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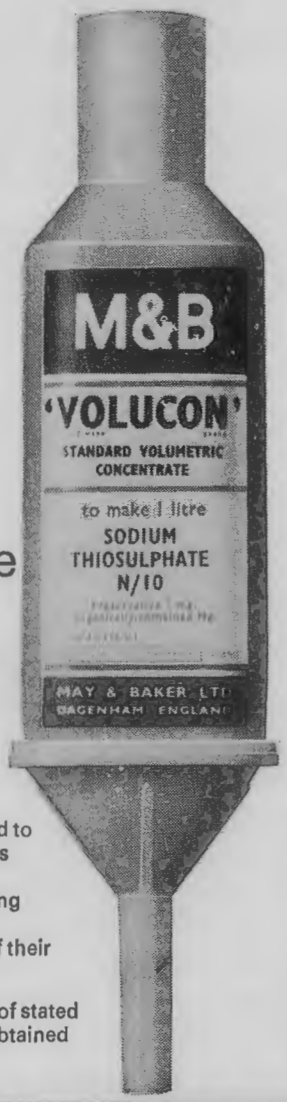

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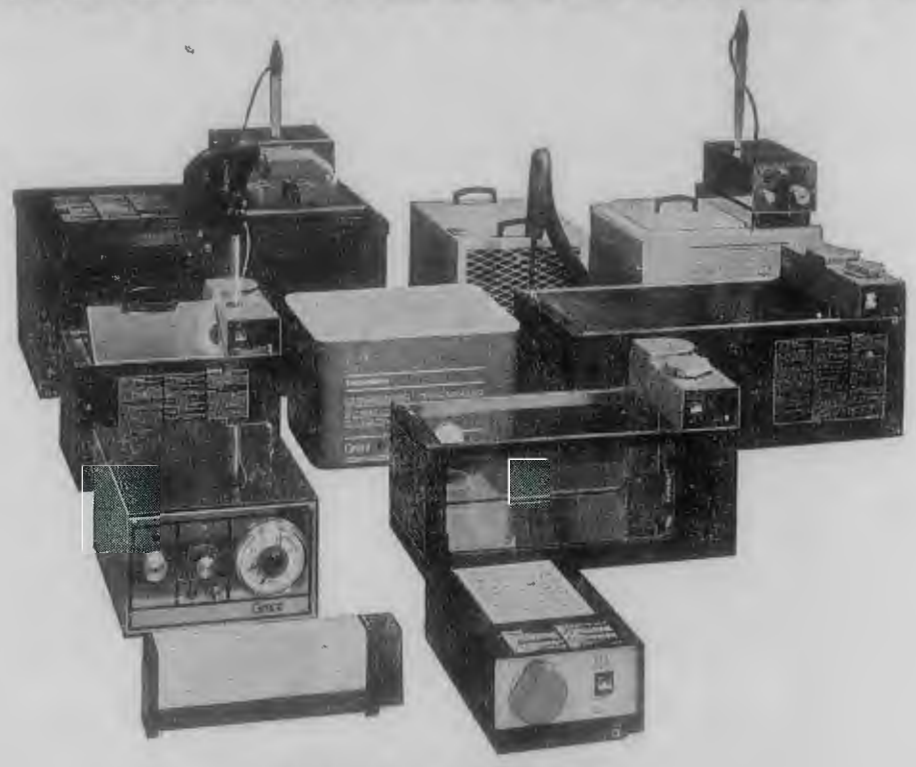

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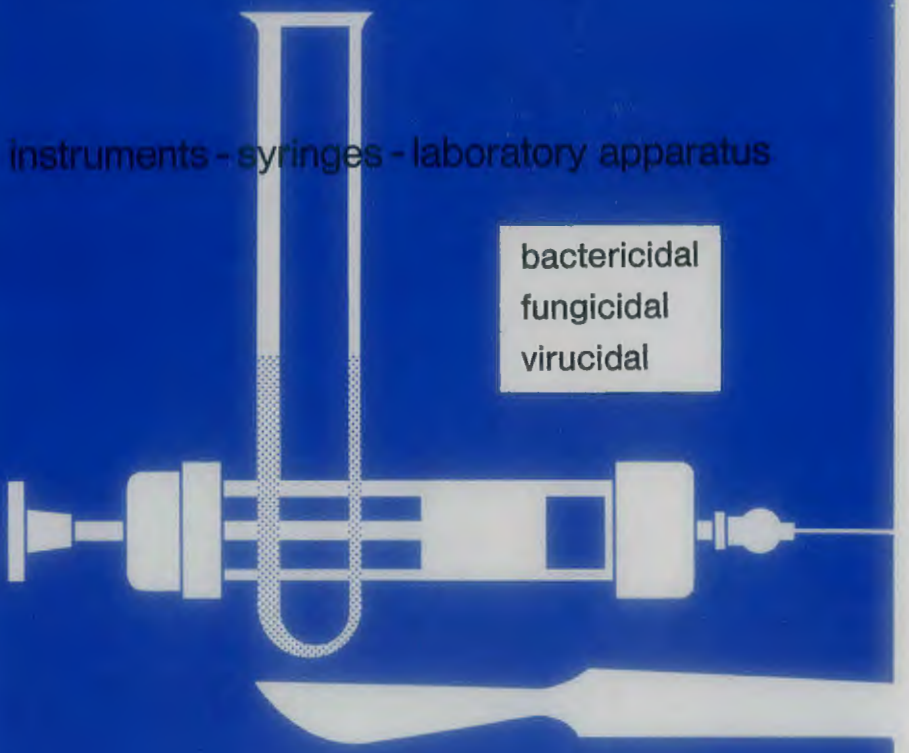

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A Comparison of Two Automated Methods for Serum Cholesterol

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Department of Clinical Chemistry Auckland Hospital

Received for publication, November 1974

Summary

Cholesterol concentration has been determined on serum from 603 healthy blood donors by a direct and an extraction automated procedure (Technicon SMA 12/60 and N-24a respectively). The regression equation for the extraction method (y) on the direct method (x) was:

$$y = 0.77x + 29.9$$

The Standard error of y on x (Sy.x) = 21.6. There are appreciable proportional and random errors between the two methods. Comparisons between the methods should be made with caution.

