cystic fibrosis and it is on this article that we have based our technique for the screening of suspected cases of cystic fibrosis.

Equipment needed for this procedure is a pH meter (an expanded scale is of value), and a micro-sodium electrode (figure I) the cost of which is $18.75 (N.Z.) manufactured by Electronic Instruments Ltd., and obtainable from Geo. W. Wilton (N.Z.) Ltd.

This method appealed for the following reasons. Most laboratories have good pH meters so the only additional cost is the electrode. After setting the pH meter temperature compensation to 37°C it only takes a few minutes for the meter to warm up to obtain all the necessary meter readings.

When not in use the electrode is stored in 0.1 M/NaCl. The electrode and pH meter are standardised against distilled water as zero and a series of standards 5, 10, 15, 20, 25, and 50mEq/L. These are brought to 37°C prior to use. The concentrations are chosen as they give a wide range of pNa values which adequately cover the normal range of 3-15mEq/L.

For each patient the standards are tested and a graph drawn plotting pH readings against sodium concentration (figure II) and the average of a series of five pH readings from the patient is then used to calculate the patient's parotid sodium level.

In between taking pH readings the electrode is sterilized using a solution of 70%

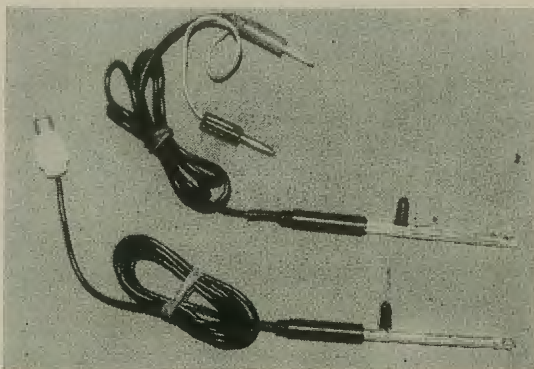

Figure I. Micro-sodium electrode. Bottom with dual glass/calomel reference connection and top converted to single glass and calomel reference connections.

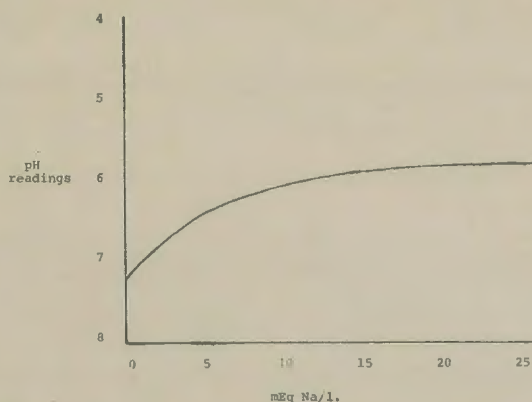

Figure II. Graph showing typical curve drawn from pH readings against sodium concentrations.

Isopropanol. In the original article the electrode was subjected to various broth cultures of bacteria and various sterilizing agents used on the electrode, after which a culture was made to determine the effectiveness of the respective sterilizing agents. By comparison it was proved that 70% Isopropanol was the most reliable and this is what we are using in our laboratory.

First step is the reading of the standard solutions, then after sterilizing the electrode it is introduced into the patient's mouth near the Stensen's duct which is in the upper region of the cheek next to the second molar tooth.

Care must be taken to ensure that the ceramic plug on the electrode is uppermost and tilted at an angle of 45° to avoid any airlocks that may form beneath the ceramic plug.

Five readings are normally taken, three from one side of the mouth and two from the other. The average of the five readings is then taken and the parotid sodium concentration is then calculated. Originally it was stated that there should be a minute interval in the taking of the readings, but over the period that I have been taking them no significant change in readings has been observed between taking the readings continuously and waiting the stipulated minute.

While taking the readings it is important to avoid hyperventilation by the patient but with some infants it is unavoidable. Medications, toothpaste, lollies and agents which may cause a pH change in the mouth should also be avoided.

In setting up this method I have tested many of the laboratory staff and the results range from 5-10mEq/L. One of the pleasing features of this method is the reproducibility of the graph.

Established normal range for this method is heterzygotes 5-15emEq/L, Homozygotes 12-52mEq/L.

In the time that this technique has been in operation in our laboratory there has been a small percentage of suspected heterzygotes and one definite homozygote and it is her case I would like to present.

Case History: K.S. F/9

This patient had been previously diagnosed by the Gibson and Cooke[2] method and by our technique had a parotid sodium level of 38mEq/L. Her physical appearance was one of marked undernourishment.

In review she was a classic homozygote who first presented to her doctor with respiratory problems, *Staphlococcus aureus* infection of the lungs, a history of large bulky fatty stools and a rectal prolapse.

Pancreatin had been given to make up for her deficiency in pancreatic enzymes, and after ceasing pancreatin treatment for some days and putting her on a normal diet she had a three day faecal fat content of 108gm total fat.

Her treatment up till the time of her death was (1) low fat diet using medium chain triglycerides for ease of absorption (2) antibiotics to control pathogenic bacteria in her lungs and respiratory tract (3) pancreatin in place of her pancreatic enzymes (4) mist therapy. An ultrasonic nebulizer was used to provide a sufficiently moist atmosphere for the patient to breathe while in an oxygen tent. (This alleviates a lot of the respiratory problems caused by the mucus membranes secreting too viscous a mucus). Propylene glycol can be used to minimize evaporation of the water molecule during the transition from room temperature to body temperature.

Several times previously the patient had nearly died of broncho-pneumonia and it was in spite of all treatment that death occurred as a result of broncho-pneumonia.

Acknowledgments

I would like to thank Mr Brian Barry for his help and advice and Dr D. H. Pullon for encouragement and case history notes.

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Hepatitis Associated Antigen: A Current Comment on the Infectivity of Clinical Material

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Introduction

Two epidemiologically and immunologically distinct forms of viral hepatitis have now been defined[6]. Infectious hepatitis (MS-I, IH. epidemic jaundice) has a short incubation time while serum hepatitis (MS-2, SH, homologous serum or inoculation jaundice, transfusion hepatitis) has a characteristically longer incubation period. Since the discovery of hepatitis associated antigen (H.A.A.) by Blumberg and his associates in 1965 the close relationship between this particle and the aetiology of serum hepatitis has been established. Needle-transmitted jaundice was probably first recorded by Lürman[7] in 1885 after an outbreak of serum hepatitis in Bremen shipyard workers following vaccination with "lymph" of human origin. That this type of hepatitis can be transmitted by needles, lancets, cuts and abrasions, dental instruments, contaminated blood and blood products, etc., has since been confirmed. Until recently, in contrast to infectious hepatitis, serum hepatitis has been generally assumed to be transmittable only by the parenteral route and that those infected do not shed the agent in their faeces, urine or nasopharyngeal secretions, etc. However, the observation by Mirick and Shank[9] of an outbreak of serum hepatitis caused by inoculation of human plasma that was associated with many secondary cases in uninoculated intimate contacts, together with the findings of Krugman et al[6] that serum hepatitis can be transmitted orally as well as parenterally, suggests that the potential infectivity of clinical material in addition to blood be recognised.

Consideration at this juncture must therefore be given to the possibility of infection via both the oral and parenteral routes through contact with urine, faeces, sputum and other biological material that may be forwarded to service laboratories for analysis.

Investigation of Clinical Materials

Shulman, in a recent review[10], noted his inability to define H.A.A. in several body fluids. H.A.A. could not be detected in saliva concentrated ten-fold, in urine concentrated one-hundred-fold or in 20% stool extracts of individuals with high complement-fixing titres in blood. Subsequently, however, Grob and Jemelka[4] have recorded their findings in eleven patients with sporadic acute hepatitis each of whom contained H.A.A. in both serum and faeces detectable by Ouchterlony immunodiffusion and/or counter - immuno - electrophoresis techniques. The antigen persisted longer in the faeces than in the corresponding sera in eight patients while the reverse was true in three patients. Latterly two patients developed anti-H.A.A. in the faeces which was not detectable in the serum. At the time of writing these findings remain unconfirmed. Gust et al[5] have been unable to detect either H.A.A. or anti-H.A.A. in 47 faecal extracts prepared from 15 hepatitis patients with H.A.A. in their serum.

Although as long ago as 1945 Findlay and Willcox[3] observed the development of infectious hepatitis in volunteers following the oral ingestion of faeces or urine from spontaneous cases of the same disease, the belief seems to have developed that urine of patients with serum hepatitis is not infectious. Blainey et al[2] have recently determined that patients with renal transplants who have become chronic H.A.A. carriers may excrete the antigen in the urine. Using, in addition to other methods, a complement fixation procedure, these workers were able to demonstrate low-titre activity in 7 samples of unconcentrated urine, and concentration of 3 of these samples by negative-pressure ultra-filtration produced high titres comparable to those seen in serum. Immune aggregates, after the addition of anti-H.A.A. to the urine concentrates, were observed using electron microscopy.

Attempts have also been made to isolate H.A.A. from other biological fluids such as bile, gastric juice and synovial fluid. These investigations have been carried out in the presence of concurrent H.A. antigenaemia. Thus, prompted by the report of Grob and Jemelka, Akdamar et al[1] aspirated samples of bile from 4 cases of hepatitis and identified H.A.A. in one using Ouchterlony immunodiffusion and counterimmunoelectrophoresis. H.A.A. could not be detected in gastric juice

collected simultaneously from the bile-positive patient.

Finally McKenna et al[8] have isolated H.A.A. from both blood and synovial fluid of a 16-year-old female with serum hepatitis and concurrent gonococcal arthritis; the patient admitted to both drug abuse and sexual promiscuity in the preceeding period Although the authors suggest that H.A.A. could have contacted the synovial fluid as a blood-borne contaminant during arthrocentesis the possibility that H.A.A. is present in synovial fluid during serum hepatitis in the absence of arthritis is recognised.

Discussion

There is little doubt that knowledge of the distribution of H.A.A. in vivo and its mode of excretion from the body during infection is still in its infancy, but a number of recent observations, some unconfirmed, suggest that auto-inoculation can be acquired in a variety of ways from a number of sources. It therefore bears emphasis that, at this time, clinical specimens of any nature from a patient with serum hepatitis should be treated on a 'high risk' basis.

Summary

Attention is drawn to the recent detection of H.A.A. in clinical material other than blood or blood products, viz. faeces, urine, bile and synovial fluid.

Acknowledgements

I am grateful to Dr P. Booth, Immunohaematologist, Christchurch Hospital, for his helpful advice and criticism and to Mrs P. Goodger for clerical assistance.

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Obituary

John Oubridge Mercer, C.B.E., M.B., Ch.B., F.R.C.P.; F.R.A.C.P.; M.R.C.P.A.

Dr Mercer died at his home in Wellington on December 30, 1971. He was director of Laboratory Services for Wellington Hospital Board until his retirement in April 1970, and was an Honorary Member of the New Zealand Institute of Medical Laboratory Technologists.

He graduated in Medicine from Otago University in 1929 and was appointed Pathologist to Wellington Hospital in 1935 holding office as President of the Medical Association of New Zealand from 1948-1952 and again from 1964-1965. He was President of the New Zealand Society of Pathologists in 1970. In 1959 he was awarded the C.B.E. for services to Medicine.

Outside the field of medicine Dr Mercer had many and varied interests in education, sport, the Church and in the Fine Arts. His active interest in the latter was shown by his association with the New Zealand Academy of Fine Arts, his term as Acting President of that body from 1959-1960, and his knowledge and interest in New Zealand artists, many of whose works are to be found in his home.

In his chosen career as Pathologist, he had frequently to give evidence at the Coroner's Court, and tribute has been paid to his depth of human understanding and his quiet words to the bereaved relatives.

He was appointed as a member of the Commission on Social Security which recently completed its report to the Government. This was a work he commenced on his retirement, and continued until the completion of the Report, attending meetings when he was far from well. He had undertaken a task and saw it through to the end, facing his illness with calm dignity.

To the end he maintained a lively interest in the activities of 'his laboratory and staff' and was keen to know the latest developments and achievements.

Those who worked with and for J. O. Mercer will remember him for his quiet manner, his unfailing courtesy, his keen and active interest in the well-being of his staff, and his sympathetic and helpful understanding of many of their problems.

Not a few have quaked when being 'summoned to see J.O.' and being quietly given 'once round the potato patch'. But many more will remember his quiet advice and help with their personal problems and his delight in their achievements and successes.

Dr Mercer is survived by his wife, son and three daughters, to whom we extend our sincere sympathy.

J.M.

Hepatitis Associated Antigen and Laboratory Precautions

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A paper presented at the 27th Annual Conference of the N.Z.I.M.L.T. in Wellington, August, 1971.

It has been known for many years that the risk of hepatitis attends the handling of blood samples, and this risk has become more apparent with the increased use of blood in such situations as by-pass surgery and renal dialysis. The increased use of communal syringes by drug takers has led to a high incidence of serum hepatitis among them, and several outbreaks of hepatitis in renal units have resulted from such patients.

The Australia antigen was discovered in 1963 and by 1966 its association with hepatitis was clear. With increased use of methods for HAA detection, particularly by blood transfusion services, the possibility that hepatitis may result from the handling or transfusion of blood has at last fairly fully entered the conscious minds of clinicians and laboratory staff concerned. This has resulted in various measures being taken, the scope ranging from sensible precautions, such as should anyway have been observed previously, to major upheavals with all and sundry at panic stations.

It is rather ironic to note that in most recommendations the identification of HAA positive samples plays a key role. Yet we know that our currently available detection techniques only pick up a proportion of carriers — probably less than half of them. Immunological tests cannot now, and probably never will detect the minute amounts of infectious material capable of causing hepatitis in a susceptible person. The carrier population may be likened to an iceberg, the tip of which has now for the first time become visible. How much more of the submerged portion we can persuade to emerge by increasingly sensitive tests is a matter of conjecture.

Nevertheless, HAA testing, being all we've got, is useful provided its limitations are not forgotten. Some figures may help demonstrate what can be expected. In most temperate countries, the HAA positive incidence has been reported at between 1 and 6 per 1,000 of the blood donor population. The variations are due partly to differing techniques used, partly to rules regarding donor acceptance in relation to past history of hepatitis or blood transfusion, and partly to differences related either to the environment or to the genetic constitution of the surveyed populations. It is perhaps appropriate here to mention that in many tropical populations, the HAA carrier rate is more like 1 in 10 than 1 in 1,000, and moreover susceptibility to antigen carriage appears to be controlled by an autosomal recessive gene. Obviously very different considerations must apply in such countries than in, for example, New Zealand.

Our HAA positive rate here among blood donors appears to be about 4 per 1,000, and already the figures suggest a much higher rate among Polynesians than among Europeans. Perhaps like the Melanesians to the north, Polynesians have a high incidence of genetic susceptibility.

About 140,000 donors are bled each year in New Zealand, so universal HAA testing of donors could be expected to identify several hundred carriers—a not inconsiderable number of 'high risk' samples, and one which should reduce the incidence of post-transfusion hepatitis significantly. By so doing, of course, the consequent risk, in the laboratory, from such patients will also be reduced.

Concerning actual precautions to be taken in a laboratory, those wishing the full treatment are referred to Percy-Robb *et al* (1970); where the precautions adopted by the Department of Clinical Chemistry at Edinburgh Royal Infirmary are set out in full. This hospital had the misfortune to suffer an extensive outbreak of hepatitis which began in their renal dialysis unit. At least two doctors, a laboratory technician and a laboratory clerk/ receptionist died, so it is not surprising that the code of practice now adopted is rather stringent. It also costs them about $3,000 per annum extra, mostly on disposable gloves and similar items. The Department has 35 staff members.

At Christchurch we have adopted an intermediate code, based upon accepted basic tenets of laboratory hygiene and the identification of 'high risk' specimens. (See Appendix).

One worthwhile additional move would be the appointment of a Safety Officer, to make sure the recommendations are actually being observed in the laboratory.

Though the evidence as to whether ad-

ministration of gammaglobulin modifies or prevents serum hepatitis is conflicting, it is considered that it should not be withheld from staff members who get blood, plasma etc. in mouths, eyes, abrasions or cuts. 20 ml of the C.S.L. product should be given intra-muscularly.

The most important and difficult part of any programme is in educating staff members about the hazards, and this requires constant supervision. Any Code of Practice fulfils a useful function by reminding staff of possible dangers. There is no evidence that precautions as described are effective, and such evidence is unlikely to be forthcoming until methods of isolating, culturing and identifying the virus of serum hepatitis are developed.

Summary:

The publicity accorded the hepatitis associated antigen now that it can, at least on some occasions, be identified, has resulted in an increased awareness of the dangers associated with the collection and laboratory testing of blood samples. The scope and value of various possible precautionary measures is discussed, and the system at present being followed in Christchurch is outlined.

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Appendix:

Pathology Services : N.C.H.B. (Christchurch Hospital) June 71

HEPATITIS

LABORATORY PRECAUTIONS AGAINST INFECTION

1. *HIGH RISK SPECIMENS*
   Specimens from *'High Risk' Patients,* (i.e. patients with JAUNDICE under investigation, with SUSPECTED HEPATITIS, and patients in RENAL DIALYSIS UNITS) to be identified by an ORANGE STICKER on the *container* and on the accompanying requisition *form.*
2. BLOOD SAMPLE COLLECTORS to wash hands following each venesection of the above patients.
3. BLOOD SAMPLES, AND FAECES SAMPLES from the above patients to be placed in plastic bags for transport to the laboratory.
4. Specimens of serum (already separated) from *Private Pathology Laboratories* from the above patients to be sent in screw capped bottles in plastic bags. Any unsatisfactory specimens to be referred to a Pathologist.
5. PLASTIC DISPOSABLE GLOVES to be available in all laboratories and to be worn when handling high-risk specimens.
6. PERSONAL HYGIENE: strict personal care and hygiene to be taken during and after handling high risk specimens.
7. CENTRIFUGING: high risk specimens to be centrifuged with cap on, or cork in tube, which should not be more than ¾ filled. Care should be taken on releasing cap or cork (risk of "aerosol" spray).
8. PIPETTING: High risk specimens must not be mouth pipetted and care should be taken with Pasteur pipetting.
9. DISINFECTION: 'Milton' solution (1:10) for disinfection of equipment and wiping of benches.
10. DISPOSAL of used specimen containers etc. by INCINERATION.
11. EATING AND SMOKING in laboratories is strictly prohibited.
12. STAFF MONITORING: 6 monthly testing of serum for HAA.
13. NOTIFICATION of both POSITIVE AND NEGATIVE HAA. Serology results to:
    - Medical Superintendents
    - Director of Pathology
    - Haematologist
    - Chemical Pathologist
    - Immuno-Haematologist
    - Microbiologist and Virologist
    - Charge Technologists all Laboratories and Units
    - Private Pathologists
    - Radiology Department
    - Radiotherapy Department
    - Radio-isotype Laboratory
    - Renal Physician

Quantitative Determination of Human Haptoglobin by Means of Immunoelectrophoresis in an Antibody-containing Gel

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Introduction

Immunoelectrophoresis was introduced by Grabar and Williams [5] in 1953, and since then has been modified in detail by many workers. [1, 10, 14, 15, 16].

In 1965, Laurell introduced a quantitative technique [13] which he called 'antigen antibody crossed electrophoresis.' In this procedure antigens are run electrophoretically through a medium containing the corresponding antibodies, the result being the formation of rocket like immunoprecipitates; hence the term 'Laurell rocket.'

By using specific antisera, single protein determinations can be made by this technique [2, 11]. The area enclosed by the peak is directly proportional to the concentration of antigen applied. [2, 17].

This paper discusses the application of this technique for the determination of the haptoglobin content of human serum.

Materials and Methods.

Apparatus

1. Gelman Sepra Tek Electrophoresis chamber.
2. Glass slides 2¼in x 2¾in.
3. Hamilton Microsyringe (1µl capacity).
4. Microtiter Dilution Apparatus.

Reagents

1. Antiserum: Rabbit antihuman haptoglobin, mixed type (Dakopatts).
2. Reference Serum: Standardised human serum O.P. No. 469, haptoglobin content 235mg/100ml (Behringwerke).
3. Protein Stain: 1% Amidoschwarz in 50% methanol: glacial acetic acid (9:1).
4. Buffer: High resolution tris barbital — sodium barbital buffer pH 8.8.
5. Agar: Ion Agar No. 2. Oxoid 8 grams of agar are mixed with 300ml of distilled water with 40mg of merthiolate added as a preservative. The mixture is heated at 100°C till clear, and when cooled to 60°C dispensed in 1.5ml aliquots in 10ml reagent bottles.

Procedure

1. To a 1.5ml aliquot 2.5ml of buffer is added and this is heated at 100°C till the agar has liquefied. It is then cooled to 50°C and 0.16ml of antiserum is added giving a 4% solution. The 4mls of agar is then poured on a glass slide on a level surface and allowed to gel.

2. In a line 10mm from one edge of the slide, 1mm holes are made at intervals of 5-7mm. The slide is then dried at 37°C for 5 minutes to remove surface moisture.

3. Applications of 1 µl are made using a Hamilton Microsyringe, taking care not to overflow the wells. The first four applications are Standard Human Serum diluted 1:4, 1:8, 1:16, 1:32. The test sera are diluted 1:4.

4. The slide is then electrophoresed at 30 V/cm (i.e. a total of 250 volts for the slide) for 90 minutes. The length of the rockets are then measured from the point of origin to the apex of the peak, using dividers and a centimetre rule.

5. A graph is then prepared on linear graph paper, plotting the values of the standards against rocket height. Values of the test sera are then read off the graph and multiplied by the dilution factor.

6. If a permanent record of the results is required, the slide may then be stained using the following procedure:

(a) The slides are washed in normal saline for 15-30 minutes to wash out any unprecipitated serum. It is then dried at 56°C.

(b) When dry, stain with amidoschwarz for 5 minutes and decolorise using a methanol-acetic acid water rinse. (See Fig. 1.)

Results

The method was compared with the haemoglobin binding method used in the Beckman Electrophoresis Manual which is a modification of the technique used by Grunbaum and Pace [6]. The results of 117 sera were compared by both techniques. (See Fig. 2).
The Correlation Coefficient of the two techniques was 0.78 when all results were included. Correlation is significant, p being less than 0.001.

Peak heights were measured instead of

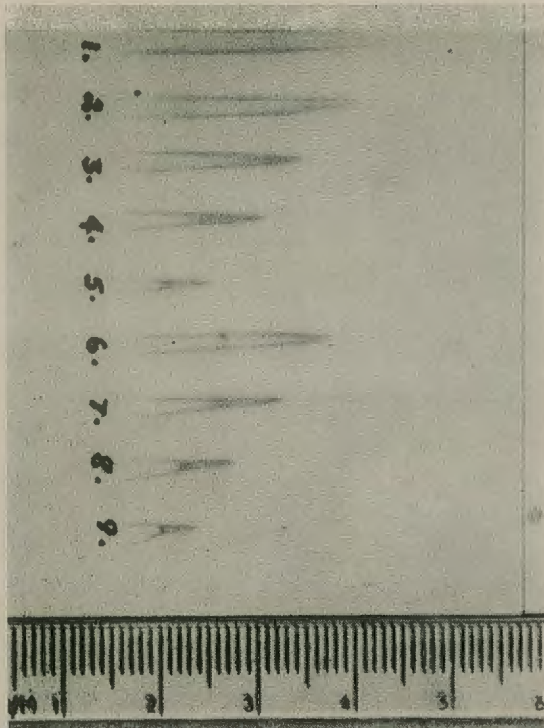

FIG. 1. Typical immunoprecipitates Nos. 1-5. Standard diluted 1:2, 1:4, 1:8, 1:16 and 1:32. Nos. 6-9. Test diluted 1:4, 1:8, 1:16 and 1:32. Note precipitate No. 1 fails to come to a sharp peak. This is a prozoning effect due to an excess of antigen.

peak areas. The peak height, unlike the area of the peak, is not directly proportional to the concentration of the antigen, but the differences are minimal for absolute values of up to 100mg/100ml of haptoglobin. (See Fig. 3).