Introduction

The two most commonly performed automated methods for determining serum cholesterol are:

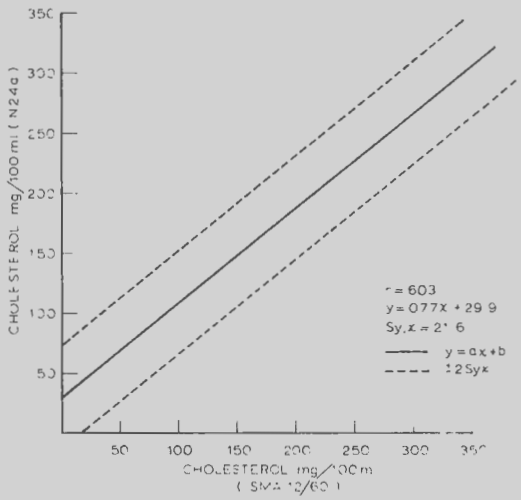
(1) The Technicon N-24a methodology, which involves a preliminary extraction into isopropanol and which uses a ferric chloride-acetic acid-sulphuric acid colour reagent, and

(2) The direct Liebermann-Burchard reaction performed on the Technicon SMA 12/60 analyser.

Because the latter is a direct procedure, and the former utilises an extraction step, it might be expected that values obtained by the SMA 12/60 procedure would be greater than those obtained by the N-24a methodology (Tietz, 1970)[3]. In an early description of the automated direct procedure (Levine *et al.*, 1967)[1] a comparison was made of the two procedures, and the following data were obtained (Table I).

It has been shown that this type of comparison between methods is unsatisfactory (Westgard and Hunt, 1973)[4]. We found the implication that the methods were closely comparable rather surprising. As these methods are so widely used, it is essential that an adequate comparison be made between them to allow for clinical and epidemiological comparisons of the results they provide. Because the opportunity presented itself during the course of a normal-range survey to make a comparison of the two methods, we decided to re-examine this point.

Figure 1



Regression of N24a Cholesterol Values on SMA 12/60 Values

Methods

The analytical procedures followed were essentially as described in the Technicon Company manuals. The N-24a method was calibrated using a commercially available preparation of purified cholesterol standardised against cholesterol obtained from the National Bureau of Standards, USA. Working standards of the commercial material were run against accurately prepared standards made from the NBS material and were found to agree within 3 percent at the level of 100 and 300 mg/100

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Procedure	Number	Mean mg/ 100ml	SD mg/ 100ml	Correlation Coefficient
Schoenheimer-Sperry		194	56	
v	58			0.97
Direct Procedure		192	52	
N-24a Procedure		199	38	
v	60			0.97
Direct Procedure		191	39	

Table 1.—Data from Levine *et al.* (1967)[1] comparing the direct automated cholesterol procedure, with the manual Schoenheimer and Sperry reference procedure and the N-24a procedure.

ml nominal value. The SMA 12/60 procedure was calibrated using Technicon reference serum, the standardisation procedure for which has been reported (Schneider and Cornette, 1973)[2]. The coefficient of variation on a pooled serum of 180 mg/100 ml mean value was 7.2 percent for the direct method and 8 percent for the extraction procedure.

The data obtained from each procedure were examined by the technique recommended by Westgard and Hunt (1973)[4]. The important parameters, as these authors have shown, are slope (the index of proportional error), the intercept on the y axis (the index of constant error) and the standard error of estimate in the y direction (the index of random errors). The data were analysed on a Monroe 1930 calculator. 603 pairs of results were processed in the first instance.

Results

The frequency distribution curves for the cholesterol values of the population obtained by the two methods were both unimodal and symmetrical, the standard deviations being very similar, namely, SMA 12/60 = 37.3 and N-24a = 37.5. The means were significantly different; SMA 12/60 = 218.4; N-24a = 195.7; ($P < 0.001$).

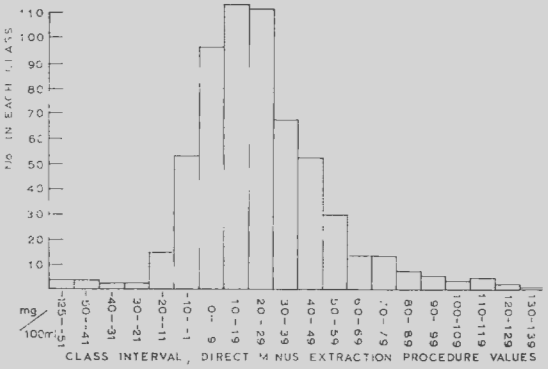
When the regression equation of N-24a cholesterol (y) on SMA 12/60 cholesterol (x) was calculated using all the paired results it was found to be:

$y = 0.698x + 43.2$ (mg/100 ml) where
x = value obtained by the SMA 12/60 procedure
y = value obtained by the N-24a procedure
The standard error for y on x (Sy.x) = 27.0
The correlation coefficient (r) = 0.694

Graphical representation of the data suggested that the relationship between the two procedures was linear but that the regression line might have been affected by a few widely discrepant results at the high and low ends as discussed by Westgard and Hunt (1973)[4], i.e., the calculated regression did not correspond well with the line of best fit by eye. Because of this possibility the difference between the two methods for individual samples were examined separately. A frequency histogram of the differences (SMA 12/60 minus N-24a) was drawn (Figure 2), and it was seen that they were distributed in a nearly Gaussian fashion, with a few high outliers. The mean of the differences was +21.9 mg/100 ml ±27.3 (SD).

It was considered however that it would be inappropriate to exclude pairs of values on the basis of this distribution of the differences, as this would tend to eliminate more values in the high regions, due to the appreciable proportional error.

Figure 2
Frequency distribution of differences between direct and extraction procedures



The regression equation was then recalculated omitting those pairs of results outside the limits ±2.5 Sy.x from the regression line. The value of the equation was now found to be:

Figure 1

$n = 580$
$y = 0.77x + 29.9$
$Syx = 21.6$
$r = 0.796$

The line was now found to fit the graphically represented data on inspection.

The linearity of each procedure over the range present was satisfactory for both methods. The range of values obtained was approximately 125 to 325 mg/100 ml.