When results of over 125mg/100ml were excluded, the Correlation Coefficient was 0.86. (See Fig. 2).

Twenty-one estimations were performed on the same sample. The mean peak height was 10.46 mm and standard deviation was 0.32.

$$\text{Coefficient of Variation} = \frac{\text{S.D.}}{\text{MEAN}} \times \frac{100}{1}$$

$$= 3.06\%$$

95% of the results lie between 9.82 and 11.11 mm

99% of the results lie between 9.50 and 11.43 mm

Discussion

The aim of this investigation was to evaluate the applicability of Laurell's method of quantitative immunoelectrophoresis to the

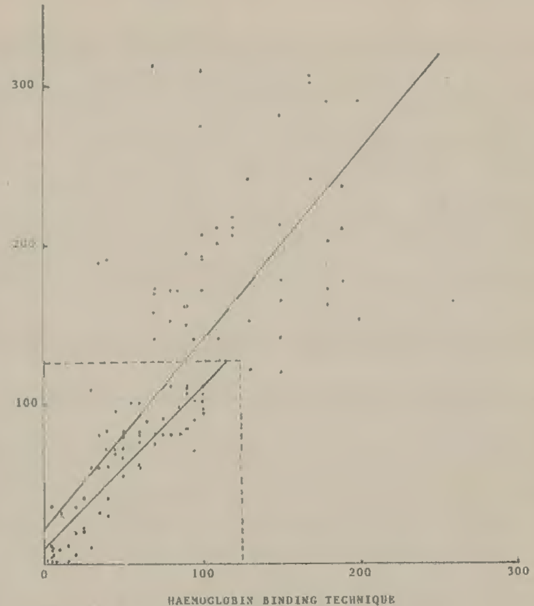

FIG. 2.

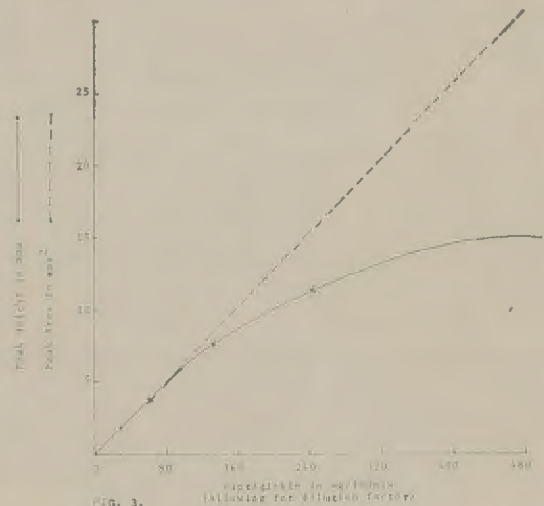

FIG. 3.

routine determination of the haptoglobin content of human serum.

In the method described the actual haptoglobin content of serum is measured and not the haemoglobin binding capacity of haptoglobin.

Several studies have shown that at saturation the binding of haemoglobin to haptoglobin is stoichometric[8, 12] i.e. 1 mole of haptoglobin binds 1 mole of haemoglobin. According to Kluthe et al[9], and Cloarec and Moretti[3], this is so regardless of the haptoglobin type. There are three main types, haptoglobin types 1-1, 2-1, 2-2, each type being genetically determined.

In E. R. Giblett's article[4] on the haptoglobin system, he states that if the molecular weight of Hp 1-1 is taken as 85,000-110,000, that 1.4 to 1.7g of haptoglobin would combine with 1.1g of haemoglobin. The molecular weights of types 2-1 and 2-2 are not yet known, but thought to be greater than the molecular weight of type 1-1[4]. This means that even more haptoglobin by weight would be needed to combine with 1 mole of haemoglobin. Hence a reason for most of the electro-immunodiffusion results being greater than the results obtained by the haemoglobin binding technique.

In the technique described, polyvalent antisera is used which reacts with haptoglobin types 1-1, 2-1 and 2-2. Also the reference serum used for the standard contains these three types. For exact determination of haptoglobin one would have to type genetically the haptoglobin and then use type specific antiserum for quantitation.

There would then be three different normal ranges for haptoglobin concentration dependent on haptoglobin type. However as this type specific antiserum is not readily available at the present time, this was not done. The purified standard available commercially is of the mixed type.

Up to now, insufficient haptoglobin estimations have been carried out to establish our own normal range for the technique. The normal range being used at present is 50-220 mg/100ml as set out in Volume 2 of Lab. Synopsis, a Diagnostic Reagents Bulletin published by Behring Diagnostics.

As can be seen in Fig. 3, by measuring peak heights instead of the area under the peaks, correlation does fall off at the higher limits. This can be overcome by diluting the specimen to 1:8, instead of 1:4, so that the results fall in the more sensitive range of the graph.

Due to the high voltage used in the procedure and the small size of the Gelman tank, the buffer can be used only once when used at room temperature. When run at 4°C however, there was less dissociation and the buffer could be re-used. When using a lower voltage the peak heights are decreased although the total area remained relatively similar; so if peak heights are used, there is a subsequent decrease in accuracy.

Dilution error must also be taken into account, but when using the microtiter apparatus this is minimal. It was suggested that a protein solution being colloidal in nature that there may be significant differences between undiluted and diluted values when dilution was made in buffer. However, when dilution was made in haptoglobin deficient sera, there was no appreciable difference in the results. Six determinations were made in each case, and there was near perfect correlation.

For laboratories doing large numbers of haptoglobin estimations, this method is ideal as up to 20 estimations can be performed on a single slide, and reasonably accurate results can be obtained in about two hours. This method is also very sensitive to small concentrations of haptoglobin and should therefore be of interest in the routine determination of haptoglobin.

Acknowledgments

The author wishes to thank Dr B. Linehan for his helpful advice and permission to publish this work performed at Hamilton Medical Laboratory; also the staff of Biochemistry and Immunology departments, who helped with the haptoglobin determinations, and Miss E. Westbrook, who performed the statistical calculations.

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Automated Blood Grouping; an Assessment of the Technicon Hospital Autotyper

E. J. McKay, A.N.Z.I.M.L.T. and D. J. Haines, A.N.Z.I.M.L.T.
Immunology Dept., Medical Laboratory, Hamilton, N.Z.

A paper presented at the 27th Annual Conference of the N.Z.I.M.L.T., Wellington, August, 1971.

Introduction

The principles for the Continuous Flow System of Haemagglutination have been described in detail in many previous publications. From these articles it is obvious that the Autoanalyser system is capable of preforming blood groups, antibody screening, quantitative antibody analysis and syphilis reagin screening with speed, accuracy and reliability hard to match by manual methods.

In the last five years, several 15 channel blood grouping machines (with a sample rating of 120 per hour) have been utilised in many large blood grouping centres. Because of its capital cost, and size, the manufacturers have developed a more compact and economical model, the Hospital Autotyper *(Figure* 1) which they consider is ideally suited for the average blood grouping laboratory.

The purpose of this paper is to give our evaluation of the machine in such an environment.

MACHINE DESCRIPTION:

The Autotyper is a two channel machine designed for (1) ABO and Rhesus typing of test cells.

(2) reverse ABO Grouping and Partial Antibody Screen on the plasma sample. It has a sample rating of 24 per hour, with an analytical time of 15 minutes. A simplified flow diagram of the machine is shown in *Figure 2.*

Dual sample probes simultaneously aspirate the cells and plasma separately for 112

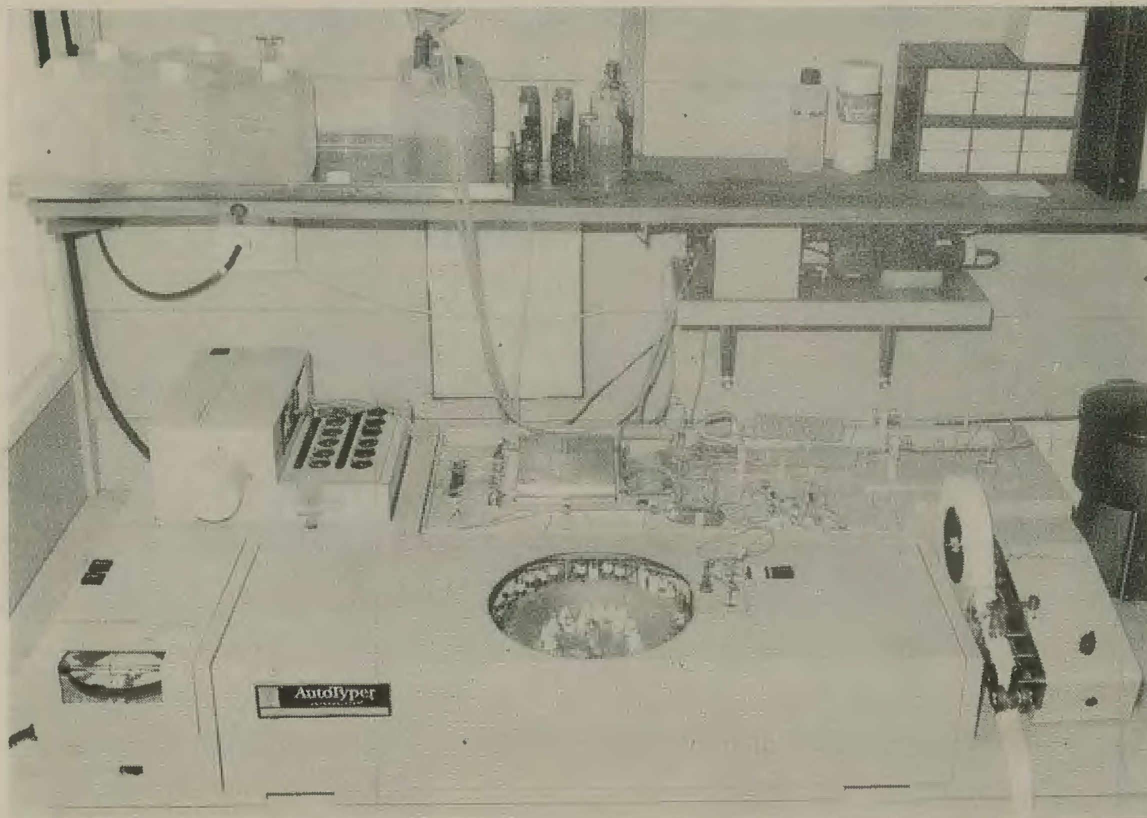

Figure 1

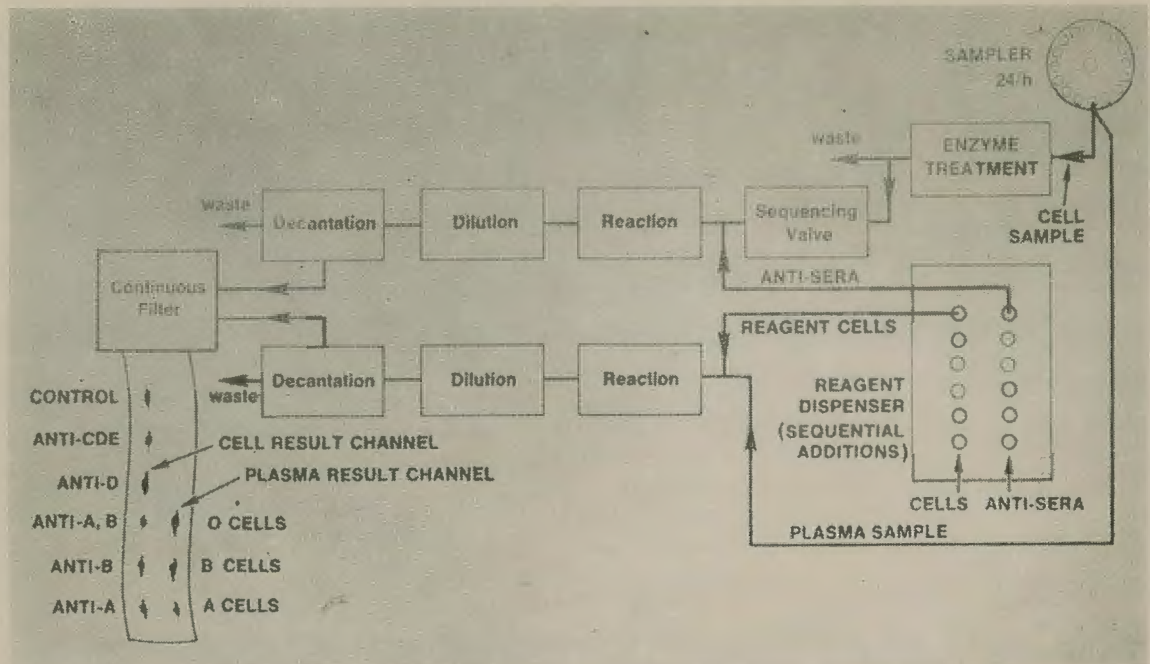

Figure 2

seconds after which there is a reverse saline wash for 38 seconds.

The cells from each test sample are automatically enzyme treated and proceed to the Sequencing Valve *(Figure 3)* where they are subdivided into 6 segments, separated by a wash of saline. Each cell segment then in turn is treated with a different typing serum which is delivered sequentially from a Reagent Dispenser. From there the reactants are mixed in a $4\frac{1}{2}$ turn mixing coil at ambient temperature, pass through a settling coil, then mixed with saline to disperse rouleaux. The agglutinates are then decanted and finally deposited on to a continuous filter strip.

The plasma from each test sample is taken to the Plasma Channel where it receives the reagent cells sequentially from the Reagent Dispenser and proceeds in a similar manner to the Cell Channel where the agglutinates are finally deposited alongside the cell results.

The Reagent Dispenser both mixes and chills the reagents, but must be critically phased to meet both cells and plasma from the test sample to achieve optimal antibody-antigen reactions and facilitate easy reading of the final results.

Methods and Materials:

Blood samples are taken in 12 x 75mm

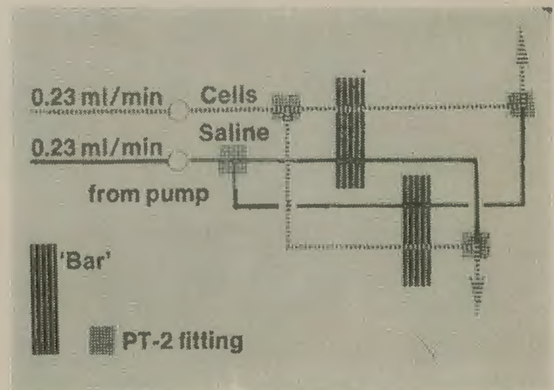

Figure 3

Vacutainers containing 1 ml of A.C.D. These are centrifuged prior to testing at 3400 R.P.M. for 4 minutes. They should be devoid of fibrin clots so as to prevent blockage of sample probes and pump lines. Because of this we have found that samples greater than 4-5 days old are unacceptable.

Each sample has a coded number and is placed in a specimen holder that contains 40 samples. A saline blank is placed at regular intervals.

The antisera used is largely produced in our own laboratory, although one commercial

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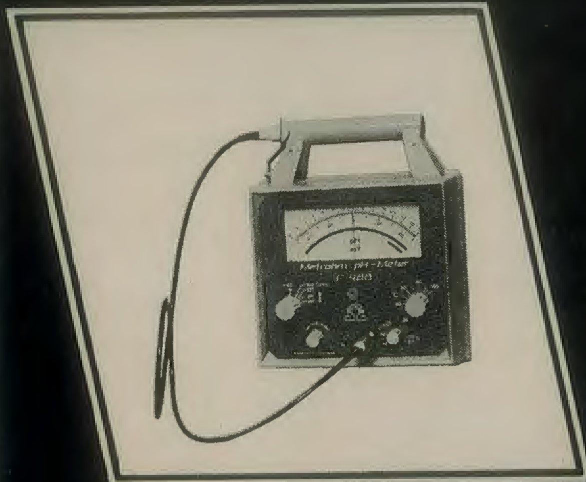

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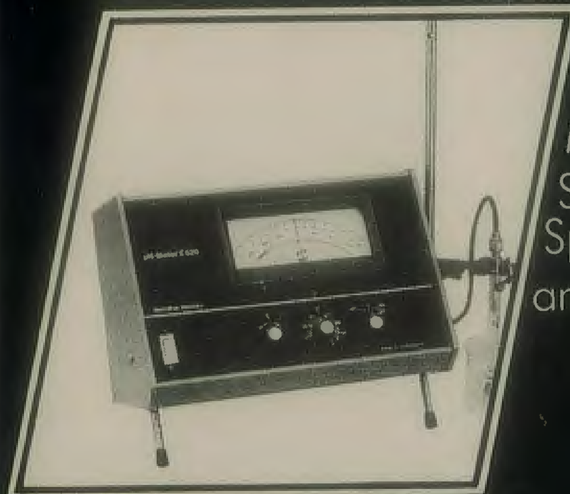

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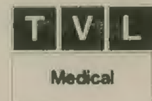

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source of anti-D (Ortho) has been found the most satisfactory. All the antisera is diluted in 0.3% methylcellulose-5% albumin mixture, the volumes vary according to the avidity of antisera used.

Albumin gland extract of the common garden snail *(Helix aspersa)* is our source of anti A. This is of very high titre and is ideally suited to automated blood typing.

The reagent cells are prepared in 0.3% methylcellulose, 5% albumin and 0.5% bromelin each day. 15 mgm fioll is added to each reagent to help prevent haemolysis occurring in the Antigen-Antibody reaction.

1.3% saline is used throughout the system as a diluent, for washing and as a rouleaux dispersant.

Modifications:

We have made several modifications to the reagents. Firstly, we increased the strength of bromelin from 0.1% to 0.5% a week after the arrival of the machine. We have since had notification from Technicon Corporation that this is a recommended alteration.

2. We have substituted 0.3% Methylcellulose for the recommended P.V.P. It is more economical, more stable, and for us, appears to give better results.

3. We have increased the concentration of albumin from 2% to 5%. This has helped in lessening the amount of carry over from one reagent to another.

4. Because Tween 20 is unstable in saline over long periods, we now make only 2½ litres of Tween 20 — 1.3% Saline at a time.

Modifications to the machine that we felt were necessary are as follows:—

1. The original sample probes gave gross carry-over of cells and plasma from one sample to another. To overcome this we have manufactured our own as shown in Figure 4. This is much simpler in design and for us works more satisfactorily. We are curious as to why such a complicated probe was originally designed.

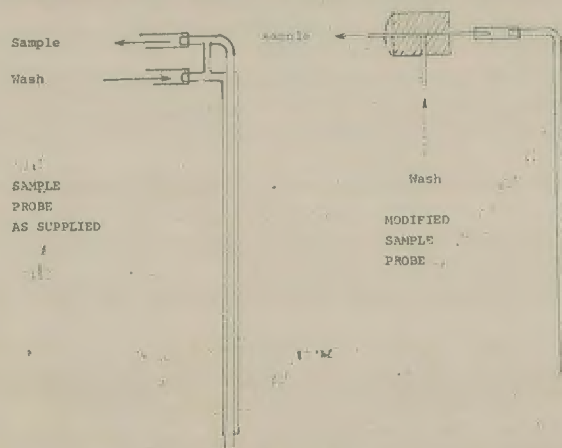

Figure 4

2. A poor bubble pattern was obtained in the machine resulting in some carry-over between reagents. This was due to the merging of three lines, each already with its own bubble pattern. So we have removed the bubbles from all saline washing lines except those to the plasma and cell sample probes. The end result is a more uniform bubble pattern with much reduced carry-over of reagents, which still separates the samples satisfactorily.

3. We have reversed the outlets on one of the wash valves so as to change the lines washing the dispenser probes and the lines going to waste. This has reduced the heating effect on the solenoid.

4. We have added two perspex blocks to raise the height of the final decanting lines for the agglutinates. This allows a greater concentration of agglutinated cells to be sampled in a smaller aliquot at the decantation joint. This results in a much neater printout.

5. In the Reagent Dispenser at least 5ml of reagents are required to bring the level high enough to come into contact with the tip of the reagent probe. We have cast small fibreglass blocks to displace this 5 mls, and so have reduced the amount of reagent required, resulting in a great saving on antisera.

6. Enzyme treatment of the test sample cells is now performed at 37°C instead of room temperature. In addition, we have added a 14½ turn mixing coil immediately after the cells and antisera meet. These two simple modifications have greatly enhanced the Rhesus typing, especially in older specimens.

Before we give our results, we have some criticism of the machine as it was delivered. Our Autotyper arrived at the end of August, 1970, and was installed by a representative of Technicon in mid-September over a period of three days. However, it took 3-4 weeks before the machine was operating to our satisfaction and one must compare this with the simplicity of installing the standard type Autoanalyser which can produce satisfactory results within a few hours.

The Reagent Dispenser has given trouble on five separate occasions due to breaking of wires from the constant stretching and flexing as the dispenser probes move back and forth. We attempted to correct this by lengthening and resoldering the harness wires. We eventu-

ally obtained a second Dispenser Module from Technicon which gave trouble once, but was quickly remedied and has been functioning satisfactorily since.

The machine was supplied without a drip tray, resulting in a leakage of saline into the machine and rusting of the chassis occurred. The whole machine had to then be dismantled to the bare chassis, treated with an anti-rust preparation, repainted and reassembled. On this occasion, the machine was out of action for three days.

Due to misadventure, one of the reaction coils worth $90.00 was broken. However, a spare was on hand and no delay was occasioned. The broken coil was repaired by a professional glass blower. The obvious solution was the construction of a perspex hood covering the whole manifold to protect the coils.

On one occasion, due to the small capacity of the waste trap in the vacuum line, liquid entered the vacuum pump which rusted and seized. We have now added a second vacuum trap in series with the first.

Results:

From October 16th to March 31st, 4,887 blood groups have been run in parallel on the Autotyper and our usual manual methods. The various discrepancies as found in this series are summarised in *Table 1*.

TABLE I

TOTAL NUMBER TESTED	TYPE	DISCREPANCIES
4887	ABO	5
4887	RHESUS	207
4887	ANTI-BODIES	7
2346	IMMUNE ANTI-A	3
1600	A.R.T.	NIL
1900	LACHLAN KAHN	2

ABO GROUPS:

Of these groups, 5 ABO discrepancies were found between the two methods. Each discrepancy was critically examined and it was found that three were due to incorrect interpretation of the paper readout. The other two were due to a technical error in the manual method.

Because the first three discrepancies were, we feel, due to lack of experience, two technologists now take separate readings of the final results. The paper readout appears to be satisfactory and accurate, in fact, for us so far, the Autotyper has been thoroughly reliable for ABO grouping.

By the methods employed, 1 ml of anti-B and anti-AB will type approximately 350-400 samples. The snail anti-A will type considerably more.