Discussion

It can be seen that there is an appreciable proportional error and constant error when the two methods are compared. This makes the comparison of the results somewhat complicated. Below a figure of 130 mg/100 ml the value for the N-24a methodology derived by this equation will be greater than the value by the direct method, but above this figure it will be less. Furthermore, due to the large value for the standard error of y on x, i.e., 21.6 mg/100 ml, the 95 percent confidence limits for the predicted value will be ±43.2 mg/100 ml.

It would appear that predictions of values by one method from a given value obtained by the other cannot be made with satisfactory reliability and clinicians should be advised accordingly. Another important implication is in the comparison of populations. The differences between the methods used need to be considered before imputing differences in the population levels of cholesterol.

Acknowledgments

We wish to thank Miss Shanti Kika for skilled technical assistance and Dr G. D. Calvert for kindly agreeing to this use of the normal range specimens.

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Plasma Enzymes in Liver Disease

Paul Hsu and Dr. M. H. Briggs

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Received for publication February, 1975

The recent paper by Dr P. S. Bhathal *et al.*[1] on the spectrum of liver disease in Australia has shown a high incidence of conditions caused by alcohol or metastatic cancer. The selection of the most appropriate biochemical tests in patients with known or suspected liver disease has always been complex and we would like to comment and present some new findings on the use of plasma γ-glutamyl transpeptidase (γGTP).

There has been considerable recent interest in the value of plasma γGTP measurements in a variety of pathological conditions[7]. This enzyme (L-γ-glutamyl-p-nitroanilide: amino acid γ-glutamyl transferase; EC 2.3.2.1) occurs in plasma as a mixture of at least four isoenzymes[5].

The major plasma source appears to be liver, though the richest tissue is kidney[5].

There is a close correlation between increases in plasma γGTP and alkaline phosphatase of hepatic origin[18]. Maximum elevations are seen in diseases which particularly affect the biliary tract. Accordingly to several reports[7, 18, 9] γGTP is a more sensitive indicator of liver disease than alkaline phosphatase in all cases and is better than aminotransferases in all conditions except viral hepatitis. A comparison with leucine aminopeptidase showed γGTP to be more sensitive in all forms of liver disease[9]. Correlations between γGTP and lactate dehydrogenase were poor in a variety of conditions[18, 19]. A comparison of γGTP with 5′-nucleotidase found that the average percentage increase in γGTP in biliary tract disease was about double that for 5′-nucleotidase, while 69 patients with other forms of liver disease showed significant changes in γGTP, but not in 5′-nucleotidase.

Aside from hepatic disorders, plasma γGTP has also been reported to be increased following myocardial infarctions and in many patients with angina pectoris. High values are also seen in patients with renal neoplasia[10], nephrotic syndrome[10], renal transplant rejection[8], pancreatitis[15], diabetes mellitus[6], certain neurological diseases[3], and following radiotherapy for cervical carcinoma[19].

A marked advantage of γGTP over other plasma enzymes is that it is not increased in pregnancy or growing children[9], nor in patients with Paget's disease, hyperthyroidism, hyperparathyroidism, bone metastases, Gilbert's disease or chronic renal failure[7, 18, 9].

High values for γGTP have been reported for new born infants in the first two weeks of life[12] and in patients with no other signs of hepatic or renal disease receiving anticonvulsants[18], warfarin[17], oral contraceptives[11], or alcoholic beverages[13].

We have measured plasma γGTP activity in a series of 123 patients at this hospital.

Enzyme measurements were conducted on heparinised plasma using a kinetic method[16]. The substrate was L-γ-glutamyl-p-nitroanilide and incubations were conducted at pH 8.6, 37°C in solutions containing glycylglycine as activator. Specimens with high values were diluted with 6 percent albumin solution and reassayed. The upper limit of normal γGTP in plasma by this method is 25 to 35 IU/l. Bilrubin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LD) were also measured on most specimens using routine AutoAnalyzer or reaction-rate kinetic methods.

Results in 65 patients with various liver diseases and in 58 patients without known hepatic involvement are summarised in Table I. It will be seen that all patients with liver disease, except two with acute hepatitis, showed raised plasma γGTP. Increased enzyme activity was also seen in some patients with pancreatic or cardiac disease.

Table II summarises abnormalities in five other biochemical tests for those patients showing high γGTP. Except in cases of acute hepatitis, these other tests showed a significantly lower number of abnormal values.

It is apparent that plasma γGTP is a more sensitive test of liver function than most other commonly used biochemical measurements, except in hepatitis. The inclusion of a plasma γGTP in a battery of early tests in patients with suspected liver disease should be of diagnostic value.

A number of precautions, however, are needed. The patient should not be taking drugs likely to activate the hepatic microsomes, especially a combination of drugs[17], while other conditions associated with raised γGTP (see above) must be excluded. Raised γGTP, often in the absence of abnormal results in other tests, is common in heavy drinkers who show no other signs of liver disorder[13, 14].

Most series are agreed that all patients with hepatic malignancies (primary or secondary) show elevated plasma γGTP, often before other tests become abnormal[7, 18, 9, 2], though one recent study[6] found normal γGTP in five patients with hepatic metastases at laparotomy. It should be stressed, however, that other tests on these patients were also normal.

We conclude that plasma γGTP measurement should be particularly appropriate to the investigation of liver disease.

Acknowledgment

This paper sponsored by Diagnostics Division of Harrisons and Crosfield (N.Z.) Limited.