RHESUS TYPING:

5% of Rh(D) Positive gave negative results with the Autotyper. On further examination, these discrepancies were either Rh. D. Positive or weak Rh(D) types. As all Rh(D) Negative samples are routinely checked for Rd(D) Variants, this is probably not a serious error. No Rh(D) Negative typed as Rh(D) Positive by the Autotyper.

Because of these discrepancies we have modified the Manifold System as mentioned previously. By enzyme treating the test sample cells at 37°C (instead of room temperature) and the addition of a further mixing coil, this discrepancy has fallen from 5% to 0.1%. We feel that this is probably within acceptable limits. Again, No Rh.D. Negative typed as Rh(D) Positive on the Autotyper.

1 ml of Anti-D (with a manual titre of 1:256) will type approximately 250 blood samples.

The Anti-CDE used for checking 'True' Rhesus Negative samples requires an Anti-C with a manual titre of at least 1:32 and the Anti-E titre of 1:16.

So far, no obvious discrepancies have been noticed.

ANTIBODY SCREEN:

From the 4,887 Blood specimens 79 Antibodies were detected by the Autotyper and 85 by our manual methods. (Indirect Coombs, Low, papain, 37°C saline and room temperature saline).

7 Antibodies, 3 Anti-Kells, 1 Auto Antibody (non specific), 2 Anti-Lewis and 1 Anti C & e, were not detected by the Autotyper, whereas an antibody, a weak Anti-D, was missed by the manual method, but was detected by the Autotyper.

In view of the inadequate antibody evaluation with the parallel running, 153 known antibodies of various specifications and strengths were assessed. As can be seen in Table II the Rhesus Antibodies again gave excellent results. However several antibody types, namely Anti-Kell Duffy, Kidd, etc., were not detected by the Autotyper System, but we consider this method superior to our manual enzyme technique.

We have recently incorporated a 37°C jacketed mixing coil into the plasma channel and attempted a low ionic strength antibody screen, from which we hope to increase our antibody range and sensitivity.

The use of high protein medium without enzyme as described by Busch and Erickson was found to be rather insensitive for use as a screening procedure.

TABLE II

ANTIBODY SPECIFICITY	TOTAL	AUTOTYPER POSITIVE	MANUAL POSITIVE
Anti-D	84	83	83
Anti-C+D	14	14	14
Anti-C	4	4	4
Anti-c	5	5	5
Anti-C^w	3	3	3
Anti-C+e	5	3	5
Anti-E	6	6	6
Anti-P_1	2	1	2
Anti-I	3	2	3
Anti-Le^a	5	3	5
Anti-Le^b	3	2	3
Anti-Le^{a+b}	3	1	3
Anti-M	1	1	1
Anti-N	1	1	1
Anti-S	1	Nil	1
Anti-s	1	Nil	1
Anti-Kell	8	Nil	8
Anti-Fy^a	3	Nil	3
Anti-Fy^b	1	Nil	1
Anti-Jk^a	2	Nil	2
Non Specific	5	3	5

IMMUNE ANTI-A & B SCREEN:

As 80% of our groups are from Ante-Natal patients an Immune-Anti-A & B antibody screen is essential. Dr G. H. Tovey has described previously the use of pig red blood cells for the detection of such antibodies. Following this line, we have used enzyme treated pig red cells in excess complement.

A negative result gives non specific agglutination by the heteroagglutinin. If the test is positive, the Immune antibody will combine with complement and so lyse the pig cells.

In 2,346 blood samples so far tested, this is quite promising as 15 Immune Anti-A's of 1:32 or greater have been detected. One of these was missed by normal manual method. 3 Immune Anti A's greater than 1:32 were not detected by the Autotyper.

SYPHILITIC REAGIN SCREEN:

Approximately 3,500 blood samples have been tested for reagin antibodies on the Autotyper. 1,980 were screened using a modified Lachlan Kahn antigen as described by W. J. Lockyer. 10 positive results were obtained besides several known positive control sera (6).

12 positive results were obtained in the series by the manual V.D.R.L. test. Extreme care in preparation of the antigen is essential for gross precipitation may occur resulting in probable loss of antigenic activity.

The Lachlan reagent is prepared by using a fat dye (Sudan IV) with Kahn Reagent (Sylvana). Tincture of Benzoin is added to stabilize the antigen, and ammonium sulphate (saturated) is added finally. This preparation seems stable for several weeks.

1,600 sera only have been tested using the A.R.T. Charcoal Reagent (Hynson, Westcott & Dunning). These results obtained are also most encouraging for although only two positive results were obtained in this series by both A.R.T. and V.D.R.L. the 4 control sera gave clear reproducable results. This is despite having a 4½ turn mixing coil where a 28 turn coil is prescribed. We have found mixing occurs in the settling coil and clear tear drop shapes appear on the readout.

Where three-quarters of the antigen is wasted by the recommended A.R.T. method, virtually no wastage is obtained by using the Autotyper. Although it is not as economical as Lockyer's method, 10 mls of A.R.T. reagent will perform 200 tests on the Autotyper.

The end product produced by the Lachlan method makes reading of results very easy. Unfortunately, for us, there is difficulty in standardisation of antigen preparation, whereas the A.R.T. reagent has already been standardised and is ready for use without further preparation.

Conclusion:

The Autotyper has now been evaluated over a period of 9 months. Some modification to both the mechanical and haemagglutination aspects were needed for us to obtain satisfactory results.

We now feel confident the results for ABO and Rhesus D-typing are at least as good as the manual method employed at this laboratory.

The antibody method however, is not as sensitive as was originally hoped, but with further experimentation in the future this may be overcome. It must be emphasised this antibody screen is supplemented by an Indirect Coombs technique to ensure adequate antibody detection.

Added to this, the inclusion of reagin antibody and haemolysin techniques the machine provides a labour saving, highly

standardised, economical laboratory tool in Immunohaematology.

Acknowledgments:

The authors wish to thank the Pathologists of Medical Laboratory for allowing them to present this publication; Dr M. R. Fitchett especially, for his helpful criticism and advice. Also thanks to Miss J. R. Still for her technical assistance.

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Letters to the Editor

Sir,—I read G. B. Winder's article on *M. distortum* published in the November issue of the Journal with interest. May I take the liberty of amending the references? References to the numbers of isolations in the Otago District are from the papers **Animals as a Reservoir of Human Ringworm,** Smith, J.M.B., Rush-Munro F.M. and McCarthy M.D., *Aust. J. Dermat* (1969) X,3, 169-192 and **Isolations of *Microsporum distortum* from skin lesions of Humans and Cats in Otago.** Fitzgerald N.W., McCarthy M.D. and Taylor B.B., *N.Z. med. J.* (1969) 70, 450, 320-321. I have no unpublished information on *M. distortum.*

Michael McCarthy,
Medical Laboratory,
Dunedin.
November 16, 1971.

Sir,—May I comment on the interesting paper by D. G. Bolitho on "Quality Control for the smaller Microbiological Laboratory," N.Z. J. med. Lab. Technol. 1971, 25, 3.

While in agreement with his attitude to control I feel that he has not been made aware of the excellent external control/Standards system recommended by the N.Z. Society of Pathologists to all its members in 1969. This system is that of the College of American Pathologists and is developed, produced and collated by Hyland Laboratories.

In the Comprehensive Laboratory Survey series for Bacteriology-Mycobacteriology-Mycology-Parasitology-Serology two specimens/cultures in each section are provided and identification must be completed as fully as possible in all sections within six weeks. These results are then returned through the standing Committee on Education of the N.Z. Society of Pathologists to Hyland Laboratories where they are computed, analysed and results returned.

This system solves any problems of putting through unknown specimens and the assessments of results is certainly an independent organisation.

Michael McCarthy,
Medical Laboratory,
Dunedin.
November 16, 1971.

Sir,—In reply to Mr McCarthy's comments on my recent paper concerning quality control in the microbiology laboratory I would offer the following explanation.

I was aware of the N.Z. Society of Pathologists' scheme. I in fact commented on the scheme at the end of my paper. The paper was, however, written in February/March of 1971, before the first batch of quality control material for the N.Z. Society of Pathologists' scheme had been received. This laboratory has in fact participated in the basic scheme this year and I have found this to be a useful check on our internal quality control programme although the scheme's usefulness is limited by the delay in the return of results from the U.S.A.

Whilst feeling that this material is a useful, even indispensable check on the internal quality control system it can in no way substitute for a continuing day to day quality control programme.

I was most interested to see Mr McCarthy's statement that the scheme was recommended by the N.Z. Society of Pathologists to its members in 1969. The Pathologist at Gisborne, Dr M. B. Bottrill agrees with me in stating that the first indication that the scheme was available in N.Z. was received in a letter from the N.Z. Society of Pathologists dated 11/11/70. I understand that the scheme was made available to smaller technologist directed laboratories and I would be interested to learn how many laboratories of any size, took advantage of the scheme. It is my personal feeling that participation in this scheme should be compulsory for all laboratories and the results should be made available to the Health Department.

D. G. Bolitho,
Cook Hospital,
Gisborne,
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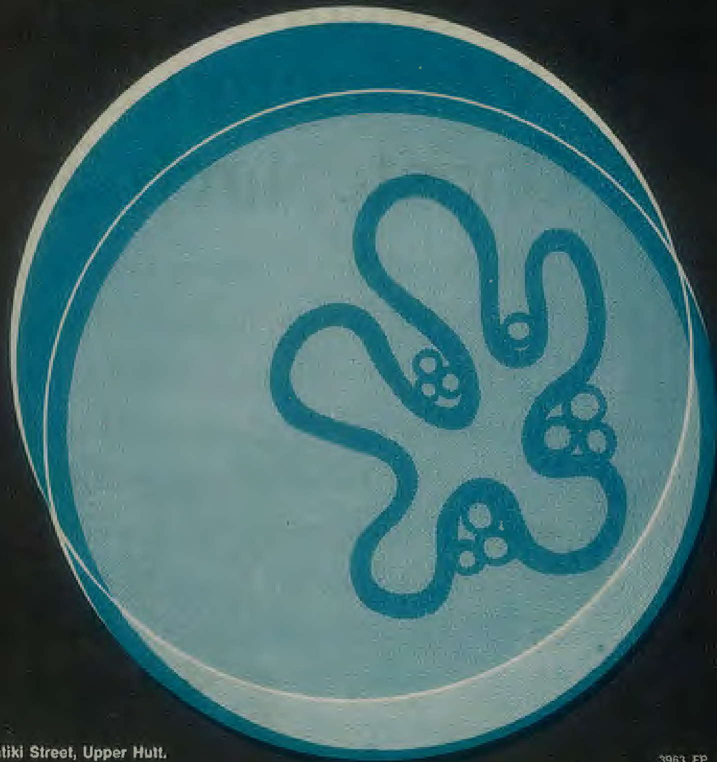

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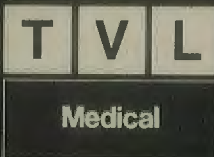

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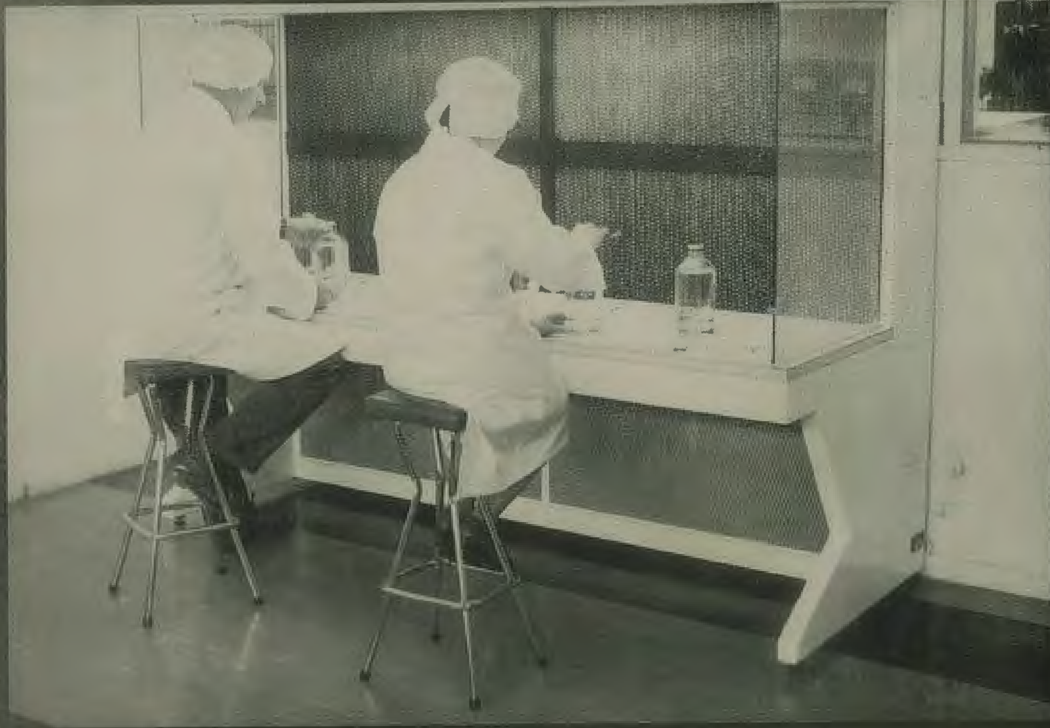

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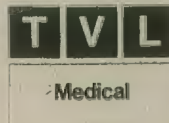

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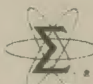

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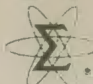

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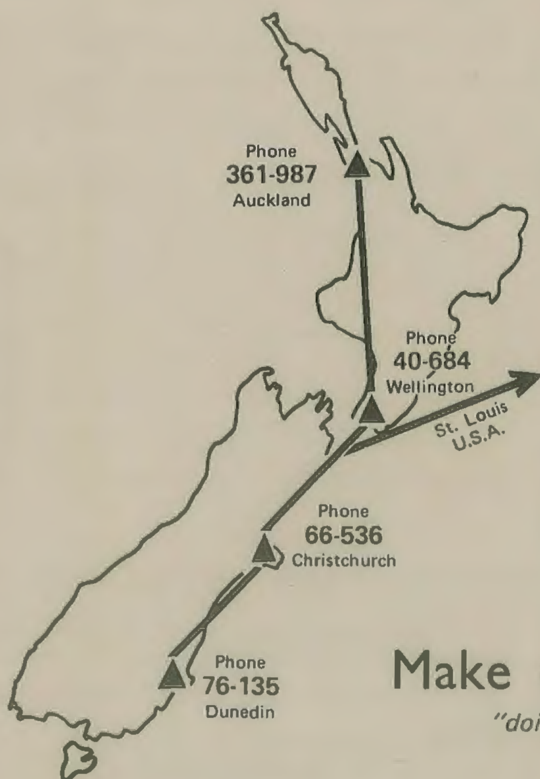

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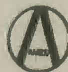

THE NEW ZEALAND JOURNAL OF MEDICAL LABORATORY TECHNOLOGY

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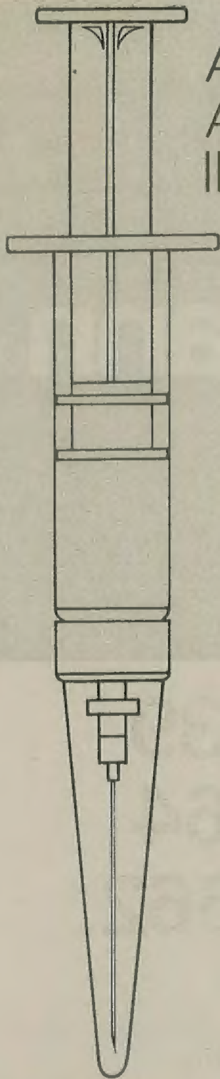

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Infectious Mononucleosis Complicated by Acute Haemolytic Anaemia

D. J. Crickett, A.N.Z.I.M.L.T.
Medical Laboratory, Hamilton.

Received for publication December 1971.

Introduction

Infectious mononucleosis is a relatively common acute infectious disease predominantly found in children and young adults. It is characterised clinically by fever, sore throat and gland enlargement, and haematologically by an absolute lymphocytosis with the appearance of a typical lymphocytes in the peripheral blood. A heterophile antibody which agglutinates sheep cells is usually present and is detected by the Paul-Bunnell test.

Infectious mononucleosis is a benign disease lasting a few weeks. Certain complications may arise, and it is said varying degrees of hepatitis occur in many cases (Baron et al, 1965)[1]. Occasionally more serious complications may arise namely myocarditis (Fish and Barton, 1958)[4], neurological involvement (Broughton, 1970)[2], rupture of spleen (Hoagland and Henson, 1957)[5], thrombocytopaenia and haemolytic anaemia (Casey and Main, 1967)[3].

Case History

This paper describes the case history of a patient who developed an acute haemolytic anaemia secondary to infectious mononucleosis. The patient, a European male aged 33 years, clinically presented with a sore neck, nasopharyngeal congestion, sweats and urinary frequency, all of which had been present for several weeks. He had no glandular enlargement, but his spleen was just palpable. Haematological investigations found a haemoglobin of 10.1 g%, reticulocytes 4.0%, total leucocytes 14,000 per c.mm with 28% neutrophils, 20% lymphocytes, 4% monocytes, 1% eosinophils and 47% atypical lymphocytes. The differential Paul-Bunnell test after absorption with guinea-pig kidney showed a titre of 1 in 640. The erythrocytes showed macrocytosis, increased polychromasia, autoagglutination and an occasional erythroblast. The ESR was 34mm/hour.

In view of the low haemoglobin level and raised reticulocyte count, a repeat blood sample was requested, and five days later a haemoglobin of 8.5 g%, reticulocytes of 4.8%, a strongly positive direct anti-globulin test and serum haptoglobin level of less than 10 mg/100ml was found. Serum bilirubin was 2.0% but the amount of indirect acting bilirubin was not estimated. These results are consistent with a haemolytic process.

The following day a sternal bone marrow aspiration was performed to confirm the presence of a haemolytic anaemia. By this time the patient's haemoglobin had dropped to 7.1 g%, and although only 13% of atypical lymphocytes were reported, the leucocyte differential showed a total of 61% lymphoid cells. Serum bilirubin was 1.2 mg% of which 1.0 mg was of indirect acting type. The marrow aspirate was found to be moderately hypercellular, with an erythroid:myeloid ratio of approximately 0.5:1. The marrow storage iron showed a moderate increase in the granular form. There were a few sideroblasts present but no abnormal 'ring' forms. The erythroid series showed a normoblastic hyperplasia with a shift to the left. There were mainly basophilic erythroblasts and numbers of nests of erythroblasts were present. The granulocytic and platelet series appeared normal. There were occasional lymphoid cells present but no infiltration. The marrow findings confirmed the diagnosis of an infectious mononucleosis with acute haemolytic anaemia.

Other incidental tests which were performed in the laboratory throughout the course of our investigations showed normal platelet counts, normal serum iron and iron-binding capacity which indicates that the increased marrow storage iron was derived from increased erythrocyte destruction, and the serum lactic dehydrogenase was increased in keeping with a haemolytic disorder. Agglutination tests for *Brucella abortus*, toxoplasmosis and leptospirosis were all negative. Urinary haemosiderin was negative. ESR was raised above that normally found in infectious mononucleosis. Liver function tests indicated an hepatic involvement with thymol turbidity of 10 Shank-Hoagland units, serum GOT 38 Karmen units and serum GPT 53 Karmen units.

The direct antiglobulin test was strongly positive using both γ and non-γ AHG fractions. Both serum and eluate were tested ex-

clusively against a panel of Group O cells of known antigen types in an attempt to identify the antibody. However, all testing proved fruitless, and the antibody was labelled as a non-specific warm reacting autoantibody. Anti-i has been implicated in the majority of cases of haemolytic anaemia associated with infectious mononucleosis (Troxel et al)[6] but no evidence of the antibody could be found in this case.

Therapy

The patient began therapy of 60 mg prednisone per day one week after initial presentation at the laboratory. His haemoglobin value improved from 7.1 g% to 10.2 g% after the first week of therapy, the reticulocyte count rose rapidly and then gradually fell over the next month back to normal levels. Serum haptoglobins quickly returned to normal indicating a cessation of the haemolytic process. (See figure 1). A month after commencing therapy, the dosage of prednisone was reduced to 40 mg per day, with subsequent reductions over the next few months. At present haematological findings on this patient are quite normal.

Conclusion

This paper is endeavouring to bring to attention the possibility of a haemolytic anaemia associated with infectious mononucleosis in patients whose haemoglobin level has fallen below that of the normal range. The incidence of this disorder, particularly of a mild degree, appears to be more frequent than earlier supposed, and in our laboratory several other cases have been found with reticulocytosis, hyperbilirubinaemia and lowered serum haptoglobins. It is our view that patients presenting with infectious mononucleosis and associated lowered haemoglobin values should be screened for the presence of a haemolytic process.

Summary

Acute haemolytic anaemia has hitherto been reported as a rare complication of infectious mononucleosis. This paper describes clinical and laboratory findings of a patient who developed such a complication.

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A Micro-modification of a Method for Detection of Streptococcal Anti-hyaluronidase

E. J. McKay, A.N.Z.I.M.L.T.
Medical Laboratory, Hamilton.

Received for publication December 1971.

Introduction

The determination of Anti-hyaluronidase (AHT) Antibody levels as a useful adjunct to Anti-streptolysin titrations for the detection of Group A Streptococcal infections has been described by several laboratory workers.[1, 2, 3, 5]

The methodology is tedious, time consuming and rather complex. Reagents are expensive, relatively unstable with an appreciable variation from batch to batch. Strict adherance to a standardised technique is essential, so it was felt a simple, economical micro-method could be usefully employed.

The Microtiter System first introduced by Takatsy in 1950 and further refined by Sever[4] in 1962, is being accepted more by serologists everywhere, whether their workload be heavy or light. By using this system, I consider A.H.T. levels can be estimated with greater speed, accuracy and economy than those obtained by the conventional macromethod.

Materials and Method

'Microtiter' Apparatus (Cooke Engineering Co.).

1. 25 Microlitre diluters (calibrated, Tulip loops)
2. 25 Microlitre pipette droppers
3. 25 Microlitre Go No-Go delivery testers
4. Lucite 'U' plates (permanent type)

Reagents

Desiccated A.H.T. enzyme and substrate are reconstituted according to the manufac-

turer's (Difco) instructions.

Fresh distilled water used throughout as diluent.

2N acetic acid prepared fresh each day.

Methodology

Follows a similar pattern to that outlined by the reagent manufacturers.

Each serum to be tested is initially diluted 1:16 with distilled water. The diluted sera are then serially titrated using Microdiluters and Lucite plates, as directed by the Instruction Manual supplied with the 'Microtiter' System.

To each serum dilution 25 microlitres of reconstituted A.H.T. enzyme is added, the contents in the plate mixed thoroughly, and incubated at 37°C for 15 minutes, after which the plates are refrigerated for 20 minutes at 4°C.

75 microlitres of A.H.T. substrate is added to each well, mixed thoroughly, incubated at 37°C for 20 minutes, and refrigerated for a further 30 minutes.

25 microlitres of 2N acetic acid is added to the contents of each well, mixed thoroughly and finally read for the presence or absence of clot formation.

With each batch, a standard serum (containing known A.H.T. antibody level), substrate and enzyme controls are tested.

Results

A blind trial was performed on 30 serum samples with varying antibody levels to Streptococcal Hyaluronidase. As shown in Figure 1, the results correlate well except for two samples. By repeating these discrepancies, the Macro-method was shown to give false high results on both occasions.

Figure II shows the typical clot formation observed using the 'Microtiter' System. Results are easier to read and the end point can be determined with confidence, as all dilutions from each test serum can be scrutinised simultaneously.

By using this system, a minimum tenfold saving in the cost of reagents and technologists' time is obtained. The method has been used in this laboratory for almost two and a half years, and has certainly helped alleviate the problem of an ever-increasing workload.

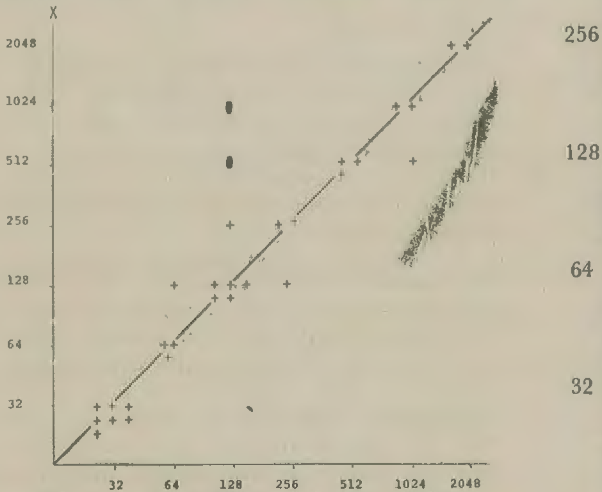

Figure I. Comparison of both methods.
● The discrepancies.
X Macro method.
Y Microtiter.

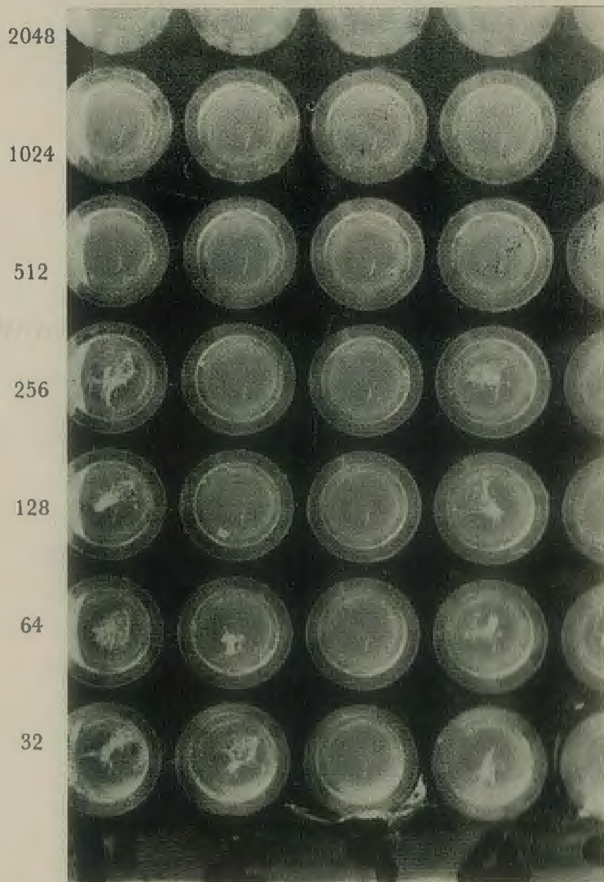

Figure II: Results of 5 sera with varying antibody levels.

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Packed Cell Volume Estimation

Sir,

For many years we have been using Vacutainers containing EDTA for the collection of venous blood samples and have always appreciated that the PCV was lowered if an inadequate draw of blood was obtained due to red cell shrinkage. However, we recently experienced difficulty in correlating PCV, Haemoglobin and MCHC results with samples of 2-3 ml volume which prompted a survey using male and female blood in volumes from 0.5ml-7ml, the latter volume being the stated draw for the Vacutainer in current use. The amount of EDTA in Vacutainers is:

2ml Paediatric Vacutainer 7.5mg
4ml Paediatric Vacutainer 6mg
7ml Adult Vacutainer 9mg

This concentration is higher than the usually accepted amount of 1mg/ml blood and in the case of the 2ml Paediatric tube is nearly 4 times the usual concentration.

The following PCV results were obtained:

ml blood	7ml Vacutainer		4ml Vacutainer		2ml Vacutainer	
	Male	Female	Male	Female	Male	Female
0.5	39	29	38	32	36	27
1.0	39	33	38.5	33	39	32
1.5	40	36	41.5	35	42	34
2.0	41.5	37	43.5	35	42	35
2.5	43	37	44	36	max. capacity	
3.0	44	38	44	37		
3.5	45	38	45	37		
4.0	44	38	44	37		
5.0	45	38.5	max. capacity			
6.0	45	39				
7.0	45	39				
	max. capacity					

These results indicate that PCV results will not be accurate if there is less than 3ml of blood in the 7ml and 4ml tubes and are not at all accurate in 2ml tubes. The haemoglobin level is not affected.

B. W. Main,
Haematology,
Dunedin Hospital.
August 5, 1971.

Questions and Answers

Answers to Questions in November Journal.

Q. In microbiology what does the O.N.P.G. test detect?

A. The O.N.P.G. (*o*-nitrophenyl-β-D-galactopyranoside) test is used to detect potential lactose fermenters which, in ordinary media, either take several days to produce acid or do not produce any acid. Lactose fermentation depends on two enzymes (i) an induced intracellular enzyme, β-galactosidase, which attacks lactose, and (ii) a permease which regulates penetration of the cell wall.

In a positive reaction the colourless O.N.P.G. is hydrolysed to the yellow o-nitrophenol.

Q. In making XLD agar which is the best method of preparation? Starting from the XL agar base and adding the desoxycholate or using XLD agar?

A. XLD agar, the complete Xylose Lysine Desoxycholate agar is prepared by heating the dehydrated material in water just until the medium boils. Excessive heating is detrimental and increases the degree of inhibition. XL agar base has the advantage that it can be sterilised by autoclaving, stored, remelted and the desoxycholate added.

Q. What type of disinfectant can I use to destroy with some degree of certainty, the hepatitis viruses?

A. A 3% aqueous gluteraldehyde solution is recommended for this purpose. It is best to leave articles in this for about one hour. As gluteraldehyde gives off noxious gases and is a skin irritant handle with rubber gloves in a fume cupboard. It is used for disinfecting autoanalyzer dialysers and may be passed through the dialyser as this is a dangerous trap for the virus. For general purposes Mucocit, which is claimed to be virocidal can be used. Autoclaving should be resorted to for all high risk discarded material to avoid risks of handling during disposal. See also the article by Percy-Robb, I. W. Proffit, J. and Whitby, L.G. (1970) J.clin.Path., 23,751.

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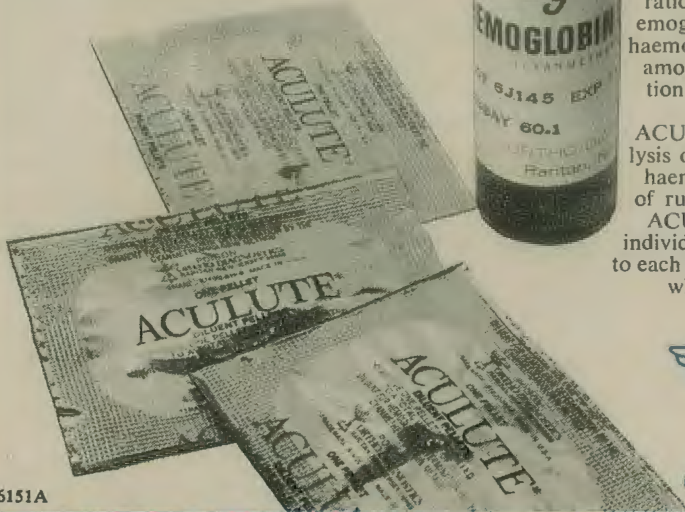

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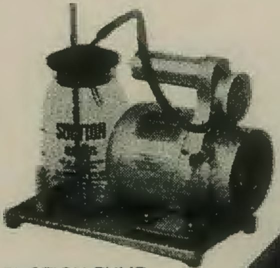

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WHO'S WHO IN THE INSTITUTE

The third article in this series, presents one of the Institute's Vice-Presidents, John Morgan. I have to thank him for providing the details of his career and letting us know something of his aims and ambitions. Once again these details are in the form of a data sheet and I would like to supplement it with a few personal observations.

John was the first person I laid eyes on when I stepped into the old Medical School laboratory for the first time. He was grinning affably. We had a pleasant conversation and I decided that the natives appeared to be friendly. He still presents an amiable appearance. I believe there is a small omission in the details provided. At one point he did leave our ranks for a short sojourn in the world of commerce but eventually returned to the fold. Although tending to be a conformist in many ways he has, in the current idiom, "done his own thing" in one respect. He is the owner of an unusual house of striking appearance without, and unusual decor within. The main building material is aluminium and he proudly boasts that the upkeep and fuel bills are negligible. There are no conventional rooms in this space-age dwelling, but activity areas. No visit to Dunedin, where John was born and bred, is complete without an inspection!

This adventurous spirit is also manifested in his interest in the Territorial Army in which he quickly gained a commission and has steadily progressed in rank and responsibility. Herewith the details.

J. D. R. MORGAN

Born in Dunedin, November 24, 1927.

Educated at Andersons Bay Primary School, Dunedin, and Otago Boys' High School.

Commenced training in Oamaru Hospital Laboratory in 1948 and then continued in the Bacteriology Department of the Medical School in the same year.

Qualified in 1953 and worked in various sections of the Diagnostic Laboratories until 1957.

In 1959, opened the Microbiology Section of the New Wakari Hospital Laboratory and was in charge of this laboratory till 1966.

From 1966, Administrative Technologist, Laboratory Services, Dunedin Hospital.

1961-69, Secretary, N.Z.I.M.L.T. and has been a Vice-President since then.

Currently Convener of the Public Relations Sub-committee and a member of the Negotiating, Awards and Fellowship Sub-committees. Also a member of the Regional Committee of the Hospital Service Welfare Society.

Married, two children.

Interests:—Territorial Army, water ski-ing and recorded music.

Joined the Territorials in 1955, Medical Corps. Assisted Professor J. H. R. Miles to design and equip the Mobile Laboratory.

Present position, Officer Commanding 9 Mobile Bacteriological Laboratory. Rank, Major.

Attended lectures in Political Science at Otago University, 1953, and was a member of the Otago University Students Association Executive. This awakened an interest in the working of Society in Institutions and Clubs and the machinery available to effect changes.

As a Past President of Otago High School Old Boys Society was considerably involved in fund raising for a new hostel and gymnasium appeal.

From 1953 onwards conducted a campaign to convince the University of Otago authorities that they should observe the Hospital Employment Regulations (Laboratory Workers) in regard to the salary scales of Medical Laboratory Technologists in their employ. This campaign was successful.

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Canta Ana, Calif., Jan. 4.—A new camera/processor unit which produces microfiche from documents as easily and as quickly as operating a copying machine—plus a companion push-button retrieval/ display unit with an unlimited cassette data base —were announced here today at low new generation pricing by Micrographic Technology Corporation, an affiliate of Varian Associates.

The system is a revolutionary low-cost method of making microfiche copies of documents and storing and retrieving them in such applications as material records, time records and materials expended records.

The new units — called the model 750 Microfiche Camera/Processor and the Model 95 Automated Microfiche Retrieval/Display device, respectively — are priced lower than conventional office copying machines and in some cases 100 times less than mass document storage and retrieval systems.

The camera/processor is priced at only $9,970 and the retrieval/display unit is only $1,990.

The new MTC units require no computers, although their data base can handle hundreds of thousands of microfiche images and can be retrieved within seconds. Unlike restrictive viewing systems, the Model 95 will not limit the size of the data base because storage of the microfiche is accomplished externally by means of specially designed cassettes. The operator never touches the individual fiche; true file integrity is maintained.

Key to the new development is the totally self-contained engineering aspects of the camera/ processor unit. All chemistry, filming, exposure control, printing and processing apparatus is entirely contained within the compact console. A file clerk or secretary only has to insert the document to be microfiched, slide a film packet into a slot and press three buttons.

AN EARLY-WARNING SYSTEM FOR DISEASE DETECTION: EXPLORATORY WORK ON CANCER

A new spectrophosphorimeter designed with support from the Science Research Council of Britain, and built at Nottingham University around two Rank Precision Industries' Monospek monochromators, has been exported to the Illinois Naval Dental Research Institute, of U.S.A.

In a four-year research programme, their principal investigator, Captain Kirk C. Hoerman, aims at developing techniques which will allow detection and identification of changes in body tissue within five minutes. This could lead to an early-warning system of body changes with far-reaching application in general and forensic medicine.

THE PROCESS IN BRIEF

A narrow waveband of light shone on the sample is absorbed and re-emitted as light of a longer wavelength. This is known as phosphorescence when the re-emission occurs in around 1/1,000,000th of a second or longer, and as fluorescence when the delay times are some thousand times shorter.

The extremely sensitive Nottingham spectrophosphorimeter allows precise selection of narrow wavebands of excitation and phosphorescent/fluorescent radiation via the Monospek monochromator, which also provides a mechanical scanning of the continuous spectrum.

Chosen by the U.S. Navy for its optical and electronic correcting system that eliminates distortion of output data, this fully-compensated spectrophosphorimeter gives phosphorescent and fluorescent excitation and emission spectra, as well as phosphorescent life-times. It is based on a design published by Drs R. B. Cundall and G. B. Evans and improved in association with Messrs W. E. Porter and R. Parsons.

The American approach is to induce low-temperature phosphorescence by shining ultraviolet light on the sample of living tissue. The three-dimension response giving visible light of different wavelengths is converted to numeric characters and compared in a computer-controlled system.

RESEARCH AT NOTTINGHAM

Dr Cundall who directs research with this instrument at Nottingham University is doing similar exploratory work on the detection of cancerous tissues.

Basically, it is a question of environment. A body cell can 'go wrong' when its environment changes. Similarly, the light emission from a sample excited by ultraviolet light depends on the environment. So Dr Cundall is using the spectrophosphorimeter, aiming to relate changes in molecular environment to changes in cell behaviour and structure.

Other materials which have been studied for the effect of changes of environment at Nottingham are the aromatic hydrocarbons and simple carbonyl compounds. Potential systems for examination are drugs, hormones and a large number of natural products that come into this important category.

The high sensitivity and low scattered light of the Monospek monochromator are particularly useful in the University's experiments with thymine, a constituent of DNA. Thymine's low fluorescent yield of about one in ten thousand is readily detected by the Nottingham spectrophosphorimeter. The be-

haviour of DNA in association with dyes and other materials is also studied. These experiments could lead to a better understanding of the genetic code —the secret of life itself. Figs. 2B and C show the results of differentiation tests on various supercooled fresh tissues.

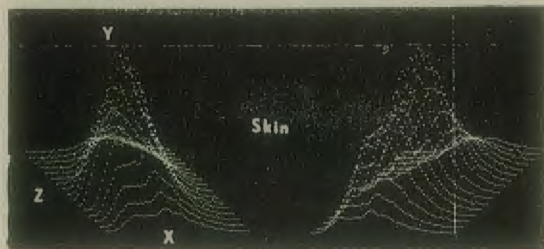

Fig 2B

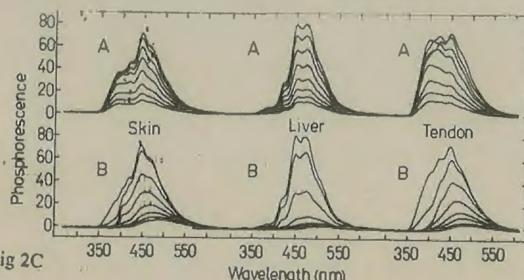

Fig 2C

NEW TIMER SIMPLIFIES REPETITIVE SWITCHING SEQUENCES

A timer which greatly simplifies repetitive switching sequences in automatic equipment (only a screwdriver is needed to install, adjust and maintain it) has been introduced by **Appliance Components Ltd.**, of Cordwallis St., Maidenhead, Berkshire, England.

Known as the TCP2 (Fig. 3), it is a compact motor-driven unit in which the switches can be readily replaced without affecting the rest of the timer. These switches, which have single-pole changeover contacts, are located in individual compartments so that any switch can be changed in a matter of seconds without tools. Any cam can be quickly and accurately positioned by micrometer adjustment without disturbing its neighbours.

Timers having up to 24 switches can be supplied, with adjustable cams or fixed-notch cams for up to 26 switch actuations per cycle. Cycle times range from 1 second to 1 month. Uni-directional or reversible motors can be fitted for operation from 24V, 50V, 220-240V 50Hz or 115V 50Hz a.c. Motors for 6, 12 or 24V d.c. operation are also available. Additional motors can be fitted to provide a choice of cycle times — up to 40 are possible with a 4-motor unit — or a rapid return to the beginning of the cycle whenever required.

The TCP2 is already being used in vending machines, machine tools, office machines, milking machines, packaging machines, dry-cleaning equipment and closed-circuit TV camera selection.

NEW MINIATURE FILTER-REGULATOR

The functions of filtering and regulating a flow of compressed air, water or non-corrosive gas are combined in a new miniature filter-regulator, the BO6, introduced by **C. A. Norgren Ltd.**, Shipston-on-Stour, Warwickshire, England. The unit, which is intended for use where space is limited, measures only 6 3/16 inches (157mm) high and 1 11/16 inches (43mm) in diameter.

The knob of the pressure regulator is fitted with a 'snap action' locking device. The filter section also has several improvements over earlier models. The bowl in which solid particles and unwanted moisture are collected is now transparent, with an optional metal bowl for higher pressures; both are threaded to simplify their removal for maintenance purposes. Louvres have been incorporated to direct incoming air into a centrifugal pattern, thereby ensuring better removal of entrained liquid. The mechanism for automatically draining collected liquid is operated by intermittent flow (causing changes in downstream pressures) instead of by changes in liquid level.

The BO6 converts primary air pressure up to 250 p.s.i. ($18kg/cm^2$) to any secondary pressure up to 100 p.s.i. ($7kg/cm^2$). Flows up to 9 c.f.m. (270 litres/min) can be handled.

ULTRA-SAFE DEWAR FLASKS

Exceptionally safe dewar flasks in rigid plastic containers, for the storage and transportation of liquid gases, boiling acids and other dangerous fluids used in laboratories and industrial processes, have been developed by **Day-Impex Ltd.**, of Earls Colne, Colchester, Essex, England. They have been designed to withstand rough handling and so remove the risk of flask explosion or breakage from the factory or laboratory bench. The standard flask is cylindrical in shape, with straight parallel sides and a hemispherical bottom. At present, three sizes are produced: 1 litre, 1.5 litre, 2 litres and 4.5 litres in capacity.

The new flasks are made from borosilicate glass, which has a lower coefficient of expansion than most other types of glass and is therefore stronger thermally and mechanically. It is also resistant to chemical action. The plastic container, which is made of polypropylene and polyethylene, has built-in cushioning of sponge-rubber rings to protect the flask against rough handling. It is fitted with a detachable handle and a vented lid or cap that prevents the build-up of pressure inside the flask. A valve in the vent enables it to be closed, and the cap is tall enough for a cork to be fitted in the neck of the flask, when the type of liquid carried makes it safe to do so. Both polypropylene and polyethylene are resistant to attack from most acids, solvents, alkalis, greases and oils. The company also makes 38 sizes of plain glass ewar flasks without containers.

OPTICAL COATING INCREASES IMAGE BRIGHTNESS

Unwanted reflections on instrument fascias and in complex optical systems are eliminated by 'HEA' anti-reflection coatings from **OCLI Optical Coatings Ltd.**, of Hillend Industrial Estate, Dunfermline, Scotland.

HEA is a multi-layer dielectric coating which, when deposited on materials such a glass, quartz and sapphire, reduces surface reflections by a factor of 10 and optimises the transfer of energy through the material over a wide spectral region.

When used on multi-element optical systems, HEA increases both the contrast and brightness of transmitted images by reducing internal light scattering and increasing the transfer of energy at each air/glass interface. On 10-element systems, for example, the image is up to 100% brighter, depending on the refractive indices of the glass.

Selected Abstracts

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

CHEMICAL PATHOLOGY

Measuring Inorganic Phosphate without using a Reducing Agent. Robinson, R., Roughan, M.E., and Wagstaff, D. F. (1971), *Annals clin. Biochem.*, **8**, 168.

The authors describe an autoanalyser method requiring no reducing agent. It uses a simple diluent and one reagent which seems to be stable indefinitely. Serum is diluted and dialysed into 1% H_2SO_4. Inorganic phosphate in the dialysate is coupled with a molybdivanadate reagent. The resulting complex is yellow and measured at 403 nm.
A.G.W.

Serial Studies of Serum Alkaline Phosphatase and 5 Nucleotidase levels in Hepatobiliary Disease. Phelan, M.B., Neale, G. and Moss, D. W. (1971), *Clin. Chem. Acta.* **32**, 95.

Serial estimation of alkaline phosphatase and 5 Nucleotidase have been carried out on patients with acute and chronic liver disease over periods up to 3 years. In acute infective hepatitis the levels of the two enzymes were similar in the 13 cases reported. In chronic liver disease 5 N.T. levels were more markedly raised than was alkaline phosphatase. The 5.N.T. is more sensitive than alkaline phosphatase in long standing hepatobiliary disease since the number of elevated 5.N.T. levels were greater than the number of abnormal alkaline phosphatases, while abnormal alkaline phosphate levels were accompanied by normal 5.N.T. levels in fewer instances.
A.G.W.

Diagnostic Value of Estimations of the C.S.F. Gamma Globulin. Paterson, C. R., Griffiths, P. D. and Craig, J. A. (1971), *Annals. clin. Biochem.*, **8**, 163.

The concentration of gamma globulin in C.S.F. has been measured by zinc sulphate precipitation in 235 patients. 52 had no organic neurological disorders. Results were distributed in a log normal manner and the normal range was 1.3 to 8.6mg/100-ml (2.S.D.).

Abnormal results were obtained in all the patients with neurosyphilis, in 10 out of 21 with multiple sclerosis and a substantial proportion of patients with cerebral infarction or cerebral neoplasma. The practice of expressing results as a ratio, gamma globulin % total protein, appears to be unjustified and this may apply also to the more specific assays of IgG content of C.S.F. Although zinc sulphate precipitation is a crude method for demonstrating the abnormality in the C.S.F. in multiple sclerosis, there is as yet little convincing evidence that specific methods for IgG have a real advantage in detecting patients with multiple sclerosis.
A.G.W.

A four day trial of Technicon S.M.A. 12/60 at Glasgow Royal Infirmary. Gardiner, M. D. (1971), *Annals of clin. Biochem.* **8**, 153.

The results were compared with routine methods and the author concluded that several channels were inprecise or inaccurate and that the system would not be suitable for routine analysis of patients' specimens without further modification. It was found that 200 specimens could be done in an 8-hour day with 2 or 3 people and a back-up of 2 or 3 trained staff.
A.G.W.

Measurement of Analytical Precision in Clinical Chemistry. Broughton, P.M.G. and Annan, W. (1971), *Clin. Chem. Acta.* **32**, 433.

Some factors affecting the precision of routine analytical methods have been investigated. The precision of urea, sodium, potassium, chloride and CO_2 with autoanalyzers were better with aqueous controls than with serum. The standard deviation increased and coefficients of variation decreased with concentration in many methods. The precision of several methods was found to depend on the arrangement of control specimens and the duration of the test period. Standard deviation and mean values of control sera found on individual autoanalyser plates often varied widely and independently. For several tests the precision determined under standard conditions for either days, weeks or months varies considerably, particularly at higher concentrations.