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TABLE I PLASMA γGTP IN VARIOUS DISEASES

Group	Diagnosis	No.	Percentage Above 35 U/l	γGTP (U/l) Mean	Range
Liver diseases	Acute hepatitis (serum or infective)	8	75	126	28-240
	Cholecystitis (cholelithiasis)	21	100	251	71-600
	Cirrhosis	15	100	138	79-198
	Alcoholism	12	100	95	83-145
	Malignancy	9	100	419	372-552
Pancreatic diseases	Pancreatitis (acute)	9	100	322	168-600
	Malignancy	5	100	309	215-386
	Juvenile diabetes mellitus	2	50	49	25-73
Cardiovascular diseases	Myocardial infarction (acute)	18	33	44	15-66
	Congestive heart failure	9	100	118	36-224
Renal diseases	Chronic renal failure	8	0	19	7-34
	Transplant	1	0	16	-
Bone diseases	Paget's disease	3	0	22	18-26
	Metastatic carcinoma	2	0	14	12-16
	Hyperparathyroidism	1	0	25	-

TABLE II CORRELATION OF RAISED γGTP WITH OTHER PARAMETERS

Diagnosis	Patients with Raised γGTP (Number)	Number with Abnormalities in Other Biochemical Tests: Bilirubin	Alkaline Phosphatase	AST	ALT	LD
Acute hepatitis	6	6	4	6	6	6
Cholecystitis	21	9	19	11	13	11
Cirrhosis	15	4	7	12	6	2
Alcoholism	12	8	6	6	4	6
Hepatic malignancy	9	6	6	9	7	7
Acute pancreatitis	9	1	6	5	3	5
Pancreatic malignancy	5	1	4	2	2	1
Diabetes mellitus	1	0	0	0	0	0
Acute myocardial infarction	6	0	2	2	2	3
Congestive heart failure	9	0	9	2	2	3
Total	93	35	63	59	45	47

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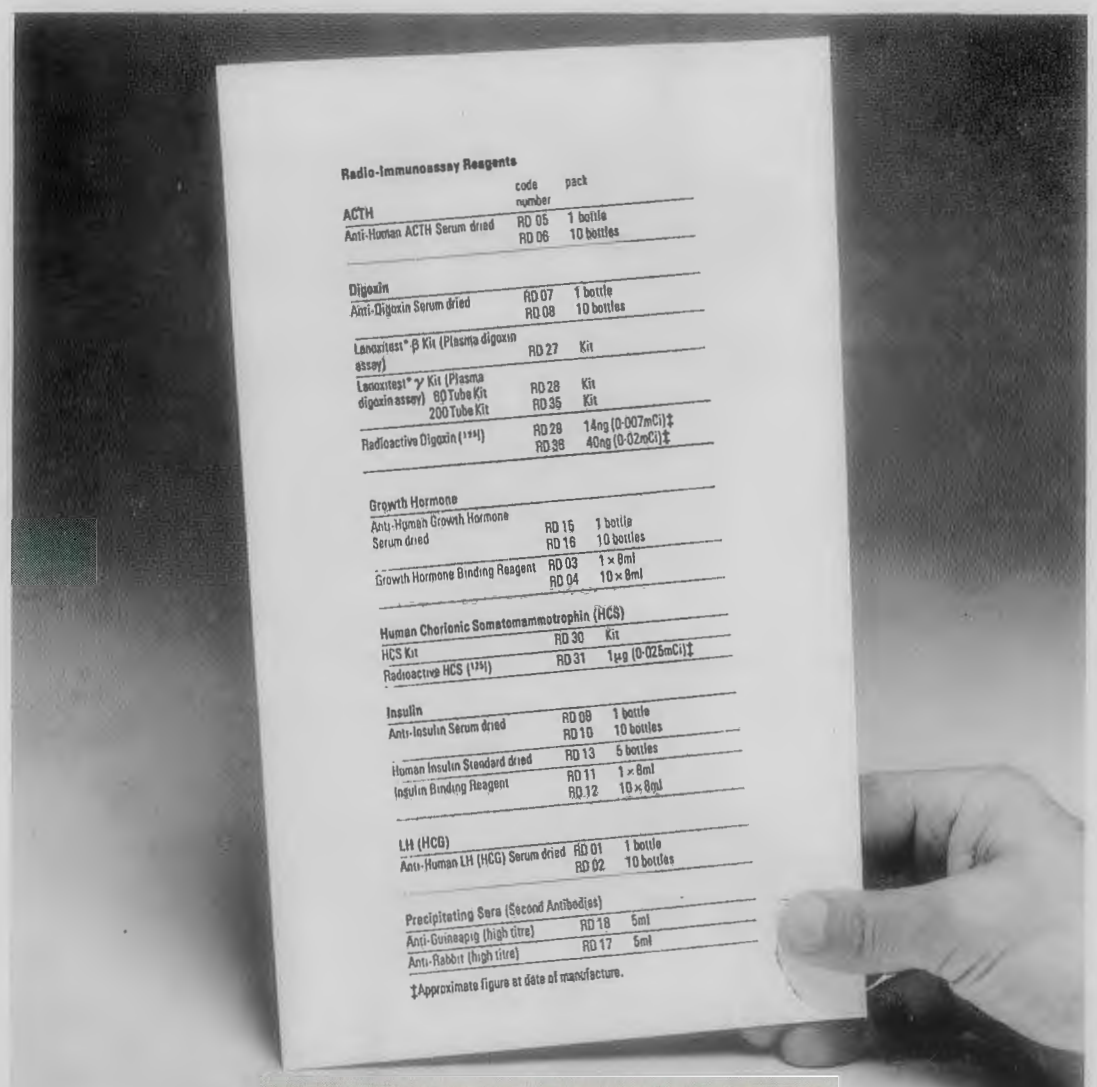

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The Use of Human Placental Lactogen in Monitoring Placental Function

M. Legge

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Based on a paper read at the NZIMLT Conference held at New Plymouth 1974

Introduction

The ideal test parameter for monitoring placental function should be present in relatively large quantities in the blood, have a short biological half life, reflect placental function alone and should have little or no diurnal variation. The use of a 24-hour urinary oestriol estimation has been widely accepted as an index of foetoplacental function. However, inaccurate urine collections, steroid therapy, glycosuria and proteinuria often produce inconsistent results. In 1967 Spellacy *et al.* observed that human placental lactogen levels ran parallel to the 24-hour urinary oestriol excretions and suggested that human placental lactogen levels might be related to placental function[36]. This was subsequently proved to be correct.

Source

Human placental lactogen was isolated from the human placenta in 1961 by Fukuschina[6] and Ito and Higaski[12]. Definite proof of placental production of human placental lactogen and the characterisation of its biological properties was shown by Josimovich and Mclaren in 1962[13]. Sciarra *et al.* in 1963, using an immunofluorescent technique, demonstrated that human placental lactogen was present in the cytoplasm of the syncytiotrophoblast of the placenta but not in the cytotrophoblast[28]. There is no evidence that human placental lactogen is produced elsewhere in the placenta.