Significant changes in accuracy were shown to occur over short periods of time and these were reflected as a long term decrease in precision. Both the analytical precision and any change in accuracy should be taken into account when assessing the significance of alterations in measured values in patients.
A.G.W.

Laboratory Hazards (1971). *Annals of clin. Biochem.* **8**, 4.

This volume includes the following titles, 'Laboratory design and chemical hazards,' 'Carcinogens,' 'Mechanical and electrical hazards and fire control,' 'The handling of infectious material in the laboratory,' 'Hepatitis in renal dialysis units,' 'Radiation hazards in laboratories' and 'Legal aspects of laboratory accidents'.
A.G.W.

An Acid Base chart for Arterial Blood with Normal and Pathophysiological Reference Areas. Siggard-Anderson (1971), *Scand. J. Clin. Lab. Invest.* **27**, 239.

A pH log (PCO_2) chart is described, with indications of the normal values for the acid base status of the blood, as well as the values to be expected in different types of acid base disturbances, indicating acute and chronic hypocapnia, acute and chronic base deficit, and chronic base excess. The scale allows conversion to hydrogen ion conc, and conversion of mm Hg to the recommended unit for pressure, kilo pascal. The chart allows estimation of the base excess or deficit of the extracellular fluid as well as the hydrogen carbonate concentration of the plasma. An example is shown.
A.G.W.

Quality control using the daily mean. Dixon, K. and Northam, B. E. (1971), *Clin. Chem. Acta.* **30**, 453.

The value of the daily mean as a quality control statistic was studied with autoanalyzers for sodium, potassium, urea, protein, albumin, calcium, alkaline phosphatase, bilirubin and aspartate transaminase Factors are discussed which minimise variation in the daily mean. These included truncation limits and the number of results used. The daily means were weighted to correct for fluctuation in the proportion of in- and out-patients. This was found to be particularly important for albumin, protein and calcium.

The daily mean was compared with conventional quality control sera as a means of detecting changes in accuracy. They found it to be more reliable for sodium, albumin and calcium, equally so for protein, potassium, alkaline phosphatase and bilirubin and less powerful for urea and aspartate transaminase.
A.G.W.

Modification of an Automated Procedure for Serum Cholesterol which permits the Quantitative Estimation of Cholesterol Esters. Siegal, A. L. and Bowdoin, B. C. (1971), *Clin. Chem.* **17**, 229.

Changes are described in A.A. N24A for automated cholesterol that increases the reaction temperature of the mixture of sample and acid colour reagent eliminating inaccuracies of the original method. The authors found 100% recovery of oleate or palmitate with the manual Zak method but only 49% for oleate and 46% for palmitate by unmodified N24A manifold.

Their modifications consisted of increasing the temperature of the 95°C bath to 100°C, placing the H_q join immediately after the exit from the bath and insulating the mixing coils. A.G.W.

"Average of Normals" used as Control of Accuracy and a Comparison with other Controls. Begtrup, H., Leroy, S., Thyregod, P. and Walloe, H. P. (1971), *Scand. J. Clin. Lab. Invest.* **27**, 247.

Hoffman's average of normals control system was evaluated. The evaluation was based on theoretical viewpoints and on a comparison with other control principles. The authors demonstrated that the "average of normals" is suitable as a daily control of systematic errors for a number of tests if each series includes 15 to 20 results or more. They concluded that the method is suitable for the control of accuracy, but not precision, and forms an excellent supplement to the reference sample method. A simple method for the determination of the sensitivity of "average of normals" is described.
A.G.W.

Management of High-Risk Pregnancies Utilizing A Rapid Estriol Determination. Curet, L. B., Carlson, I. H. and Farb, H. F. (1971), *Wis. med. J.*, **70**, 169.

The elucidation, by Diczfaluzi *et al.* (1965), of the interrelation between the foetus and the placenta in the production of oestriol has provided a means of assessing placental function. It is now known that from maternal precursors (acetate and cholesterol) which undergo conversion to pregnenolone in the placenta, the foetal adrenals produce androgenic steroids which after hydroxylation by the foetal liver are converted to oestriol by the placenta. The oestriol thus produced is conjugated by the maternal liver and excreted in the urine as oestriol glucosiduronate. Thus by measuring the 24 hr urinary excretion of oestriol by the mother an idea can be obtained of how well the foetal-placental unit is functioning.

The authors have evaluated 3 rapid means of measuring urinary oestriol and have found that enzymatic hydrolysis followed by gas chromatography offers the most reliable measurement.

As a result of data obtained on 16 high-risk pregnancy cases (diabetes, toxaemia, chronic hypertensive or renal disease, rupture of membranes) and 6 normal pregnancy cases, the authors follow all high-risk pregnancies with serial estimations of oestriol excretion. If the values are consistently above 10 mg/24 hrs and show an upward swing, the patients are allowed to go to term. If values are less than 10 mg/24 hrs but stable, the patient is delivered at 37-38 weeks.

After 33 weeks, if oestriol excretion shows a persistent tendency to go down, the pregnancy is interrupted. At least 2 successive determinations must demonstrate the declining trend.

The authors find that these determinations offer reliable and useful information for proper management. However, it is realised that this measurement alone is not the answer to complete placental evaluation, and they are currently evaluating the reliability of serum levels of heat stable alkaline phosphatase, plasma levels of placental lactogen and ultrasonic mesurement of the fotal biparietal diameter as additional indices. J.H.

Urine and Blood Serum Muramidase (Lysozyme) in Patients with Urogenital Tumors. Kovanyi, G. and Letnansky, K. (1971), *Eur. J. Canc.*, **7**, 25.

Muramidase, an enzyme which cleaves certain glucosidic linkages in mucopolysaccharides, is widely distributed in the mammalian organism. High concentrations of this enzyme are found in kidneys, tears, human milk and blood serum. In urine, on the other hand, no appreciable activity of this enzyme can be detected.

During the last few years, the significance of the muramidase determination for clinical purposes has increased rapidly. It has been a valuable tool in the diagnosis, classification, and management of patients with various forms of leukaemia, as well as in kidney transplantation and some kidney diseases.

The objective of the present study was to investigate muramidase concentrations in urine and serum of patients suffering from tumours of the urogenital tract and to evaluate the usefulness of these determinations for diagnosing neoplasms of this region. It was concluded that determination of muramidase concentrations both in urine and in serum proved to be a valuable test for this purpose.
J.H.

Immunoglobulin E in Human Tears. Brauninger, G. E. and Centifanto, Y. M. (1971), *Am. J. Ophthal.*, **72**, 558.

Immunoglobulin E (IgE) was demonstrated by a specific radioimmunodiffusion procedure and was found in detectable amounts in all tear samples studied (26 individuals) and in increased amounts in 7/9 patients suffering from presumed allergic disorders of the external eye, including all 5 cases of vernal conjunctivitis.

IgE may represent a local immune system at the mucous membrane surfaces of the eye mediating allergic phenomena and causally relating to certain allergic disorders.

A further definition of the IgE system may be of clinical importance. IgE levels in tears might someday serve as a test for the presence of an atopic component in inflammatory states and in the diagnosis of inflammations of the cornea and conjunctiva.

Tear flow was not stimulated by contact with the eye, but the subjects were asked to stare at a point without blinking. When tears began to flow the first 50 were collected in a capillary tube and stored in vials at minus 4°C for less than 1 month. J.H.

Serum Multichannel Autoanalyzers in the Detection of Hypercalcemia and Hyperparathyroidism. Mays, E. T. and Weakley, S. D. (1971), *Surgery Gynec. Obstet.*, **133**, 603.

In the authors' institution, there were only 11

patients with proven hyperparathyroidism seen in 20 yrs., leading to the false impression that this is a rare disease primarily presenting with classic bone lesions. The installation of a multichannel auto-analyser increased the number of operations for hyperparathyroidism from 4 in 10 years to 6 in 8 months. The management of asymptomatic patients with hypercalcaemia and hyperparathyroidism prior to the onset of serious complications is as yet unresolved.

The level of serum calcium considered abnormal must be reconsidered. It has become evident, with increased screening of patients, that 10.5mg/100ml should be considered the highest normal. Yendt and Gagne (1968) recently defined the 95% confidence limits as being 8.93 to 10.05mg/100ml for women and 9.04 to 10.30mg/100ml for men.

Differences of 0.1mg/100ml may mean the difference between euparathyroidism and hyperparathyroidism. In 1/3 of their patients, Yendt and Gagne found the initial concentration to be 11mg/100ml or less during hyperparathyroidism. The mean serum phosphorus levels were below 3.5mg/100ml in 53 of their 55 patients. J.H.

Laboratory Features of Rheumatoid Arthritis. Katz, W. A. (1971), *J. Albert Einstein med. Cent.*, **19**, 108.

Rarely are laboratory studies pathognomonic of rheumatoid arthritis (RA), but they will help to confirm or deny clinical diagnostic impressions. They can be used also as a guide to the severity of the disease, to the nature of the disease, and, in certain instances, to treatment. There are many misconceptions about how laboratory studies can and cannot be useful. This discussion attempts to correct any such false notions.

Most rheumatologists prefer the Westergren sedimentation rate as opposed to the Cutler of Wintrobe because of its increased sensitivity in rheumatic diseases. One case is described which had a normal Wintrobe sedimentation rate but the Westergren technique revealed a rate of 120 mm. in one half-hour — one of the most rapid rates ever seen.

Normally the sedimentation rate tends to increase with age. Normal variance for women is 4-7 mm/hr and in men 1-3 mm/hr. Elevations to 20 mm/hr, however, are usually not helpful in establishing a diagnosis. Generally, patients with RA have a sedimentation rate >30 mm/hr. It is not uncommon to see a sedimentation rate of >100 with very active disease.

Until recently the author's laboratory performed the slide latex fixation test, which is fraught with many false-positive reactions. A determination of the rheumatoid factor titre is then useful. Generally, titres of 1:80 and above are considered positive. The great majority of tests which are positive by the slide method indicate titres of $<1:20$. Occasionally a patient with no joint disease or other illness exhibits a markedly elevated test in the range of 1:5000. The author refers to such patients as having probable rheumatoid disease.

Ragocytes are inclusion bodies present in 3-100% of the synovial fluid WBCs in 95% of patients with RA. The term is derived from the raisin-like appearance of the inclusion bodies. These are also present, however, in other types of inflammatory arthritis. Studies have revealed that in patients with RA, the ragocytes contain a rheumatoid factor not present in patients with other inflammatory diseases.

Serum electrophoresis shows abnormalities in most cases of active RA. It is a nonspecific test which shows an increased α_2-globulin and usually an increased γ-globulin. Immunoelectrophoresis may show a nonspecific increase in IgM; one may also see an increase in serum hexosamines and increased mucopolysaccharides. These have been thought throughout the years to be nonspecific acute phase reactants.

Other areas discussed: antinuclear antibodies (10-15% positive in RA); anaemia of RA (present in a large percentage of patients); and LE preparations (10-15% positive in RA). J.H.

Automated Differential Isoenzyme Analysis. II. The Fractionation of Serum Alkaline Phosphatases into Liver, Intestinal and Other Components. Green, S., Anstiss, C. L. and Fishman, W. H. (1971), *Enzymologia*, 41, 9.

Since L-phenylalanine inhibition is specific for intestinal and placental isoenzymes, activity inhibited is attributed to the intestinal isoenzyme in non-gravid individuals whereas, in the pregnant woman, this fraction represents the sum of both intestinal and placental isoenzymes. Since it is assumed that intestine, placenta, bone and liver are the 4 major sources of the serum alkaline phosphatase, the moiety which is not inhibited by L-phenylalanine is regarded as a mixture of liver and bone isoenzymes.

Heat inactivation represents a different means of partially resolving the isoenzyme mixture of serum alkaline phosphatase. At one extreme the placental isoenzym is heat-stable (5 min. at 65°C.) and at the other, the bone isoenzyme is completely heat-sensitive (16 min at 55°C.); intestine and liver isoenzymes are partially stable at 16 min at 55°C., but completely heat-sensitive (5 min. at 65°C.).

The authors have exploited these properties to develop a specific method for the placental isoenzyme based on heat inactivation of the non-placental moieties.

This combination of L-phenylalanine inhibition and differential heat inactivation has been incorporated in an automated procedure which has been applied to normal sera and over 500 patients, most of them with metastases to liver or to bone or to both. Their experience indicates that these measurements are of clinical value in gauging the progress of such bone and liver disease in relation to therapy. J.H.

Evaluation of Lipid Disorders by Lipoprotein Electrophoresis. Wilson, W. B. (1971), *J. Miss. St. med. Ass.*, **12**, 287.

Each of the bands appearing in the electrophoretic pattern of lipoproteins is composed of triglyceride, cholesterol, phospholipid and protein in varying amounts. Chylomicrons, which are particles of nearly pure fat, consist mainly of triglyceride (neutral fat), and the pre-beta band is about half triglyceride. The main component of the beta band is cholesterol and the alpha band consists mainly of phospholipid and protein. The lowest density lipoproteins have the highest concentration of triglyceride, while the heavier bands contain relatively more cholestrol, phospholipid, or protein.

The fastest moving group of proteins are designated the alpha band. These have a high protein content which gives them a high density. The next fastest band is designated the pre-beta band (which has also been called the alpha-2 band), and it consists of very low density lipoproteins, with low protein content. The band behind this is designated beta,

and consists of low-density lipoproteins with medium protein content. J.H.

The Diagnosis and Treatment of Hyperlipemia. Kane, J. L. and Powell, R. C. (1971), *J. Indiana St. med. Ass.*, **64**, 819.

Arteriosclerosis is a common and serious clinical problem. Although pathogentic mechanisms are not yet understood, a number of risk factors have been established, including certain hyperlipaemic states. These hyperlipaemias may be diagnosed by measuring fasting plasma triglyceride and total cholesterol levels. Once an abnormality is established, lipoprotein electrophoresis is helpful for classification and planning a treatment programme.

Among the high risk patients who should be evaluated, the authors include those with a cloudy fasting serum.

Patients should be tested after several days of a regular or customary diet pattern.

Visual inspection of plasma is often quite helpful. Triclyceride levels above 400 or 500 mg./100 ml. are suggested if the plasma is cloudy or lactescent. With practice one can approximate triglyceride levels simply by estimating the degree of turbidity. Chylomicrons, but not endogenous very low density lipoproteins, often float to the top as a discrete creamy layer if plasma is allowed to stand overnight in the refrigerator. Hypercholesterolaemia (Type II) results in a clear or non-lactescent plasma. Carbohydrate-induced hypertriglyceridaemia (Type IV) is characterised by elevated triglyceride levels and lactescent plasma that does not form a floating top layer unless extremely turbid. J.H.

Microprocedure for Serum Triglyceride Estimation. Haux, P. and Natelson, S. (1971), *Microchem. J.*, **16**, 68.

The Von Handel and Zilversmit method, as modified by Carlson and Wadstrom, which measures the formaldehyde produced by oxidation of liberated glycerol is a commonly used procedure for triglyceride estimation. Reproducibility is difficult with this method because the procedure is time-consuming and elaborate. Procedures which employ the condensation of triglycerides with hydroxylamine and estimation of the hydroxamic acids as the ferric complex yield results which are elevated, due to interference from other esters. The fluorimetric procedure, using the condensation of acetyl acetone with ammonia and the formaldehyde liberated, also yields elevated results, as practised. This is especially true when the patient shows an elevated glucose level.

The enzymatic procedures proposed are specific for glycerol but do not solve, in a convenient manner, the problem of extracting the triglyceride free of other substances and hydrolysis to liberate the glycerol. Other procedures such as gas chromatography and thin-layer chromatography have not been shown to be practicable for quantitation, in the routine laboratory. The nephelometric procedures are unreliable.

In the procedure developed, 100 μl. of serum are shaken with a mixture of 1 N sulphuric acid and chloroform. On centrifugation, the protein precipitate appears at the interface of the 2 solvents, the triglycerides dissolving in the chloroform. Phospholipids are removed with silica gel. An aliquot of the chloroform is evaporated. Hydrolysis is carried out in the presence of a mixture of KOH and Ba $(OH)_2$ solution. The barium hydroxide is added so that on acidification with dilute sulphuric acid, the fatty acids will co-precipitate with the barium sulphate, permitting their easy removal. The glycerol formed is then determined by oxidation to formaldehyde and reaction with chromotropic acid. The procedure is readily applied in the routine laboratory and is reproducible. J.H.

CYTOLOGY

Rapid Chromosome and Sex-Chromatic Staining with Pinacyanol. Klinger, H. P. and Hammond, D. O. (1971), Stain Technology. **46**, 43.

A simple, reliable 10 min. procedure for producing uniformly and intensely stained, fade resistant, chromosome and sex-chromatin preparations using pinacyanol chloride as the dye. Slides are extracted in 5N HCI at 20-23°C for 2 min, washed in running tap water for 2min, stained in 0.25% pinacyanol chloride solution (made up using 70% methanol) for 45 sec, differentiated in Wrights' Buffer solution (pH 6.4-6.5) for 45 sec, washed again in running tap water for 5 sec, dehydrated in two changes, 1 min. each, of absolute tertiary butanol, cleaned in 3 changes of xylene 30 secs each, and mounted in a synthetic resin. The main advantage with this procedure is its permanency. W.B.

Commercial Hair Sprays as Fixatives for Haematological Cytochemistry. Kaplow, L. S. (1971), Stain Technology, **46**, 177.

Commercial hair sprays have been found to be excellent cytological fixatives for a variety of enzymatic and nonenzymatic haematological and cytochemical staining procedures.

Some varieties evaluated were not found suitable for each staining procedure tested.

With some preparations, excellent leukocyte morphology and preservation of reaction product was obtained after reactions to demonstrate the following; — Carbohydrates (PAS), lipid (Sudan Black), nucleic acids (methyl green—pyronin), peroxidase, M-nadi oxidase and alkaline phosphatase.

These spray preparations are inexpensive, easily stored, stable and easy to use.

The properties of fixation of these preparations is probably related to their content of both polyvinylpyrrolidine and alcohol. W.B.

HAEMATOLOGY

Decreased Haemoglobin A_2 Concentration in Iron Deficiency Anaemia. Steiner, J., Marti, H. R. and Dean, D. (1971), *Acta Haemat.* 45, 77.

One hundred patients with iron deficiency anaemia had HbA_2 determinations done by starch gel electrophoresis with quantitation by elution with spectrophotometry. These patients showed a significant decrease in HbA_2 but no correlation between the amount of HbA_2 decrease and the degree of anaemia and hypochromia could be demonstrated. M.J.G.

Detection of Serum Fibrinogen and Fibrin Degradation Products: Comparison of Six Techniques using Purified Products. Marder, V. J., Matchett, M. O. and Sherry, S. (1971), *Amer. J. Med.* 51, 71.

Six assay systems for serum fibrin degradation

products (tanned red cell haemagglutination inhibition immunoassay (TRCHII), staphylococcal clumping test, Fi test, flocculation, immunodiffusion and anti-coagulant assay) were compared for reactivity with plasmin digests of fibrinogen and fibrin, with purified degradation products and with serum obtained from patients suspected of having circulating degradation products. The TRCHII reacts best with undigested fibrinogen and with the intermediate degradation fragments X and Y. Although less reactive with purified fragments, the staphylococcal clumping test is as reliable for the assay of test serums, and both react with about 90 per cent of test serums. This suggests that circulating complexes of degradation products with fibrin monomer or with fragment X may be responsible for the positive results of test serums. M.J.G.

An improved Cytochemical Method for Nitroblue Tetrazolium Reduction by Neutrophils. Hicks, R. G. and Bennett, J. M. (1971), *Amer. J. Med. Technol.* **37**, 226.

Previous methods have required too much blood or have had poor cell preservation leading to difficulties in morphological examination or do not allow any form of quantitation. This paper gives a simple and more rapid method requiring as little as 1.5 ml. blood for the diagnosis of chronic granulomatous disease of childhood or for the differentiation of bacteria from non-bacterial infection. (Normal people show a low level of spontaneous reduction of the dye by their neutrophils). M.J.G.

Anaemia in Renal Failure. Penington, D. G. and Kincaid-Smith, P. (1971), *Brit. Med. Bull.* **27**, 136.

A superb up-to-date coverage of most aspects of anaemia associated with renal failure. The peripheral blood pictures are presented together with assessment of haemopoiesis and red cell life-span in this type of anaemia. The mechanisms of haemolysis in such cases is discussed and there is a full coverage of miçroangiopathic haemolytic anaemia. M.J.G.

Atypical Mononuclear Cells in the Blood. Leading article (1971), *Brit. Med. J.* **3**, 549.

A short but important survey of atypical mononuclear cells and their relevance to various conditions in the human body. These conditions range from malignancy and infections to immune and auto-immune states. The quantity of mononuclear leucocytes in the peripheral blood at any moment is easily discovered but it is difficult, if not impossible, to establish the quality (or nature) of such cells and the word 'atypical' is hardly explanatory. M.J.G.

Postnatal Changes in Some Red Cell Parameters. Matoth, Y., Zaizov, R. and Varsano, I. (1971), *Acta. Paediat. Scand.* **60**, 317.

The red cell blood picture was studied in detail from birth to 12 weeks. The low point in the red cell count was reached in the seventh week, which represented the turning point from a negative to a positive balance between cell production and destruction. From the dynamics of the total number of circulating red cells it was concluded that, although erythropoiesis slows down after birth, a considerable amount of red cell production takes place during the first few postnatal weeks.

The MCHC increased significantly over the first 5-6 postnatal weeks and remain constant thereafter.

Macrocytes predominated at birth. Their percentage distribution decreased after the second week with a corresponding increase in microcytes. M.J.G.

Auto-immune Thrombocytopenic Purpura and the Compensated Thrombocytolytic State. Karpatkin, S., Garg, S. K. and Siskind, G. W. (1971), *Amer. J. Med.* **51**, 1.

This paper reviews current data supporting the theory that idiopathic thrombocytopenic purpura (ITP) is due to platelet antibodies but also suggests that in 65% of such cases, the I.T.P. is no longer tenable and should be replaced by 'auto-immune thrombocytopenic purpura' (ATP). Five criteria for such a diagnosis were listed. The possible aetiology of the other 35% of cases of ITP is discussed.

It also suggested that there may be some people with compensated thrombocytolysis plus antiplatelet antibody and that those with ATP are only the top of the iceberg. M.J.G.

Lymphocyte Function. Annotation (1971), *Brit. J. Haem.* **20**, 139.

An excellent survey of a rapidly developing field of knowledge, this paper discusses the definition of 'lymphocyte' and then proceeds to deal briefly with the current state of knowledge of at least twelve functions of lymphocytes. However, these functions fall into five main categories: antigen-recognising cells, immune effector cells, memory cells, precursor cells and 'other roles'.

In summary, the annotator H. E. M. Kay sees that the essential role of the lymphocyte is to amplify required responses and perhaps to extinguish unwanted ones. M.J.G.

Smoking and Leucocyte Counts. Corre, F., Lellouch, J. and Schwartz, D. (1971), *Lancet.* **2**, 632.

A study of 4,264 men showed that the number of leucocytes is increased in smokers, notably in those who inhale. The increase is about 30% for a heavy smoker who inhales, compared with a non-smoker. Investigation of a sub-group of 483 men of the same population confirmed this finding and revealed that the increase was in granulocytes, lymphocytes and monocytes. The differential leucocyte-count showed no real change. M.J.G.

The Significance of Fibrin Degradation Products in the Blood of Normal Infants. Chessells, Judith M. (1971), *Biology Neonate.*, **17**, 219.

Disseminated intravascular coagulation (DIC) has been reported in the newborn as a complication of virus infections and in the sick infants of toxaemic mothers. DIC may also occur in infants with severe rhesus iso-immunization. The possible role of DIC in the respiratory distress syndrome has also been the subject of recent debate. However, diagnosis of DIC in the newborn is not easy since accepted adult values of coagulation factors are not always applicable in the neonatal period. Since the presence of fibrin or fibrinogen degradation products (FDP) in the serum has been used as an index of intravascular coagulation it is important to know the significance of this finding in the newborn. A survey of 63 babies was, therefore, undertaken to define the incidence of FDP in the neonatal period and to determine if their presence was related to any other detectable disturbance of haemostasis.

FDP were estimated in thrombin-treated plasma using the tanned red cell haemagglutination inhibition technique with antifibrinogen prepared in the author's laboratory. FDP were detected in 4 babies.

It was concluded that FDP are present in low titre in a small proportion of newborn babies. Diagnosis of intravascular coagulation in a neonate

must be based on additional evidence: alterations in platelet count, a low level of plasma fibrinogen and a screening test or assay of labile coagulation factors. J.H.

Clinical Experience with Determination of Fibrinogen Degradation Products. Hedner, Ulla and Nilsson, Inga Marie (1971), *Acta med. scand.*, **189**, 471.

The last 5 years have witnessed a remarkable and sustained surge of interest in the occurrence, chemistry and properties of fibrinogen degradation products (FDP) in various conditions. The degradation of fibrinogen and fibrin by plasmin occurs over several intermediate stages. The degradation results initially in the formation of high molecular weight split products designated as X (Mol. Wt. 270,000) and Y (Mol. Wt. 165,000). Complete breakdown of the fibrinogen results in the formation of several fragments. Two of them, the D (Mol. Wt. 80,000) and E (Mol. Wt. 50,000) components have antigenic determinants in common with fibrinogen. In normal persons FDP have been found in an amount <10 μg/ml.

The authors determined the FDP in the serum of 3,075 patients by Nilehn's immunochemical method in order to ascertain the possible diagnostic significance of their presence.

Blood samples were collected with ε-aminocaproic acid (EACA) to prevent fibrinolysis *in vitro*. Antiserum against the D-fraction of the degradation products was applied to agarose gel. On high voltage electrophoresis serum migrates into the gel. If FDP are present, they will produce precipitation peaks. The height of such peaks is measured and related to a standard of high molecular weight substances.

FDP were found in the sera of 61% of patients with maligant disease. In several, FDP appeared in the serum before the diagnosis had been recognised in any other way. Such products were also found in the serum in chronic renal disease (37%), acute renal disease (78%), shock due to sepsis (100%) or postoperative bleeding (100%), acute stage of thromboembolism (72%) and in the acute stage of collagen diseases (38%). In patients with toxaemia or hepatosis [*sic*] or other complications of pregnancy FDP were found in 61% and 81% respectively.

The authors feel that the constant demonstration of FDP by the method used is a sign of a pathologic condition requiring further investigation. Routine examination for FDP in certain cases may be of value as a screening method. In patients with suspected malignant diseases, renal diseases, postoperative complications and sepsis, and during pregnancy, determination of FDP seems to give valuable information. J.H.

Coagulation Defects in Burned Patients. Gehrke, G. F., Penner, J. A., Niederhuber, J. and Feller, I. (1971), *Surgery Gynec. Obstet.*, **133**, 613.

Twenty-three patients with burns in excess of 40% of their body surface, admitted to a Burn Unit, were studied.

Thrombocytopenia, abnormal platelet function and plasma factor deficiencies were demonstrated in addition to a circulating anticoagulant with properties similar to those of heparin.

In 11 patients there were 21 episodes of bleeding, 14 of which were treated with specific blood component therapy or protamine sulphate. Five patients had a thrombin-clotting time in excess of 3 min.: all died from their burns.

Evaluation of haemostasis should be an essential part of burn management. J.H.

Fibrinemia in Medical Patients Screened by the Ethanol Test. Kierulf, P. and Godal, H. C. (1971), *Acta med. scand.*, **190**, 185.

The significance of disseminated intravascular coagulation (DIC) as a pathogenic mechanism in several clinical conditions is becoming increasingly appreciated. Massive DIC is usually readily detectable because of severe depletion of platelets, fibrinogen and other coagulation factors. In the absence of frank defibrination, however, platelet counts and clotting time tests frequently offer little help in establishing this diagnosis since similar results may occur in several disease states, *e.g.* liver insufficiency, malabsorption, multiple transfusions.

Demonstration of soluble fibrin in plasma is a more sensitive and reliable indicator of DIC. Plasmas from cases with DIC are gelled by ethanol at room temperature. This ethanol test was performed on 948 patients admitted to the authors' Dept. of Internal Medicine.

Blood was collected in the fasting state, with a minimum of stasis, preferably from an antecubital vein, through an 18 ga., disposable needle, discarding the first few ml. Samples from poorly executed punctures were discarded. Nine parts of blood were collected into a precooled polystyrene tube containing 1 part of anticoagulant (3.13 gm. of sodium citrate dihydrate dissolved in 100 ml H_2O). After thorough mixing the sample was centrifuged within 1 hr. (during this period kept at 4°C.) at 3,000 r.p.m. (1,200 *g*) for 30 min. at 4°C. The plasma was pipetted off into polystyrene tubes and tested within 2 hr.

Plasma, 0.5 ml., was transferred to a glass tube 70 x 10 mm. and equilibrated for 3 min. in a waterbath at 20°C. Using a blow-out pipette, 0.15 ml. of ethanol solution 51 ml. of 96% ethanol + 49ml. H_2O) was added. The tube was immediately shaken thoroughly, then left undisturbed for 10 min. The tube was then tilted once slowly to the horizontal position and thus inspected for the presence of a gel.

Plasmas containing no grossly visible particulate matter and plasmas showing discrete granules or floccules only, were considered negative.

A total of 108 patients, when first examined, demonstrated gelation. A new blood specimen, obtained within 24 hr was colected in 86 of these patients. A positive test was again demonstrated in 54 of these 86 patients, suggesting that fibrinaemia may be transient in some patients.

There was a highly significant difference ($p < 0.0005$) in the disease pattern of the patients having a positive and those having a negative test. A preponderance of malignant neoplasms, pneumonia, and diseases of the blood, and blood vessels, including pulmonary embolism, was found among the patients with a positive test. A highly significant increase in the number of deaths ($p < 0.0005$) was found among patients with a positive test. Thus low-grade intravascular coagulation may contribute to a fatal outcome. J.H.

Assessment of Iron Deficiency in Children Without Anaemia. Lytton, D. G., Godbole, V. K. and Lovric, V. A. (1971), *Pathology*, **3**, 87.

Iron deficiency anaemia in children aged between 6 months and 3 years still presents a common problem in paediatric hospital practiee. There is reasonable circumstantial evidence linking the usually

clinically unsuspected iron deficiency anaemia with childhood morbidity.

A method is described for studying iron deficiency in children with or without anaemia, using the fragiligraph or a colorimeter. The results indicate useful clinical application in making possible the detection of iron deficiency before the appearance of anaemia.

The fragiligraph model D2 was used to study the behaviour of RBCs in a dilute buffered hypotonic saline solution. The instrument contains a dialysis chamber which was filled with 0.2% buffered saline. A metal frame surrounded with dialysis tubing is inserted into this chamber and acts as a cuvette when filled with a dilute blood suspension; dialysis is then commenced. The instrument has an integrated recorder, which will register decreased optical density over a specific period of time which is concomitant with haemolysis. The "fragiligram angle" was measured on the automatically recorded changes in optical density by drawing a tangent along the straight section and measuring the tangent-abscissa angle. Within the normal group of 30 children, the minimum recorded fragiligram angle was not less than 55°, whilst all 30 children with iron deficiency anaemia recorded angles of less than 45°.

A standard filter colorimeter (green filter, peak absorbance 540 mm) was also used for the purpose of investigating fragiligram angles. The fragiligraph frame was introduced into the colorimeter cuvette containing 0.2% buffered saline and changes in optical density recorded at intervals. Results were similar to those obtained with the fragiligraph.

J.H.

The Place of the Erythrocyte Sedimentation Rate (ESR) in Oral Surgery. Juniper, R. P. (1971), *Br. J. Oral Surg.*, 8, 183.

If its limitations are understood, that a low reading does not mean necessarily that a patient is free of serious disease, the ESR is still a valuable investigation.

Determination of the "normal" ESR has been a problem. Westergren originally suggested that the upper limit of normal should be set at 3 mm. in the first hour for men and 7 mm. for women, but amended this later to 5 and 12 respectively. However, since that time, many investigators have shown that there are wide variations in the ESR in perfectly normal persons. There is a well-established increase in the ESR with age. It will be seen, therefore, that to establish a "normal" for the ESR is no easy task and many factors have to be taken into consideration. A recommended upper limit of normal is as follows: below 50 yrs., men 15 mm./hr., women 25; above 50 yrs., men 20, women 30. J.H.

Consumption Coagulopathy: Practical Principles of Diagnosis and Management. Nalbandian, R. M., Henry, R. L., Kessler, D. L., Camp, F. R., Jr. and Wolf, P. L. (1971), *Hum. Path.*, 2, 377.

Consumption coagulopathy, with its multiple aetiologies, is a common mechanism of death. If the disease is properly diagnosed and managed, life can often be extended over several days, yielding sufficient time for the diagnosis and treatment of the underlying disorder. With the correction of the primary disease the threat of consumption coagulopathy will spontaneously remit. Determination of the type of coagulopathy is critical since the disease may be lethal if the wrong treatment is given.

Consumption coagulopathy is a distortion of normal coagulation and fibrinolytic mechanisms. There is a growing view that under normal conditions there is a precisely balanced, dynamic equilibrium between continuous coagulation by the intrinsic coagulation system in the arteries and arterioles, and continuous fibrinolysis by the fibrinolytic system, primarily in the capillaries. In various diseases the 2 basic counterpoised activities of clotting and lysis can be accelerated, either singly or concomitantly. Thus there are 3 types of consumption coagulopathy.

The authors' panel of tests includes: "plasma" fibrinogen level; platelet count; prothrombin time; Glueck modification of the ethanol gelation test of Breen and Tullis; plasma protamine paracoagulation test; euglobulin lysis time; partial thromboplastin time; and fragmentation of RBCs, noted in a peripheral blood film.

The panel is repeated as frequently as is necessary, or at least at 12 hr. intervals. By observing the values of the several coagulation factors as they shift toward serum or toward plasma, the clinician can evaluate the effectiveness of treatment and adjust the dosage of the indicated drug. Thus a small group of simple tests suitable for hospital laboratories will yield patterns of differential diagnostic and therapeutic significance. J.H.

Differential Diagnosis of Hypochromic Anemias. Linman, J. W. (1971), *Postgrad. Med.*, 49 (4), 88.

Anaemia characterised by hyprochromic RBCs is one of the most frequently encountered disorders in clinical medicine. Hypochromia due to a factor other than iron deficiency is relatively rare but must be considered in the differential diagnosis.

Haemoglobin normally makes up 30-36% of the wet weight of an RBC. If the concentration of haemoglobin is <30%, the RBC is termed hypochromic. The mean corpuscular haemoglobin concentration is clinically useful as a rough indicator of haemoglobin concentration; however, the erythroid values from which mean cell measurements are obtained are subject to errors that may lead to erroneous conclusions. Furthermore, these indices are averages that reflect cells of different size and haemoglobin concentration (*e.g.* large cells may be counteracted by small cells). Consequently the presence of hypochromia requires additional documentation from a stained peripheral blood film. Whereas each of the erythroid measurements is subject to error, a well-made, well stained peripheral blood film is not; microcytes cannot be transformed artifactually into macrocytes, and hypochromia cannot be hidden from an experienced observer's eye.

Because of the importance of the blood film in diagnosing hypochromic anaemia, the reliability and reproducibility of this technique should be considered.

Much has been written about the difficulties associated with recognising hypochromic RBCs. Detection of minimal hypochromia (a few slightly hypochromic cells in a blood film in which normochromic RBCs predominate) requires a technically excellent preparation and an experienced examiner. Consequently, observers of varied competence may not agree consistently on the presence or absence of minimal hyprochromia in blood films of different technical quality. However, this should not discredit this simple means of detecting an abnormality that has important diagnostic connotations.

In most clinical situations, examination of a blood film is the easiest and most reliable way to detect impaired haemoglobin synthesis. J.H.

Evaluation and Comparison of Serological Procedures for Hepatitis-Associated Antigen (H.A.A.). Escobar, M. R., Dalton, H. P. and Allison, M. J. (1971), *Va. med. Mon.*, **98**, 494.

This study demonstrates the usefulness of the counter-electrophoresis method for routine screening of blood donors where results are needed rapidly. The immunodiffusion technique may be helpful if results are not required quickly, and it could be utilised for confirmatory tests in reference laboratories. The complement-fixation procedure should also serve as a reference test or it may be used routinely in the diagnostic virus laboratory where quantitation is essential.

The sensitivity of immunodiffusion in this study was somewhat higher than that obtained by other workers, probably due to certain factors found by Schmidt and Lennette to affect the sensitivity of this test, such as the titre of the antiserum as well as the placement of sera adjacent to wells containing positive reagents.

J.H.

MICROBIOLOGY

Some Observations on *Clostridium tetanai*. Williams, K. (1971), *Med. Lab. Technol.*, **28**, 399-408.

71 strains of *Clostridium tetani* were tested for gelatinolytic and proteolytic activity and it was found that all were gelatinase positive but none produced a proteolytic enzyme. Over 50% of the strains produce a fibrinolytic enzyme and a deoxyribonuclease. The production of a renin-like enzyme is described and the use of commercial horse tetanus antitoxin serum to inhibit swarming of *Cl. tetani* in plate cultures is described. D.G.B.

The Rapid Identification of Gram-Negative Bacilli. Roberts, G. (1971), *Med. Lab. Technol.*, **28**, 382-384.

A rapid method for the identification of Gram-negative bacilli is presented. It uses only 8 media including the complex motility and inositol fermentation medium of Donovan (1966). The method can be criticised on the grounds that it uses a dichotomous key, these are liable to lead to erroneous results, particularly when attempting to identify members of the Enterobacteriaceae. D.G.B.

Sensitivity of *Pseudomonas aeruginosa* to Sulphamethoxazole. Dalton, A. C. and Mayers, S. A. (1971), *Amer. J. clin. Path.*, **56**, 371-374.

The authors have examined 92 strains of *Pseudomonas aeruginosa* for sensitivity to sulphamethoxazole and sulfixazole. The authors' findings were that 40 of these isolates were inhibited by concentrations of sulphamethoxazole achievable in blood and 48 of the strains by concentrations achievable in urine. This was not the case with sulfixazole. They suggest that sulphamethoxazole may well have a place in the therapy of *Pseudomonas aeruginosa* infections, particularly in the urine, and that it is worthwhile carrying out sensitivities to sulphamethoxazole routinely on *Pseudomonas aeruginosa* isolated from this source. They point out the dangers associated with the antimicrobials normally used for the treatment of *Ps. aeruginosa* infections, namely, toxicity and the necessity for systemic administration. Full technical details of methods used are given.

D.G.B.

Fatal *Chromobacterium violaceum* septicaemia. Johnson, W. M., Disalvo, A. F. and Steuer, R. R. (1971), *Amer. J. clin. Path.*, **56**, 400-406.

A fatal case of septicaemia due to *Chromobacterium violaceum* is presented. A detailed case presentation is given and discussion of the taxonomic position of *Chromobacterium violaceum* is given. A list of the 70 human infections with this organism so far discovered is given. D.G.B.

A Systematic Programme of Quality Control in Clinical Microbiology. Glaser, L., Boxley, G. S. and Boring, J. R. (1971), *Amer., J. clin. Path.*, **56**, 379-383.

A microbiological quality control programme is presented. The system uses the type of charts normally used in the biochemistry laboratory. Commercially prepared lyophilized bacterial cultures were used and the results logged on the chart. Although an interesting approach the method has limitations (as do all control schemes proposed for microbiology so far), but it appears to be a useful contribution to the accumulating literature of quality control in this field and should be studied by all persons concerned with the implementation of quality control schemes in microbiology laboratories. D.G.B.

Isolation of L-forms in Recurrent Urinary Tract Infections. Crowe, C. C. and Koblasz, K. K. (1971), *Amer. J. Med. Technol.*, **37**, 9-367.

A modified Abraham's medium is described for the isolation of L-forms from urine. A discussion of the role of L-form persisters in recurrent urinary tract infection is given. The numbers of isolates in the authors' series is very small, 14 in all, which are further divided by the authors into two groups, half of which were currently receiving antibiotic therapy and half receiving no antibiotic therapy. The authors' use of percentage figures for these low numbers is, I feel, apt to be misleading.

D.G.B.

A Simple Apparatus for Reading Antibiotic Disk Zones. Gerdes, J. A. and Maddern, J. F. (1971), *Amer. J. clin. Path.*, **56**, 369-370.

This short note describes the construction of a simple piece of apparatus which is an aid to reading zone size charts using the Kirby-Bauer or similar methods of antibiotic sensitivity testing.

D.G.B.

Observations on *Aeromonas hydrophila* Septicaemia in a Patient with Leukaemia. Abrahams, E., Zierdt, C. H. and Brown, J. A. (1971), *J. clin. Path.*, **24**, 491-492.

This paper describes a case of septicaemia due to *Aeromonas hydrophila* and gives the reason for its original incorrect diagnosis as an *E. coli* infection. The authors appear to rely on antibiograms to a considerable extent as a diagnostic aid.

D.G.B.

The Evaluation of Coagulase Mannitol Test Strips. Brainson, D. (1971), *Amer. J. clin. Path.*, **56**, 375-378.

The recently introduced coagulase mannitol paper strips for detection of bound coagulase and mannitol oxidation in Micrococcacaeae isolates is critically examined in this paper. Using these strips coagulase can be read in one hour and mannitol oxidation in 6. The author used 188 isolates of Micrococoecaceae and found an incidence of false positives and negatives so high, compared to classic

methods, as to indicate that these strips are insufficiently accurate for clinical use. D.G.B.

Sterility Testing Procedures in a Pharmaceutical Factory. Wilson, D. A. (1971), *Med. Lab. Technol.*, **28**, 351-354.

A detailed description of methods used for sterility testing in the pharmaceutical industry is presented. Details of technique and culture media are given. D.G.B.

The Disk Sensitivity Test. Waterworth, T. M. (1971), *Med. Lab. Technol.*, **28**, 385-393.

This important paper discusses sources of error and methods of control of sensitivity testing. Reasons for the author's preference for interpretation of sensitivity and resistance by comparison with a control organism rather than by zone size interpretation as suggested by Kirby and Bauer are presented.

The paper gives details of both techniques and media, and the limitations of both as used in sensitivity testing.

Details of methods for the detection of methicillin resistance and detection of penicillinase producing staphylococci are given. Miss Waterworth's considerable expertise in this field must be respected. D.G.B.

Aerobatic Bacterial Counts on Human Skin after Bathing. Holt, R. J. (1971), *J. med. Microbiol.*, 4, 319-326.

This interesting paper reviews the previous work on bacterial counts on human skin and attempts to resolve the paradoxical findings of previous workers, namely, that large numbers of bacteria are removed from human skin by washing with dilute solutions of surface active agents and the contrasting observations that after washing the impression counts of bacteria on skin show higher counts than before washing. The author's findings confirm the correctness of both observations. He suggests that this can be explained by the fact that on the undisturbed skin, most of the bacteria are present as fairly large aggregates of bacteria (micro colonies) which are broken up by the mechanical actions of scrubbing and the surface active effect of soap and thus spread more widely over the skin surface to give a larger number of colony forming units consisting of a smaller number of bacterial cells than the colony forming units present before washing. D.G.B.

A New Group of Enterobacteria, Possibly a New Citrobacter Species. Leuth, E. V. and McDonald, S. (1971), *J. med. Microbiol.*, **4**, 329-336.

The authors describe the biochemical and serological characteristics of 40 strains of a new species of Enterobacteriaceae isolated in a Canadian hospital over a 15 month period. Serological studies showed no cross reactions between the 40 strains and other members of the Enterobacteriaceae. Biochemically the organisms appeared closest to the Salmonella-Citrobacter-Arizona group The most striking difference being that all isolates were indole positive. D.G.B.

A Preservative for Urine Specimens in Transit to the Bacteriological Laboratory. Amies, C. R. and Corpas, A. (1971), *J. med. Microbiol.*, **4**, 362-365.

The authors describe a mixture of sodium chloride and polyvinyl pyrrolidone (P.V.P.) as a combined bacteriostatic agent and a protective colloid. The paper gives full details of the protective action of this compound and the techniques used to determine the optimum concentrations of both sodium chloride and P.V.P. Optimum concentrations were found to be 9 g/100 ml of urine of NaCl and a P.V.P. concentration of 1 to 5%. The authors' final formula being sodium chloride 30 g, P.V.P. 3 g in 100 ml of water. The authors' claim the compound is self sterilizing but it can be autoclaved if required. In use the preservative was dispensed in 3 ml volumes in 10 ml vials and urine added to fill the vial. The authors presented evidence that the preservative will maintain bacterial cells in a viable condition with no increase in total counts for up to 7 days. D.G.B.

Comparison of Hoyle's Medium and Billing's Modification of Tinsdale's Medium for the Bacteriological Diagnosis of Diphtheria. Jellard, C. H. (1971), *J. med. Microbiol.*, 4, 366-369.

The author concludes that Hoyle's medium is better for the rapid diagnosis of *Corynebacterium diptheriae* infection than Tinsdale's medium but that Tinsdale's medium gives better differentiation. Hoyle's media is recommended for routine use where experienced staff are available. D.G.B.

***Haemophilus influenzae* Infections of the Genital Tract.** Farrand, R. J. (1971), *J. med. Microbiol.*, **4**, 357-358.

This paper discusses a case of acute vaginitis' and of salphingitis caused by non capsulate strains of *Haemophilus influenzae*. The author discusses the influence of predisposing abnormalities and the fact that some damage to the mucous membranes must properly occur for this organism to obtain a foothold. D.G.B.

Frequency of Salmonellae in Wild Ducks. Mitchell, T. R. Ridgewell, T. (1971), *J. med. Microbiol.*, **4**, 359-361.

A two year survey of 477 samples of duck droppings collected at an English reservoir showed that the faecal excretion rate in wild ducks is just over 4%. The commonest serotype being *Salmonella typhimurium* but *Salmonella para typhi B* and *Salmonella emek* were also isolated. D.G.B.

A New Cetrimide Medium for the Detection of *Pseudomonas aeruginosa*. Mossell, E. A. A. and Lourdes, I, (1971), *J. med. Microbiol.*, **4**, 380-382.