Synonyms

Human placental lactogen has a number of synonyms; these are:

Human Chorionic Somatomammotrophin (H.C.S.)

Human Chorionic "Growth Hormone Prolactin" (C.G.P.)

Purified Placental Protein (P.P.P.)

At the Ciba Foundation Symposium on Lactogenic Hormones in 1971[44] it was decided that the simplest and most acceptable term was human placental lactogen (HPL).

Structure

HPL and human growth hormone (HGH) are both polypeptide hormones with strikingly similar structure, being identical in 85 percent of corresponding amino acid positions and having 190 amino acids each[21, 32]. The molecular weights of both hormones is 21,500 and both demonstrate immunological similarity when tested by agar gel diffusion against antisera directed towards HGH[13, 32]. The amino acid sequence and immunological properties are similar to those of ovine prolactin, and it has been suggested that the three molecules may have derived from a common ancestral source[21, 23, 32].

Production

The daily secretion of HPL is in the order of one to three grams per day, in contrast with

the normal secretion of HGH which is approximately 1 mg per day[15].

The HPL concentration in maternal plasma rises progressively during pregnancy, and at term its concentration is approximately three-hundred fold that found in umbilical venous blood[10]. The concentrations of HPL in the umbilical artery and vein have been shown to be equal, suggesting that the hormone is not metabolised by the foetus[5, 10, 33]. HPL has also been demonstrated in relatively low concentrations in the amniotic fluid and maternal urine[3, 5, 12, 33, 37].

The biological half life of HPL is estimated at between 14.5 and 30 minutes, a mean of 20 minutes is usually accepted[10,25,33].

Plasma HPL levels are constant throughout the day and are unaffected by meals, activity, posture or diurnal variation[25, 41, 45].

Action

The apparent overall effect of HPL is to make the maternal metabolism more dependent on fat as an energy source, thereby, ensuring the supply of glucose to the foetus[31, 43]. It may also play an important role in the immunological maintenance of the foeto-placental unit during pregnancy by modifying maternal lymphocytes[4, 17].

Assay

Plasma HPL has not yet been measured by bioassay.

Since the development of radioimmunoassay, a number of techniques for the detection of HPL have been developed. In all fourteen different methods have been published since 1964. The methods all vary in the use of antibody concentrations, plasma dilutions, duration of assay and separation techniques. The method of Letchworth *et al.* is now the most popular technique[18]. Their method employs no initial plasma dilution, uses an antibody concentration of 1 in 5,000 and the duration of the assay takes approximately one and a half hours. This technique can be easily semi-automated[18].

Other immunological techniques such as complement fixation (CFT)[46], immunodiffusion[22] and haemagglutination inhibition (HI)[11] have also proved satisfactory for the assay of HPL and require less sophisticated equipment.

Antisera to HPL usually show a weak cross reaction with HGH, however, the dilution that occurs during the test is sufficiently high to remove any contribution from HGH.

There is no international reference standard for use with this assay, although a partially purified standard is available.

Plasma HPL retains its immunological activity at room temperature or +4°C for three days or six months when stored at —20°C. Serum and plasma will yield the same assay results[18, 34].

Clinical Significance

Normal Pregnancy

HPL can be first measured at above five weeks of pregnancy in amounts of a few nanograms per ml[7, 24]. Thereafter it gradually rises to peak levels around 36 weeks and then shows a decline[38] (Figure 1). During the last four weeks of pregnancy the values show more variability about the mean than during any other stage of pregnancy. A number of HPL normal ranges are reported in the literature and it is recommended that the HPL levels in an individual should be followed as these tend to follow a trend either above or below the population mean.

There is a highly significant correlation between HPL and placental weight[19, 27, 29, 30] and foetal weight[30]. Some workers report a poor correlation for both placental and foetal weight with HPL during labour[33, 34, 35].

Prolonged Pregnancies

In pregnancies which go beyond 40 weeks, without foetal distress, no significant differences of HPL levels have been reported as compared with the levels of normal pregnancy at term[9].

Multiple Pregnancies

Levels of HPL are usually, but not invariably, raised in multiple pregnancies. This is thought to be due to the increased placental mass[9, 27].

Abnormal Pregnancies

Rhesus Isoimmunisation

Plasma HPL levels in severe untreated cases of Rhesus isoimmunisation fall steadily with a drop prior to foetal death[9]. Generally plasma HPL levels in Rhesus negative mothers of Rhesus positive infants are usually normal or raised[9, 14, 32, 33]. The placental weight in Rhesus isoimmunisation is often increased, and when corrected for placental weight HPL levels are usually low[14].

Work by Ward *et al.* in 1973 indicates that HPL levels rising sharply above the normal range in the second trimester may well be an unfavourable sign[42].

Intrauterine death has been reported with high, normal or low levels of plasma HPL[9, 26, 33, 41]. High levels of HPL were reported when the foetuses were hydropic.

Threatened Abortion

In patients whose pregnancies survive, plasma HPL levels are usually normal or slightly low[9, 26, 33, 41]. Even if subnormal, HPL levels show a normal increase with time when abortion is averted. In inevitable abortions, HPL levels are usually low and serial observations show them to be decreasing[7, 24, 38]. Abortion may occur in the presence of normal HPL levels, probably because the placenta itself may be still healthy[8, 39].

HPL may be used to predict abortion in the first trimester and has a use in the diagnosis of inevitable abortions[7, 24, 38].

Placental Insufficiency

This is a poorly defined condition and may depend simply on a clinical impression of intrauterine growth retardation.

In all uncomplicated cases of placental insufficiency studied plasma HPL levels failed to show a progressive rise after approximately 28 to 30 weeks[9, 26, 27, 33]. A plateau was formed which persisted until delivery. There is a poor correlation between individual foetal weight and HPL levels[18].

Foetal Distress and Neonatal Asphyxia

Letchworth and Chard in 1972 demonstrated that three or more levels of plasma HPL below 4 μg/ml between the thirty-fifth and fortieth weeks of an apparently normal pregnancy indicated a 71 percent risk of foetal distress in labour or neonatal asphyxia. HPL levels above 5 μg/ml were associated with a very low frequency of these complications[20].

Toxaemia and Hypertension

These particular conditions have been studied by a number of workers in considerable detail and on the basis of their results a foetal danger zone was defined which includes all toxaemic and hypertensive subjects who have levels of less than 4 μg/ml after the 36th week of pregnancy[41] (Figure 1). If the level was moved further down the scale to 30 weeks gestation, it was found that, with only one exception, all women studied had stillbirths. In cases of maternal hypertension the placenta has been shown to be reduced in volume and low or decreasing

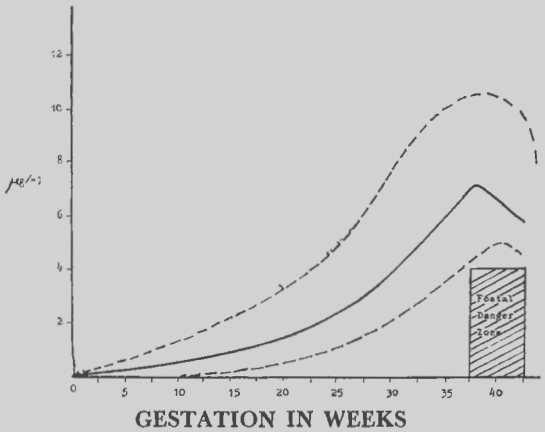

Figure 1.—Normal range of HPL, showing foetal danger zone. — = Mean. — — = 2 SD.

plasma HPL levels have been shown in every patient in whom foetoplacental distress was diagnosed[1, 16].

Diabetes Mellitus

Plasma HPL levels are usually normal or upper limit of normal, but when related to placental weight they are generally low[2, 9, 26, 27, 33]. Foetal death occurs in the presence of normal or low levels of HPL[9, 27, 38].

Intrauterine Foetal Death

There are conflicting reports on the usefulness of HPL in predicting foetal death. A number of workers have demonstrated normal HPL levels for up to three weeks after the presumed death of the foetus[9, 27, 39]. This emphasises the fact that foetal and placental development do not necessarily run in parallel.

Conclusion

The use of HPL has a number of advantages over other tests of placental function, (1) no 24-hour urine collection, (2) single blood sample, (3) rapid results, and (4) easily automated. These points make the analysis ideal for large numbers of samples to be processed.

In all investigations made into the usefulness of HPL as a placental function test the overall result has been the same; namely, HPL is extremely accurate in predicting severe placental dysfunction. However, HPL only indicates placental function and not foetoplacental function. The best overall test for foetoplacental function is still urinary oestriol as this analysis has a lower incidence of false results in normal foetoplacental function.

In all cases where intrauterine death has occurred, HPL levels have remained normal or low normal, but urinary oestriol levels have fallen quickly. The ideal combination therefore, appears to be urinary oestriol as a test of foeto-placental function and plasma HPL as a test of placental function.

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Lecithin Sphingomyelin Ratios in Amniotic Fluid

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Summary

The need for monitoring "at risk" pregnancies with foetal maturity tests to prevent Respiratory Distress Syndrome is outlined.

The method used in determining lecithin sphingomyelin ratio is adapted from the original method preposed by Gluck and Kulovich in 1970[2]. Mention is made of the useful, if carefully performed, shake test as a screening test for foetal maturity.

Two very brief case histories give evidence of the importance of monitoring "at risk" pregnancies by serial LS Ratios to determnie the best time for obstetric intervention without exposing the infant to Respiratory Distress Syndrome.

Introduction

In 1970 Gluck and Kulovich[2] stated the most important current need in assessing the foetus is a simple procedure to provide reliable information about the degree of pulmonary maturity.

In high risk pregnancies premature delivery is often undertaken in an attempt to prevent intra-uterine foetal death but the prime disease to which the prematurely born infant is heir to is Respiratory Distress Syndrome.

Respiratory Distress Syndrome is characterised by:

(a) breathing difficulty,
(b) radiological changes in lung,
(c) inability to maintain adequate PO_2.
(d) expiratory grunt.

These problems are caused by a lack of surface active alveolar lining which normally stabilises the fine alveolar air spaces. In the mammalian lung, the alveoli are lined with a material with unique surface tension activity which prevents alveolar collapse at small lung volumes. This surface active material is a phospholipid-lecithin.

The phospholipids in the lung fluid contribute to the amniotic fluid so the lung level of phospholipids that is lecithin is reflected in the amniotic fluid.

Lung lecithin is synthesised by two pathways[3].

Pathway 1

$$\text{Choline phosphate} + \text{diglyceride} \xrightarrow[\text{transferase}]{\text{phosphocholine (cytidyl)}} \text{Dipalmitoyl lecithin}$$

This is the major pathway and becomes active during week 34-36 at which time lecithin levels in the lung and amniotic fluid rise sharply. The lung is then mature and RDS will not occur.

Pathway 2

$$\text{phosphatidyl ethanolamine} + 3\ CH_3\ \text{(from S-adenosyl-L-methionine)} \xrightarrow[\text{Transferase}]{\text{Methyl}} \text{palmitoyl myristoyl lecithin}$$

In this pathway by the action of methyl transferase methionine is utilised as methyl donor. Methyl groups are transferred to the non surface active phospholipid precursor phosphoethanolamine. This produces palmitic myristic surface active lecithin identifiable from week 22-24.

This helps the premature infant but the methylation pathway is highly sensitive to changes in pH (See Fig. 1).

In normal development, sphingomyelin concentrations are greater than those of lecithin until gestation of week 26. After this the levels are approximately equal until week 34 where pathway 1 produces a rapid surge in the lecithin levels. This rapid surge indicates a mature lung.

Present methods for estimating foetal age are:

(1) Menstrual history—very reliable if recorded very early.
(2) Clinical examination.
(3) Radiological examination.
(4) Sonar cephalometry — fairly good with serial measurements.
(5) Amniocentesis.