A differential cetrimide medium for the detection and direct counting of *Pseudomonas aeruginosa* is described. The medium is known as GMAC (glycerol, mannitol, acetamide, cetrimide agar). The medium consists of 0.2 g peptone, 10 g K_2SO_4, 1.4 g $MgCl_2.6H_2O$, 0.3 g cetrimide AR, 5 ml glycerol AR, 5 g D mannitol AR and 15 g agar added to 900 ml distilled water, pH adjusted to 7.0 and the mixture sterilized for 20 minutes at 121°C. The base medium is brought to a temperature of 50°C and 100 ml of a solution containing 10 g acetamide, 12 ml phenol red adjusted pH 7.0 sterilized by filtration are aseptically added. The medium is then dispensed and has a faint yellow-orange colour. The medium is designed for incubation at 42°C. 444 of 450 isolates of *Pseudomas aeruginosa* grew prolifically and changed the colour of the slants to cherry red. Some Gram-negative rods grew but with the exception of 1 strain of Alkaligenes did not change the colour of the medium to red.

To use the medium in plates to recognise individual colonies of *Pseudomonas aeruginosa* from sewage and other material, it was necessary to add 0.04M phosphate buffer of pH 7 to GMAC agar. This restricted the size of the red zones around individual colonies. D.G.B.

Postoperative Urinary Infections Caused by *Escherichia coli*. Bettelheim, K. A., Dulake, C. and Taylor, J. (1971), *J. clin. Path.*, **24**, 442-443.

This paper is a survey of the serological and biochemical characteristics of 20 strains of *Escherichia coli* isolated from patients who developed a urinary infection after gynaecological surgery and the insertion of a self retaining catheter. The survey demonstrated that in 13 out of 20 patients the infecting organism was present in either the vagina or rectum of the patient before surgery. The authors stress that the mechanism of infection and route of spread is still obscure. D.G.B.

Search for Serratia. Black, W. A. and Hodgson, R. (1971), *J. clin. Path.*, **24**, 444-448.

This paper describes a systematic search conducted over a four month period for *Serratia marcescens* in clinical specimens. The findings suggested that the presence of this organism in clinical material is commoner than is generally realised. The authors are at a loss to explain the fact that opportunist outbreaks of infection caused by this organism of the type described in the U.S.A. literature have not occurred in Britain. D.G.B.

Suphamethoxazole/Trimethoprim: The First Two Years. Reeves, D. S. (1971), *J. clin. Path.*, **24**, 430-437.

This paper comprises a review of the mode of action of sulphamethoxazole/trimethoprim and the difficulties of assessing combined activity. The pharmacology and therapeutic use of the drugs is examined. The many pitfalls of disk diffusion susceptibility tests with this combination of chemotherapeutic agents is reviewed. D.G.B.

Thoughts on Screening Tests in Bacteriology. Lapage, S. P. (1971), *J. clin. Path.*, 24, 404-408.

The author briefly discussses a wide range of screening methods currently available and discusses possible future development. The treatment is general and ranges over the whole field of screening procedures from composite media to the use of the auto-analyser and computer in bacteriology. The author provides a useful bibliography for those who wish to pursue the subject. D.G.B.

Clinical Uses and Control of Rifampicin and Clindamycin. Phillips, I. (1971), *J. clin. Path.*, **24**, 410-418.

A review of the pharmacological properties, methods of microbiological assay and methods of in vitro sensitivity testing of these two antibiotics is presented. The available evidence suggests that disk testing is not a reliable guide to M.I.C. in the case of rifampicin. In the case of clindamycin correlation is good. A discussion of the most suitable methods for the determination of bacteriostatic and bactericidal activity is presented and clinical indications for the use of these antibiotics discussed. D.G.B.

The Importance of Group B Streptococci as Human Pathogens in the British Isles. Harper, I. A. (1971), *J. clin. Path.*, **24**, 438-441.

This paper presents a case history of a case of meningitis in a neonate due to a group B streptococcus, the organism was also isolated from the vagina of the mother. The author also reviews other work implicating group B streptococci as a human pathogen. It is suggested that group B streptococci are more dangerous and more common infective agents than is generally assumed and that they are particularly hazardous to neonates. D.G.B.

Uses and Control of Cephalosporins. Eykyn, S. (1971), *J. clin. Path.*, **24**, 419-429.

The paper presents an exhaustive review of the pharmacology, methods of assay and clinical uses of the cephalosporins. The problems of a vitro susceptibility testing are also discussed. D.G.B.

A Selective Differential Medium for Histotoxic Clostridia. Ellner, P. D. and O'Donnell. E. D. (1971), *Amer. J. clin. Path.*, **56**, 197-200.

A new selective differential medium for histotoxic clostridia is described. The medium is simple to prepare and supports the growth of all commonly encountered histotoxic clostridia. The medium contains lecithin, lactose, neomycin and sodium azide as selective and differential agents.

D.G.B.

Brain Abscess with *Corynebacterium haemolyticum*. Report of a Case. Washington, J. A., Martin, W. J. and Spiekerman, R. E. (1971), *Amer. J. clin. Path.*, **56**, 212-215.

A fatal mixed infection with *Corynebacterium haemolyticum* and *Fusobacterium necrophorum* is described. No route of infection was found at post mortem. D.G.B.

Infection due to *Actinobacillus actinomycetemcomitans*. Meyers, B. R., Baltone, E., Hirschman, S. Z., Schneierson, S. S. and Gershergorn, K. (1971), *Amer. J. clin. Path.*, 56, 204-211.

This paper presents two cases of bacteremia due to *Actinobacillus actinomycetemcomitans*. The cultural characteristics of this rarely encountered organism are fully described and a review of the literature pertaining to infection with this organism presented. D.G.B.

Rapid Antibiotic Sensitivity Testing Utilising Zone Size Methodology. Melia, J. L., Cook, E. C. and White, R. S. (1971), *Amer. J. clin. Path.*, **56**, 59-63.

This paper describes a modified single disk antibiotic sensitivity test using zone sizes as in the Kirby Bauer method and incorporating haemoglobin in the medium as an indication of growth. The advantage claimed by the authors is that of speed, results being read 4 hrs after inoculation of the sensitivity plates. At a time when efforts are being made to standardise on either the Kirby-Bauer or Stokes method of sensitivity testing this method has little else to recommend it. D.G.B.

Comparison of Enterotubes and Routine Media for the Identification of Enteric Bacteria. Marton, H. E. and Maraco, M. A. (1971), *Amer. J. clin. Path.*, 56, 64-66.

This paper assesses the efficiency of the Enterotube system in identifying Enterobacteriaceae in comparison with classical methods.

Enterotubes, a proprietary device containing 8 culture media arranged vertically in compartments and inoculated by stabbing through the whole lot. The authors found 82.3% agreement between Enterotubes and classical techniques, they suggest that the inclusion of ornithine in the Enterotubes would raise this to 97.2%. The system is not available in N.Z. as far as I know and would probably be very expensive. D.G.B.

Taxonomic Status of Facultative and Strictly Anaerobic '*Corroding Bacilli*' that have been Classified as Bacteroides corrodens. Jackson, F. C., Goodman, Y. E., Bel, F. R., Pui Ching Wong and Whitehouse, R. L. S. (1971), *J. med. Microbiol.*, *4*, **2**, 171-183.

This paper critically assesses the taxonomic status of the various corroding bacilli described

at various times over the past 20 years. The authors conclude that the facultative group are not Bacteroides species and probably require the creation of a new genus to accommodate them. The anaerobic organisms studied also differ from classical Bacteroides species, further work is required to decide their true taxonomic position. The authors conclude that the name *Bacteroides corrodens* has been bestowed on a wide range of organisms whose only similarity is their ability to digest agar. D.G.B.

Contrasting Growths on Different Peptone. Laws, G. J., Inghorn, H. R., Crone, P. B. and Slater, D. A. (1971), *J. med. Microbiol.* *4*, 2, 289-292.

This short paper describes the interesting observation that some bacteria isolated in a laboratory grew on media containing one type of peptone and not on another. Eight other peptones were examined and two of these were found to also enhance the growth of these fastidious bacteria. It was noted that those peptones enhancing the growth of the fastidious organisms contained aerobic spore bearing bacilli. The authors describe the steps taken to prove that growth factors provided by these contaminants were the reason for the enhancement of growth of the fastidious organisms. The fastidious organisms were five *E. coli* and two Streptococcus strains. D.G.B.

Comparative In Vitro Sensitivity of Nocardia species to Fusidic Acid and Sulphonamides. Black, W. A. and McNellis, D. A. (1971), *J. med. Microbiol.* *4*, 2, 293-295.

An assessment of the in vitro sensitivity of 12 strains of Nocardia to fucidin and sulphonamides is presented. The authors conclude that fucidin may well have an important role to play in the treatment of this complaint in the future. D.G.B.

In Vitro Sensitivity of *Flavobacterium meningosepticum* to Antimicrobial Agents. Altman, G. and Bogoskovsky, B. (1971), *J. med. Microbiol.* *4*, 2, 296-299.

A survey of the in vitro sensitivity of *Flavobacterium meningosepticum* to 20 antimicrobial agents is presented. The Flavobacterium strains examined were sensitive to only 6 of these. The authors suggest that advantage should be taken of the organisms outstanding resistance in designing selective media. D.G.B.

Erwinia Species from Human Sources. Tilton, R. A., Murphy, J. R. and Soestberger, A. D. (1971), *Amer. J. clin. Path.*, **56**, 186-192.

This paper discusses 7 organisms characterised as Erwinia species, isolated from man. The authors conclude that although at present these organisms appear to play an opportunist role, that clinical laboratories should be aware of them. The authors also note the heterogeneous nature of the organisms grouped in the genus Erwinia. D.G.B.

A Comparative Study of Media Used to Detect Shigella. Bhat, P., Shanthakumari, S. and Myers, R. M. (1971), *Amer. J. clin. Path.*, **56**, 193-196.

This paper compares the relative efficiency of desoxycholate citrate agar, McConkey Agar and Salmonella Shigella Agar in the isolation of shigellae. The authors conclude that D.C.A. is the most effective of the three media but should be used in conjunction with the less inhibitory agar. D.G.B.

Urinary Candidiasis Following the Haemolytic Uraemic Syndrome. Hobday, J. D. (1971), *Aust. paediat. J.*, 7, 114.

The presence of yeast cells on microscopy, or Candida on culture, in the urine of children with a normally functioning urinary system is usually assumed to result from contamination of the urine specimen. This report documents persistence for 7 months of *Candida albicans* in "clean catch" and catheter specimens of urine in a male child. J.H.

Gonococcal Arthritis in Two Patients with Active Lupus Erythematosus: A Diagnostic Problem. Edelen, J. S., Lockshin, M. D. and LeRoy, E. C. (1971), *Arthritis Rheum.*, **14**, 557.

These patients developed polyarthritis which was due to gonococcal infection. One patient had LE cells and intracellular diploccoci in her synovial fluid. The difficulties of differential diagnosis, the possibility that these 2 types of polyarthritis might coexist in other patients, and the therapeutic importance of making the correct diagnosis are discussed. J.H.

A New Clinical Entity: Human Infection with Yersinia Presenting as an Acute Abdomen. Vilinskas, Juliet, Tilton, R. C. and Kriz, J. J. (1971), *Am. Surg.*, **37**, 568.

A case of enteric infection with *Yersinia [Pasteurella] enterocolitica* simulating a surgical abdomen is reported. This infection is recognised in Europe with increasing frequency but is almost unknown in America. It is important to be aware of this entity since it is often indistinguishable from acute appendicitis, terminal ileitis, colitis, and mesenteric adenitis.

The diagnosis of this entity can readily be made by testing sera for rising agglutination titres to Yersinia antigen; these are usually present within a few days of onset of symptoms. Culture of stool, blood, or biopsied lymph nodes may provide additional evidence of the presence of this infection.

In the case described in this article, mesenteric lymph nodes were cultured both anaerobically and with CO_2 on 10% sheep blood agar, MacConkey's agar, chocolate agar, and thioglycollate broth. After 48 hours at 35°C. a single colony (<0.5 mm. diameter, nonhaemolytic, convex, smooth, glistening, translucent, grey with a beaten copper appearance) appeared on both the blood agar and on the MacConkey plate incubated with CO_2. Each colony was subcultured and a series of biochemical tests performed. The thioglycollate broth showed diffuse growth after 48 hours and was also subcultured on blood agar and on MacConkey's agar. A Gram stain of a lymph node showed rare Gram-negative coccobacillary forms. A Gram stain of the isolated colonies revealed 2 types of morphology: a small (0.8-1.2 micron) coccobacillary Gram-negative form with bipolar staining and a larger (3-5 micron) Gram-negative rod with uneven staining. Biochemical tests and morphology were consistent with *Yersinia enterocolitica* biotype 1. The organism was sensitive to tetracycline, streptomycin, ampicillin, colistin, cephalothin, kanamycin and gentamycin. J.H.

Genito-Urinary Tuberculosis: A Study of the Disease in One Unit Over a Period of 24 Years. Gow, J. G. (1971), *Ann. R. Coll Surg.*, **49**, 50.

Before any patient was accepted for treatment, *Mycobacterium tuberculosis* had to be isolated from their urine, either by culture or by guinea-pig inoculation, hence the diagnosis and, more important, as assessment of success or failure of treatment depended on the efficiency of the methods used for the detection of the organism in the urine, an organism which is notoriously difficult to isolate be-

cause it is excreted infrequently in small numbers.

Examination of 10,029 urine specimens by culture and guinea-pig inoculation demonstrated that, although guinea-pig inoculations give a greater proportion of positive results than culture, it is essential that the latter method be used in conjunction with guinea-pig inoculation if the highest number of positive results is to be obtained. Positive cultures were obtained with 301 specimens which were negative by guinea-pig inoculation.

Reliance on the presence of an elevated number of WBCs in the urine deposit as a screening test for genito-urinary TB should no longer be accepted. It was shown that 12.8% of 164 urine deposits which were positive for *M. tuberculosis* had no increase in leucocytes (>3/HPF in males and >6/HPF in females). J.H.

***Vibrio parahaemolyticus:* A Case Report.** Roland, F. P. (1971), *Clin. Med.*, **78** (8), 26.

This patient had intravascular coagulation and gangrene of 1 leg which developed following an infection by a marine halophilic vibrio. Failure to isolate this vibrio from the stool at the time of an active gastroenteritis may have been due to the use of media without added NaCl. It is possible that isolation from vesicles on the leg was made because it was the only organism present.

Vibrio strains in the routine laboratory will confront the microbiologist with the always difficult problem of identifying an uncommon Gram-negative bacillus. If one has been fortunate enough to isolate a halophilic marine vibrio on the ordinary media (without NaCl added) used in routine bacteriologic work — and only a few will grow without added NaCl — the clue that it belongs to the Vibrio genus is the demonstration of a single polar flagellum, but very few laboratories engaged in "routine" bacteriologic work do stains for flagella. Once a vibrio is suspected, identification of the species should not be too difficult.

In laboratories located near the seashore it would be advisable to use media for direct isolation of *Vibrio parahaemolyticus*. The 2 media available are bromthymol blue-teepol agar and thiosulphate-citrate-bile-sucrose. Identification is based on finding a motile, pleomorphic, Gram-negative rod possessing a single polar flagellum and giving a positive oxidase test.

Sensitivity tests are mandatory on media with 3% NaCl. Sensitivity to erythromycin and resistance to polymyxin B are characteristic. J.H.

Primary Amoebic Meningoencephalitis: A Potential Problem in the South-eastern United States. Weng, N. K., Wagner, W. and Parker, J. C., Jr. (1971), *Sth. med. J., Nashville,* **64**, 691.

Primary amoebic meningoencephalitis, an apparently rare disorder, must be considered as a possible cause of purulent meningitis, especially when bacteria are not demonstrated in the cerebrospinal fluid (CSF) and there is a history of swimming in fresh water. The disease is caused by free-living amoebae *(Naegleria* and *Hartmanella)* that are distributed widely throughout the world.

The CSF WBC count varies from 300-21,000. About 73-95% are neutrophils. RBCs have been seen in a few instances. The protein ranges from 90-170 mg/100 ml, while the glucose was found to be 10-56 mg/100 ml.

Examination of the CSF is essential to the prompt establishment of the diagnosis of primary amoebic meningoencephalitis. One does not have the luxury of waiting for a culture report. With experience these pathogenic amoebae can be readily recognised in an unstained wet preparation of the CSF, but they are easily confused with macrophages. *Naegleria* is very motile, while *Hartmanella* is sluggish. Using wet preparations, it was found that a drop of buffered (pH 6.5) 1% cresyl fast violet added to a drop of infected CSF on a slide enables easy recognition of the amoebae. With this technique the amoebae show a single, central or eccentric nucleus bounded by a distinct, homogeneous, purple nuclear membrane. A central karyosome which appears as a 1-2 μ diameter purple dot, is seen in each nucleus and distinguishes the amoebae from CSF inflammatory cells. In some organisms the clear hyaline purple ectoplasm can be differentiated from the granular purple endoplasm.

With prompt diagnosis early treatment with amphotericin B, the only currently available effective drug, can be achieved. J.H.

Vibrio parahaemolyticus: Smith, M. R. (1971), *Clin. Med.*, **78**, (8), 22.

Vibrio parahaemolyticus, an organism associated with food poisoning which follows the eating of raw seafood, is more widespread than has been supposed. Statistics show that this vibrio has been the aetiologic agent in 60-70% of the summer diarrhoea reported in Japan. There is reason to believe that this bacterium is as prevalent in other parts of the Far East and South East Asia.

Persons with the severe diarrhoea associated with these organisms often do not excrete any coliforms, just as if they had been "washed out", but do excrete pure cultures of vibrios (these remarks also hold true for *V. cholerae*). Also, neither *V. parahaemolyticus* nor *V. cholerae* grows well or at all on EMB agar or M-Endo agar (or broth), and neither vibrio produces acid or gas from lactose. Therefore, the standard methods for examination of water or food, using coliform counts as indices of contamination, are of little or no value with this organism.

The organism is selectively cultivated on a special medium, thiosulphate-citrate-bile salts-sucrose (TCBS). A scheme for identification is given.
J.H.

Infections Caused by Achloric Algae (Protothecosis). Tindall, J. P. and Fetter, B. F. (1971), *Archs Derm.,* **104**, 490.

Species of colourless algae of the genus *Prototheca* have recently been recognised as pathogens, with 5 cases being described. Algae are capable of growing in skin, subcutaneous tissue, bursal sacs, and lymph nodes.

Prototheca grows readily at room temperature on Sabouraud's glucose agar, blood agar, beef infusion broth and brain heart infusion agar. Colonies are produced in 1-2 days, provided cyclohexamide is avoided as it inhibits growth of some strains. The colonies are yeast-like, white to cream coloured and may be smooth, wrinkled, or pasty, depending on the strain. Differentiation from Candida and Cryptococci can be made on the basis of lack of budding of the *Prototheca*, and the characteristic septations resulting from the formation of autospores in the mature forms. Species vary both in the number of autospores and the size of the organisms. J.H.

Diagnosis and Treatment of Gonorrhoea. Kvale, P. A. (1971). *Henry Ford Hosp. med. J.,* **19**, 83.

The disease is so different in the 2 sexes that it should almost be considered 2 separate entities.

In the male, a Gram-stained smear of urethral exudate is adequate for diagnosis as it affords 98-99% accuracy. In the early post-treatment assessment of cure, the Gram-stained smear is inadequate and cultures should be done. This is because there may be a temporarily reduced number of organisms in the discharge; consequently the organisms cannot be seen. Later in the post-treatment course, the organisms will multiply and the quantity of exudate will usually increase. Once again the smear becomes fairly reliable to assess the results of treatment. If results are doubtful or if exudate is present after treatment, greater accuracy can be obtained with a culture.

In the female with gonorrhoea the Gram-stained smear of vaginal or cervical exudate is grossly unreliable. In studies conducted in the Philippines less than half of the readings were correct with a Gram-stained smear. Because of the volume of secretions and the presence of many kinds of bacteria there will be false positive as well as false negative readings with the Gram-stained smear. Culture remains the best means for diagnosing gonorrhoea in the female.

A small but significant number (7%) of infected females will yield a positive result only in the anal canal.

Thayer-Martin (T-M) medium is currently employed for maximum growth of *Neisseria gonorrhoea.* T-M medium is an enriched chocolate agar to which has been added a nutrient supplement called Isovitalex and the antibiotics vancomycin, colistimethate and nystatin. Direct plating gives the optimum yield but is usually not practicable in a hospital setting. A suitable alternative is the use of transport medium.

Inocula on T-M medium are incubated in candle jars under 10% CO_2 tension. Typical gonococcal colonies are picked after 24-48 hr and Gram-stained for their morphologic characteristics. Oxidase reactions will confirm the diagnosis and sugar fermentations usually need not be done. Except for the pathogenic neisseria, almost all organisms are inhibited by T-M medium, including the saprophytic neisseria.

Since 1959 fluorescent antibody staining by the delayed technique has also been used to diagnose gonorrhoea. The author believes it is no more accurate than culture using the T-M medium; it is less accurate in assessing cure, so it is seldom used.

Development of serologic techniques for the diagnosis of gonorrhoea is under way but these are not clinically available at the present time. They should broaden the scope of diagnosis in the future.

J.H.

Neonatal *Hemophilus vaginalis (Corynebacterium vaginalis)* Infection. Platt, M. S. (1971), *Clin. Pediat.*, 10, 513.

Haemophilus vaginalis (Corynebacterium vaginalis) should be added to the growing list of organisms which affect the newborn. Data from 10 cases strongly suggest that the organism is a maternal, foetal, and neonatal pathogen.

One might speculate that small numbers of these organisms reside in the vagina. Mucosal irritation and alterations in local cellular metabolism or pH (conditions which frequently occur in pregnancy) may provide the background for rapid microbial replication and invasion in the mother and foetus. The organism was usually isolated from blood cultures obtained from maternal or cord samples.

The vaginitis has been characterised as having an odorous, greyish vaginal discharge with a pH range of 5.5-6.5. Clusters of organisms are found in and above squamous cells when wet mounts are examined; these cells are called "clue cells".

Identification of this organism is complicated. It is not well-known, usually being considered a diphtheroid. It is nonmotile, nonencapsulated, nonsporulating, pleomorphic, Gram-variable rod. Club forms are occasionally seen. Metachromatic areas are found when stained by Albert's method.

The organism is reported to be sensitive to penicillin, tetracycline, chloramphenicol, erythromycin, kanamycin and bacitracin. It is frequently resistant to neomycin, colistin and sulphisoxazole. L-forms have appeared following penicillin therapy. Thus antimicrobial sensitivity studies should parallel the isolation procedures. J.H.

Meningitis Caused by *Listeria monocytogenes*. Birt, C. A. (1971), *Br. J. clin. Pract.*, 25, 466.

For many years, *Listeria monocytogenes* infection has been thought to be very rare. More recently, however, several cases have been described, mainly of meningitis and septicaemia at the extremes of life and in those suffering from debilitating diseases.

In this case report, the turbid cerebrospinal fluid contained 600 WBCs/cu. mm. 95% of which were polymorphonuclear neutrophils with 5% lymphocytes. The protein was >500 mg/100 ml with increased globulin, and the sugar was 30 mg/100 ml. Culture on blood agar produced growth of a delicate, nonsporing, β-haemolytic, Gram-positive rod. This organism grew on potassium tellurite, was motile at room temperature and fermented glucose, maltose and salicin, but failed to reduce nitrates, produce urease or to ferment mannite. Agglutination tests showed the organism to belong to *Listeria monocytogenes* Type 4.

The importance of early diagnosis has been stressed if complications are to be avoided, and this can be particularly difficult in pregnant women and babies. Hydrocephaly seems to be the commonest sequel to listeria meningitis, particularly in children.

J.H.

Subacute Brucellosis — The Diagnostic Dilemma. Davidson-Lamb, R. and MacDonald, A (1971), *Br. J. clin. Pract.*, 25, 367.

Subacute or persisting brucella infection is one of the important, if geographically limited, causes of ill-health in man but there is surprisingly little information about its incidence or about the validity of criteria for diagnosis. Proof of infection, which is more readily made in the acute illness, requires isolation of the infecting organism but this is seldom achieved in the later stages of infection.

Serum agglutination tests are of limited value, even when present in comparatively high titre, as supporting evidence in the diagnosis although they are useful particularly when rising titres can be demonstrated in an acute attack. Of several serologic methods available as aids to diagnosis, those like complement fixation (CF) or anti-human globubin (Coombs') tests, which reflect IgG activity are the most valuable.

The newer types of serologic examination, CF and 2-mercapto-ethanol agglutination tests, which indicate that immunoglobulin of the IgG and IgA types are being produced are considered to be of greater value in diagnosis. There are good reasons

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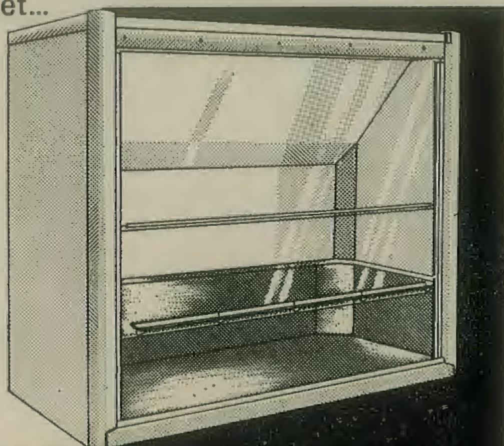

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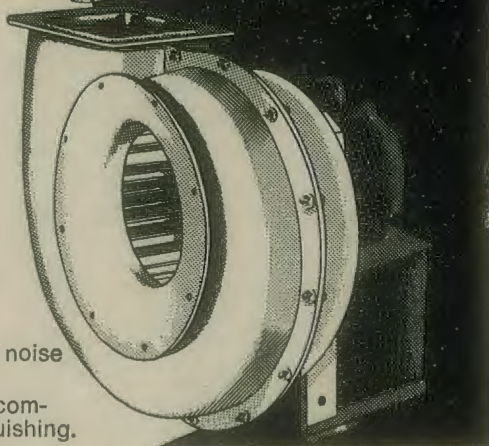

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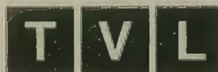

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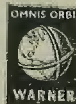

for believing that specific IgG production indicates activity of Brucella infection, but this does not imply that positive CF or other antibodies which reflect IgG are to be taken as proof that a patient's symptoms are necessarily caused by brucellosis. In the group studied in this work, a CF titre of 1:64 or over was considered of particular value.

While repeated CF tests are at present the most useful diagnostic aids in subacute brucellosis, it is unlikely that further advances will be made until more information is obtained about the pathogenesis of brucellosis in man. J.H.

Diagnosis of *Cryptococcal Uveitis* with Hypertonic Media. Grieco, M. H., Freilich, D. B. and Louria, D. B. (1971), *Am. J. Ophthal.,* **72**, 171.

The patient described in this article developed cryptococcal uveitis. A routine cerebrospinal fluid examination revealed increased lymphocytes and protein with decreased glucose and chloride but the aetiologic agent escaped isolation by routine culture.

One spinal fluid specimen cultured in hypertonic sucrose medium (M/0.3 sucrose in brain-heart infusion broth, enriched with 10% horse serum) to explore the possible presence of microbial L forms showed tiny colonies of 2 types when subcultured on blood agar, 1 appearing microscopically like cells of candida and the other appearing diphtheroid-like. Both were considered contaminants and were placed in a refrigerator. Two months later the colonies were again examined. The colonies containing candida-like cells all now showed typical encapsulated cryptococci. The diphtheroid-like colonies were unchanged; 10 of these were transferred to blood agar on different days; each of these, on subculture, appeared as large mucoid colonies consisting of typical encapsulated cryptococci. Subsequently, this isolate was identified as mouse-virulent *Cryptococcus neoformans.*

It has been emphasised that cryptococci may be isolated with difficulty from some patients with cryptococcal meningitis and that it is important in these cases to inoculate a large volume of spinal fluid on each of several small flasks of agar. Use of a flask of agar permits a long incubation period without undue evaporation of water from the medium. The value of cultivating for wall-deficient microbial variants by using hypertonic media which protect the organisms from osmotic stress has also been shown to be useful in another yeast infection. In 3 instances, wall-defective forms of candida have been recovered from blood.

The usefulness of the latex slide agglutination test for detecting cryptococcal antigen is pointed out. J.H.

Report of a Case of *Nocardia asteroides* Keratitis. Newmark, E., Polack, F. M. and Ellison, A. C. (1971), *Am. J. Ophthal.,* **72**, 813.

Ocular infection caused by *Nocardia asteroides* is rare. External infections have been reported infrequently, but more recent reports deal with intraocular endogenous infection.

In this case, Gram and Giemsa stains of corneal scrapings for fungus and a KOH wet preparation did not reveal bacteria or fungi. Three days after plating the culture, minute mealy white colonies appeared along the inoculation streak on a blood agar plate. The organism was a Gram-positive and acid-fast filamentous bacterium, identified as N. asteroides. Nocardia are usually sensitive to sulphadiazine and frequently to tetracycline and pencillin. However, sensitivity tests are warranted to determine the sensitivity of each new strain. J.H.

Book Reviews

Blood Coagulation Simplified. 2nd Edition. F. Nour-Eldin Ph.d., M.B., B.Ch., L.M.S.S.A., M.R.C. Path. 196 Pages. Butterworth & Co. (Publishers) Ltd., London, 1971. Price in U.K., £2.00.

The second edition of this useful book contains an additional 36 pages. These are largely devoted to a new chapter on Thrombosis and Antithrombotic therapy and control, and revision of the sections on Fibrinolysis and Haemophilia.

Reference is made in the fibrinolysis section to fibrin degradation products and their detection by the "Fi" test and the haemagglutination inhibition immuno-assay. The chapter on Thrombosis contains a discussion on the current theories of blood coagulation, deals with normal haemostasis, haemostasis after trauma and vascular occlusion, followed by a section on anticoagulants and their control.

As in the first edition the 4th chapter containing exercises and hints for examinations is worthy of note by technologists in training.

The author's practice of describing a test followed by interpretation and then any further steps required for identification of defects in coagulation makes for easy reading and assimilation of facts.

The author claims on page 17 that barium sulphate does not absorb Factors II, VII, IX and X from citrated plasma. However from experience a concentration of 200mg $BaSO_4$/ml in citrated plasma, is effective.

All in all this is a very useful book which students of haematology would do well to acquire. B.W.M.

Experimental Methods in Modern Biochemistry. George Rendina, PhD., Chemistry Department, Bowling Green State University, contains 333 pages including illustrations. 1971, Publishers W. B. Saunders Company, Philadelphia, London and Toronto. Price, $8.50.

I suppose if one is trying to sell a book there are two methods one can adopt. The most obvious one is to put a naked woman on the cover. This method would probably not work very well for a book on biochemistry. The second method, which is the one adopted by the publishers (and perhaps the author) of this book, is to give it an impressive-sounding title. I would have expected a book entitled 'Experimental Methods in Modern Biochemistry' to deal with some of the more sophisticated research methods currently in use in biochemistry laboratories. This book is in fact an elementary laboratory manual in biochemistry suitable for second year medical students. No doubt it would not have sold nearly as well had it been so entitled.

As an elementary practical manual in biochemistry this book is quite good. It follows fairly traditional lines in that the early chapters deal with simple physico-chemical techniques such as pH measurements and the elementary chemical properties of materials of biochemical interest. The later chapters cover in a rather sketchy fashion some aspects of enzymology and the more metabolic aspects of biochemistry. The book contains information that would be quite useful for anyone

planning an elementary practical course in biochemistry for medical or science students. There are however several experiments which I think are of little value and there are experiments that I would use instead of the ones in the book if I were planning such a course. I would not recommend the book to either medical students or science students. The only people likely to find it of any value are those planning practical courses for students, and in any case they should be able to obtain a free copy from the publishers.

J.G.T.S.

Cell Structure. Second Edition. P. G. Toner and Katherine E. Carr. Churchill, Livingstone, London (1971). 130 pages of text, 124 pages of plates and captions. Price in U.K., £2.50.

This book is essentially an introduction to the structure and function of the components of cells as seen in the electron microscope. It consists of three sections. The first part is a detailed description of cell membranes and surfaces along with the means of cell contact and adhesion. The structure and function of the cell components such as the nucleus, ribosomes, endoplasmic reticulum, etc., is described followed by the ultrastructure of cells with specialised functions such as secretory cells, gastric parietal cells, cells which carry out absorption phagocytosis protection, contraction and communication.

The second part consisting of approximately twenty-five pages briefly describes E.M. processing techniques, conventional and unconventional electron microscopes, tracer techniques plus a chapter on the critical examination of electron micrographs, including the recognition of processing and technical artefacts.

The third part of the book consists of just over one hundred electron micrographs illustrating cell components and basic cell types.

The description of the cell components is presented in a clear and simple manner making it easy and interesting reading. I would recommend this book to anyone who is entering the field of electron microscopy or teaching medical biology.

D.T.

Haemolytic Disease of the Newborn. Published by Ortho Diagnostics (Obtaining from Johnson & Johnson Ltd., P.O. Box 11-125, Ellerslie, Auckland.)

This book is the third in a series issued by Ortho Diagnostics. The two earlier issues are The ABO and Rh System and Compatibility Testing.

The first nine pages contain excellent stylised illustrations which clearly show the sequence of events leading to haemolytic disease and the action of Anti-Rh Immunoglobulin in prevention of primary immune response.

The main body of the book deals with all aspects of Haemolytic Disease in sections covering Aetiology, Prediction, Diagnosis, Treatment and Prevention. The material is a very clear yet concise digest from many major works on the subject, and provides the reader with all the necessary information in one compact book.

The techniques associated with the prediction and diagnosis are given in a separate section at the rear of the book, and are easily followed. Although Ortho products are named throughout this section, the basic techniques can be applied whatever reagents are used.

The whole book is presented in a spiral bound form which makes it well suited for quick reference, although gentle handling is required if it is to be kept intact for long.

It is ideal as a reference book for trainee technologists studying for the N.Z.C.S. or Part I Certificate of Proficiency examinations, and would be useful revision for candidates for higher examinations. The colour illustrations lend themselves admirably to visual aids for lecturers in Immunohaemotology.

In common with the other books of the series, Haemolytic Disease of the Newborn is available at only $1.25. At this price it should be an essential part of every trainee's and tutor's library.

D.S.F.

Clinical Chemical Pathology. Sixth Edition. C. H. Gray. 244 pages, illustrated. Price in U.K., £2.00.

Revisions of this handbook appear at intervals of about three years and in this wav it never lags far behind the current trends in chemical pathology. One of its attractions, in these days of massive technical tomes, is the compactness which the author and publisher are at pains to preserve. It is in the nature of things that there must be some increase in size and I note that the earliest edition I still possess, the third, contains 196 pages.

The preface states that the chapters on renal function, biochemical tests in endocrine disease and biochemical genetics have been virtually rewritten and that a new chapter on the chemical pathology of the lipids has replaced that concerning miscellaneous topics. All except four of the remaining chapters have been revised. The illustrations, although approaching the limits of legibility, are well chosen; those relating to the countercurrent osmotic multiplier, the Davenport diagram, and those in the chapter on Biochemical Genetics are particularly useful.

The chapter on Plasma Proteins provides a good basis for understanding the immunoglobulins and immunological disorders and provides recent references for further reading. The chemical pathology of the lipids is also adequately dealt with but although it is not the function of this book to dwell on technical matters, one felt the illustrations were perhaps a little idealised. The chapter on Diabetes was possibly one of those not revised as no reference is made to the hyperosmolar non-ketotic varieties of the disease which have recently become prominent in the literature. In relation to the C.S.F. it is stated that certain neurological diseases lead to abnormalities of the outdated Lange Colloidal Gold Curve. It would be helpful to add that quantitation of gammaglobulin or IgG provide more direct information particularly in multiple sclerosis. These are minor points. It is surprising how much information is packed into these pages. This is achieved by avoiding discussion on unresolved issues and hypothesis. In this regard the book fulfils a useful function as an initial introduction to clinical chemical pathology which is of course the author's intention.

The book provides an excellent background for Technologists preparing for their final examinations and in fact, few of us engaged in chemical pathology will want to be without this modestly priced volume.

R.D.A.

Textbook of Biochemistry. Tenth Edition. A. Mazur and B. Harrow. 727 pages, profusely illustrated. 1971. Publishers, W. B. Saunders Company, Philadelphia, London and Toronto. Price in N.Z., $15.00.

The title, "Textbook of Biochemistry" does little to indicate the contents of a book of this

nature. Nowadays the field is so large and varied that it is necessary to ascertain whether the contents are animal, vegetable or microbiological. This however is an established textbook first published in 1938 and bears its traditional title although it is perhaps better known by the authors' names. It is intended for use in an introductory course in biochemistry for 'undergraduate seniors' of City College, City University of New York. These students will have completed courses in biology, physics, mathematics, general chemistry and organic chemistry.

This is not a simple introduction; there is no attempt at sweetening the pill, no evasions nor concessions. Where mathematical treatment is called for it is given. Two significant advances in biochemistry pervade this textbook. These are the clarification of the cellular macromolecules, protein and nucleic acid and the identification of the coded base sequences of the amino acids. This is exemplified in the chapter on biochemical genetics. It also brings the reader into the forefront of current investigation in regard to the phenomenon of feedback inhibition of enzyme activity at a cellular level. Indeed most of the material is discussed to the point of hypotheses.

To illustrate the depth of treatment given to the various subjects, protein is subdivided into chemical properties and structure. The latter section deals with primary, secondary, tertiary, quaternary and quintary structures; that is from the simple amino acid to the aggregated multiprotein systems such as the mitochondria. The size, shape and function and how these were determined, is explained in each instance. The quaternary structures are exemplified by the haemoglobins and the isozymes of L.D.H. and their structures are illustrated.

Very little is taken for granted and preliminary matters are generally discussed. From a revision point of view terseness is legitimate. In the chapter on enzymes the Michaelis-Menton equation and its modifications are derived from first principles. The current concept of enzyme action is described. This is the close approximation of the active sites of the enzyme to the substrate and is brought about by the folding of the polypeptide chain forming a three-dimensional structure which "fits" the substrate.

The physiological chapters generally refer to mammalian tissues although some examples bear on other life forms. Many of the illustrations are familiar and the blood normal concentrations are from Bodansky and Bodansky 1952. A reference to 0.2% glucose would not please the International Union of Chemists! Another minor point, I did not find a reference to calcitonin in the hormone section.

The final chapter on immunochemistry is worthy of note providing a useful survey of its various aspects and characteristically providing an illustration of the amino acid sequence of Bence Jones proteins.

To sum up, this is not a textbook for the uninitiated but would certainly provide an excellent reference text for N.Z.C.S. candidates and those preparing for finals in Chemical Pathology.

R.D.A.

Laboratory Assistants Examination Review Book. Vol. 1. 1st Edition. Edited by Alvin M. Ring, M.D. Medical Examination Publishing Co. (Inc.) New York, 1971, 178 Pages. Price in N.Z. approx. $7.70.

In reviewing a book of this nature there is a great temptation to launch forth on a review of the advantages and disadvantages of multiple choice questions and of 'true or false' type questions. It is unfortunate that degrees of rightness and wrongness are eliminated in such questions; no judgment or personal opinion of any kind enters into it. They are wholly objective and can be answered at speed. Therefore bearing in mind that these types of questions impose an artificiality and a rigidity on the questions, this book can still be very useful in certain circumstances. It was compiled in a realistic environment of students; this is certainly a point in favour of the volume.

The purpose of such a book of examination questions is debatable but this book is recommended for students who require a method of revision prior to examinations. Some of the questions would be suitable for actual use in examinations, but examiners would be better advised to set their own style of question. Readers will probably be familiar with the series to which this book belongs. Two previous volumes have been of some use to Medical Technologists (i.e. Medical Tech. Exam. Review Vol. 1 and Vol 2). Presumably the standard of questions in those volumes is meant to be higher than this present volume for Laboratory Assistants. However, the present volume is not really suitable for New Zealand conditions as our Technical Assistant Examinations are specialised and this book is in fact spread over six different subjects in Medical Technology and as an edited volume, each section was compiled by a specialist in that field.

The fields covered are as follows: 250 questions on Urinalysis; 148 questions on General Laboratory Techniques, ranging over various subjects including Quality Control; 200 questions on Microbiology and Parasitology; 400 questions on Haematology; 200 questions on general chemistry applied to clinical biochemistry, so in actual fact the Chemistry is somewhat of a misnomer; 150 questions on Serology as applied diagnostically; 150 questions on Blood Banking.

The reference given beside each question refers to books listed at the back of this volume, books that are not in common usage in New Zealand. (In fact of the 24 references only about eight are in common usage in N.Z.). This review book naturally has the answers to the multiple choice questions set out in tabular form at the back of the volume.

The Instructions which precede each section of groups of different types of multiple choice questions are not very clearly marked so that often there are many pages to be thumbed through to search for instructions pertaining to questions. This of course would be overcome by using the book sequentially. Apart from multiple choice questions there are also such items as 'select the most closely related item'. Many of the multiple choice type questions are very original but it is doubtful if these are about important aspects of the subject. Some of the answers given are too rigid, and occasionally none of the choices from which selection is to be made is truly correct.

The characteristics of good test questions are variable. The item must discriminate between examinees who are more competent and those who are less competent with respect to the particular point in question. The item must be phrased in such a way that it probes the knowledge of students without making them wonder what the question really means. The item should not merely be a

memory-recall type of question. The item must be clear and concise. Most of the items in the examination review book would in fact be acceptable under the above categories of acceptable questions. Snap checks on the correctness of the answers failed to reveal any mistakes but in past volumes such as the Medical Tech. Exam. Review Vol. 1 or 2 there were, in fact, a few incorrect answers listed.

For any laboratory with Third Year Trainees or Technical Assistants about to sit qualifying examinations this is a useful book to have on the shelf to enable students to do some revision on their own accord and test their own knowledge before arriving in the examination room. This volume is also a challenge to the qualified technologist and will serve as a stimulus to further reading by those who tend to rest on their laurels!

M.J.G.

Medical Parasitology. J. Walter Beck, B.S., M.S. Ph.,D. and Elizabeth Barrett-Connor, B.A., M.D., D.C.M.T. (London), 210 pages with 150 illustrations. C. V. Mosby Co., St Louis (1971). Price in U.S.A. $10.95.

This book is written for the teaching of medical parasitology primarily to medical students, but also as a guide to technologists and practising physicians. The authors' objective has been to provide a study programme for the student. At the end of each chapter is a series of review questions and a list of references.

As to be expected the clinical aspect, particularly treatment, figures largely. However, the technical side is not overlooked; a useful appendix gives details of diagnostic laboratory procedures.

Content of this book is divided into three major categories:—

I Protozoology — amoeba, flagellates, ciliates, sporozoa and also a fifth division including protozoa of undetermined nature.

II Helminthology — including roundworms, flatworms and spiny-headed worms.

III Arthropology — covering centipedes, tongue worms, crabs, crayfish and copepods, spiders, scorpions and mites, ticks and insects.

For convenience of study the content of each section is arranged from the simple to the complex.

Although beautifully illustrated I feel that for detailed study of ova and parasite morphology diagrams could be better labelled.

This book, well presented and easily read, has a definite place in the laboratory library although the information presented can be obtained in more detailed form from standard texts. M.J.

Introduction to Blood Banking. Robert M. Greendyke, M.D., and Jane C. Corner, B.S., M.T. (A.S.C.P.) Medical Examination Publishing Company, Inc., New York. 1970. Price in New Zealand $8.00. Pages: 184.

The book is loose-leafed in a plastic binder and consists of twenty-one chapters and a glossary. Only six of the twenty-one chapters are devoted to blood group systems (46 pages).

The text as a whole leaves a lot unsaid. In trying to make the subject easy for a beginner (indeed it is an Introduction to Blood Banking), it makes some errors by over simplification. At the end of each chapter there are several books recommended for reading. If in fact these were all read by the student, he would be rich in knowledge on the subject.

The first chapters deal with the composition and function of blood and disorders associated with blood and circulatory function, followed by general principles in Immunohaematology and Genetics. The ABO, Lewis and Rhesus blood group systems are each allotted a chapter but the next three chapters contain all the other blood group systems mentioned. Practical considerations in methodology; routine blood typing; compatibility testing and identification of irregular antibodies are dealt with moderately well although the crossmatch technique would be considered inadequate in New Zealand.

The chapter 'Complications of Blood Transfusion' is worth merit. Contained therein is a list of checks for a technologist to perform in the event of a haemolytic transfusion reaction. To the 'on call' technologist at 3 a.m. this could be invaluable.

'Haemolytic Disease of the Newborn', is covered reasonably well and includes a list of desired blood groups for exchange transfusion in the event of Rhesus antibodies being present.

Leucocyte Antibodies and Histocompatibility testing is briefly mentioned and the interested reader is referred to Journals to obtain full information.

The chapter on Blood Donors is quite comprehensive for any technologist having contact with donors giving blood and being required to select donors on their medical history. It is in fact pleasing to find the information set out in an easily referable manner.

'Blood storage and Processing of Blood Components', precede a chapter on the use of blood components. Finally a chapter on Technical and Administrative Controls reminds the reader of the importance of strict adherence to the rules for anyone working in a Blood Bank. I was particularly impressed with a paragraph on working conditions which points to distracting influences that have no place in a Crossmatch Laboratory.

The Glossary would be inclined to convey false impressions to a student who wished to use this for examination purposes. Again the tendency to simplify to the point of falsity makes me hesitant about the value of this book as a recommended text for reading. 'Occuring without air' to the enlightened is a vacuum — not as the authors state 'anaerobic'.

This book is not entirely without merit but should not be relied upon to give accurate information for examination purposes. L.W.

Book Received

Fundamentals of Clinical Haematology by Leavell and Thorup. 3rd Edition, 659 pages with illustrations (1970). Price in N.Z. $18.00, from N. M. Peryer Ltd.

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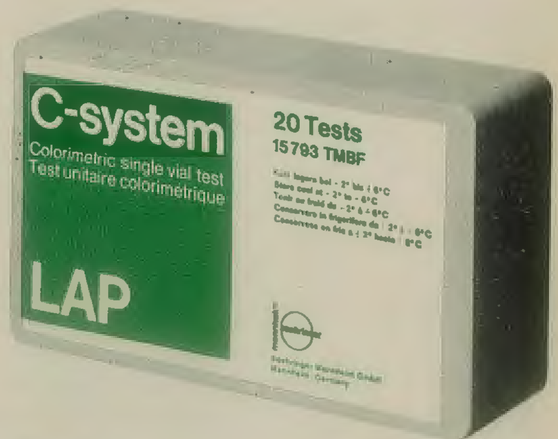

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