The first four parameters give varying results

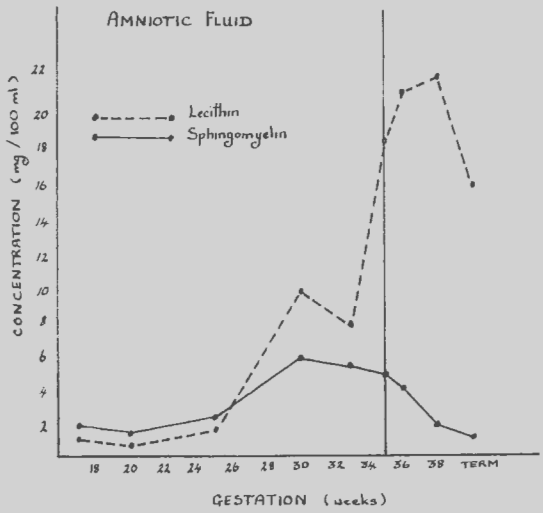

Figure 1.—Mean concentrations in amniotic fluid of sphingomyelin and lecithin during gestation. The acute rise in lecithin at 35 weeks marks pulmonary maturity.

in different hands and never provide a really accurate assessment.

Different biochemical parameters have been suggested for estimating foetal age:

(1) Creatine increases with foetal age.
(2) Osmolality decreases.
(3) Sodium decreases.
(4) Urea increases.

Of these the creatinine has been the most widely used but the range at any given gestational age is too wide to be accurate.

The Nile Blue staining of foetal squamous cells has been used with some success giving a moderately reliable index.

The Lecithin Sphingomyelin Ratio has been termed an almost unerring index of foetal maturity[4].

Method

Reagents

(A) Solvent — cholorform : 32 ml
methanol : 10 ml
ethanol : 2.5 ml
water : 2.0 ml

The side of the tank is lined with filter paper soaked in solvent to aid equilibration.

(B) Location Spray — 50 percent sulphuric acid

(C) Standards

(i) Lecithin No. L-0629 from Sigma. 5 mg/ml solution in chloroform.

(ii) Sphingomyelin No. S-3251 from Sigma. 5 mg/ml solution in chloroform.

(iii) L:S Standards. Combine the both standards to make a 2:1 and 1.5:1 and 1:1 Lecithin — Sphingomyelin standards.

Sample Preparation

The amniotic fluid must be centrifuged within 30 minutes of amniocentesis and the supernatant transferred to another tube. The specimen must be sent to the laboratory on ice where it is placed in the refrigerator if analysis is to be done that day. If not performed on the same day the specimen must be deep frozen.

Procedure

To 2.0 ml of centrifuged amniotic fluid add 2.0 ml of methanol—shake; add 4.0 ml chloroform—shake.

Centrifuge, to separate layers (4000 rpm — 5m.).

Aspirate off top layer and discard.

Filter the chloroform layer (bottom layer) through Whatman No. 1 Phase Separating Paper (P.S.) into a clean tube.

The chloroform is then evaporated to dryness on a rotary evaporator at 60-65°C.

Add 50 μl of chloroform to dissolve the phospholipids.

On to a prewarmed silica gel plate 7.5 cm $\times$ 7.5 cm (Helana Foetal Maturity plate, CAT. No. 8002), spot a 5 μl and 10 μl aliquot of chloroform extract and 5 μl of each of the standards.

Run till solvent front is approx $\frac{1}{2}$-inch from end of plate (10-15m.). Air dry.

Spray with 50 percent sulphuric acid. Char in hot air oven 120-140°C.

Photograph plate for permanent record.

The area and density of the sphingomyelin and lecithins spots are evaluated either visually or by densitometer and results reported as:

Less than 2:1 suggests immature foetal lung function.
2:1 suggests foetal lung development not yet complete.
Greater than 2:1 suggests mature foetal lung function (See Fig. 2).

Notes

(a) Delay in Processing: If specimens are allowed to stand at room temperature the phosphodiesterase activity of amniotic fluid will destroy lecithin.

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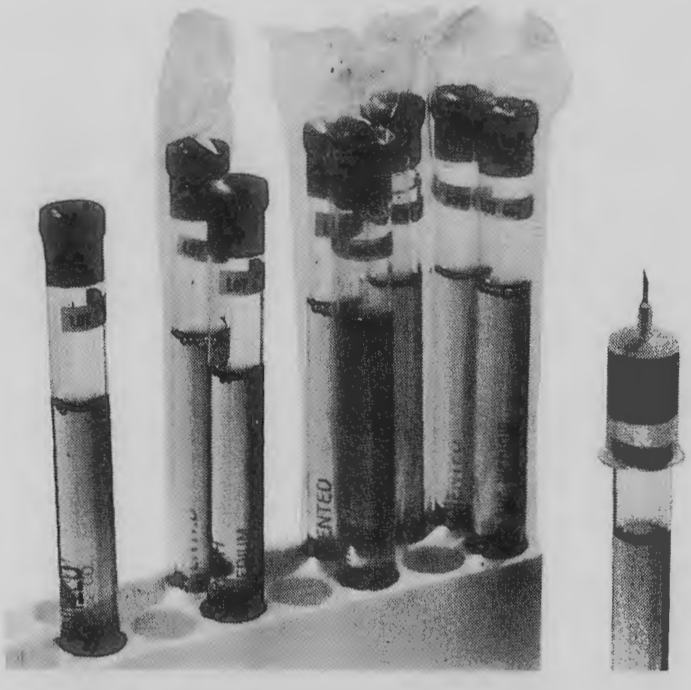

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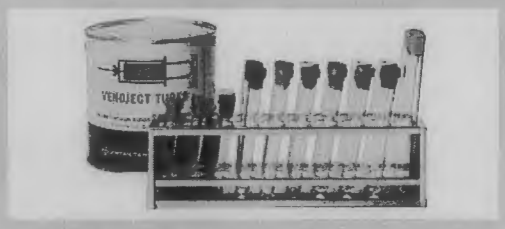

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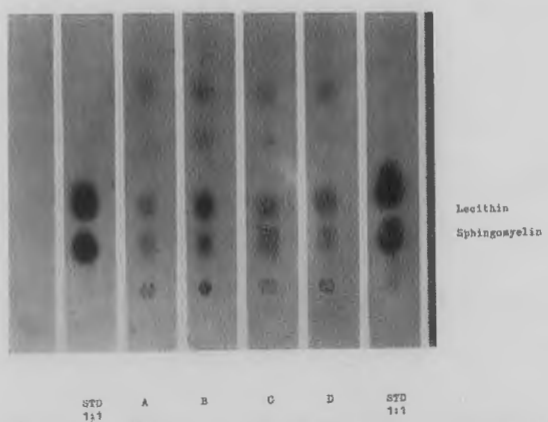

Figure 2.—Thin lay chromatography of amniotic fluid phospholipids. The relative concentrations of lecithin and sphingomyelin are obvious to inspection. Patients B and D show mature patterns (L:S Ratio greater than 2:1); C shows maturity not fully developed (Ratio 2:1); A shows immature pattern (Ratio less than 2:1).

(b) Blood in Amniotic Fluid: This may give false positives due to the presence of serum phospholipids.

(c) Meconium: Obscures extraction and tends to lower results. (Faecal bowel cells contain sphingomyelin.)

Shake Test

For those lacking facilities for T.L.C., a screening test has been used giving close correlation with L/S Ratios[5].

A 1:1, 1:1.3, 1:2, 1:4, 1:5 dilutions are made of the amniotic fluid in saline. An equal volume of ethanol is added to each tube. They are then shaken vigorously and allowed to stand for 15 mins.

N.B. The dilutions must be exact and the tubes scrupulously clean. The tubes are examined for the presence of a complete ring of bubbles—this constitutes a positive result. The quantity and size of bubbles diminish through the series of dilutions. The tube with the highest dilution of liquor giving a positive is recorded.

If it is positive in the dilution of 1:2 or more this indicates the presence of foetal pulmonary surfactant and a mature lung. The test requires care in interpretation and a doubtful shake test should be confirmed by determining the L:S Ratio.

Results

Standards quantitated by densitometric scanning and integration gave the following readings:

(1) a 1:1 standard gave: 1.0:1.0, 1.1:1.0, 1.1:1.0, 1.0:1.0

(2) and a 2:1 standard gave: 1.9:1.0, and 2.0:1.0

Out of 180 amniocenteses

1 patient with a ratio indicating maturity developed moderate RDS and lived.

1 patient with a ratio of less than 2:1 developed severe RDS and died.

Two interesting cases are presented below:

Patient 1

At 40 weeks gestation by dates presented with probable intra-uterine growth retardation. Clinical examination showed a "small for dates baby". The ultrasound Bi parietal diameter gave a gestational age of approx. 34 weeks.

On 13.9.73 the 1st amniotic fluid had L:S Ratio of less than 2:1; 26.9.73 the 2nd amniotic fluid had L:S Ratio of less than 2:1; 3.10.73 the 3rd amniotic fluid had L:S Ratio of greater than 2.1.

On induction she delivered a small healthy baby wtih no RDS.

Patient 2

Was admitted at approx. 36 weeks by dates with a falling oestriol level (down to 6 mg/24 hr). Examination showed a "small for dates baby". On 24.5.74 1st amniotic fluid L:S Ratio less than 2:1; 5.6.74 2nd amniotic fluid L:S Ratio less than 2:1; 14.6.74 3rd amniotic fluid L:S Ratio greater than 2:1.

On induction delivered a small baby with no RDS.

Conclusion

The L:S Ratio of foetal respiratory maturity is a valuable aid in determining the time when obstetric intervention can occur without the risk of RDS in the newborn infant.

At Christchurch Women's Hospital, planned inductions for patients thought to be over 40 weeks gestation, 5-7 percent were found to be less than 36 weeks.

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The Use of Polybrene in Manual Antibody Detection

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Summary

A method of detecting red cell antibodies by direct agglutination, without pre-treatment of panel cells or subsequent manipulation of the serum/cell mixture, is presented. The method is adapted from automated antibody procedures, utilising induction of rouleaux for maximal cell contact with subsequent dispersion of the agglutinates, except in the presence of antibody, in which case dispersion does not occur or is delayed. The resultant sedimentation pattern in the hemispherical base of the test tubes after a set time interval denotes the presence or absence of antibody. Titrations may also be carried out by this method.

Introduction

Following a tour of several blood bank laboratories, an attempt was made to adapt current automated antibody screening techniques to manual methods. During the course of several months various modifications were tried, the one presented being the simplest and most sensitive to date.

Much of the earlier experimentation was based on the principle of rapid antibody binding in a low ionic strength medium[3, 5] coupled with induction of rouleaux by the addition of polybrene (hexadimethrine bromide)[1], and subsequent dispersion of the agglutinates by addition of excess ions, which occurs except in the presence of antibody. However, identical results were obtained using polybrene alone, and in view of the fact that some antibodies react poorly by L.I.S. methods (notably some anti-Duffy and anti-Kidd[5]), it was decided to omit this step.

In the presence of antibody dispersion of rouleaux is prevented, or in the case of weaker antibodies, delayed, upon addition of excess ions. The remaining aggregates rapidly settle to form a characteristic button or ring, thereby revealing the presence of antibody directly without the need of enzyme treatment or the performance of a Coombs test.

Materials

1. Glycine 2.885 percent aqueous.
2. Stock Polybrene 10 percent aqueous. Store at 4°C. (Aldrich Chemical Co. Milwaukee, U.S.A.), (N.Z., Geo. Wilton and Co.).
3. Working Polybrene 0.02 percent. 10 microlitres to 5 ml Glycine. Made fresh daily.
4. Resuspending solution, Stock: a. 1 molar tri-Sodium Citrate; b. 3 molar Sodium Chloride.
   Working Solution: 10 ml of b, 4.5 ± 0.5 ml of a, distilled water to 100 ml (pH 7.7).
5. Panel of Cells: Selectogen I and II (Ortho Diagnostics) are used in this Laboratory; these cells are in a 6 percent suspension.
6. Neutral AB serum.

Method

To each of two tubes labelled I and II respectively, is added the following:

2 drops of patient's serum; 1 drop of the appropriate cell suspension.

After agitation the tubes are incubated for a minimum of 30 minutes at 37°C; two drops of working strength Polybrene solution are then added to each tube. The tubes are incubated for a further 20 minutes preferably with a second agitation after 10 minutes. All tubes will show agglutination, though with minor variation of degree. Add 0.2 ml of resuspending solution to each tube, shake well to fully resuspend the cells and immediately transfer the tubes to a glass or perspex based tube rack. Stand this in a vibration free area for 10-15 minutes, no longer.

Examination of the characteristic sedimentation patterns is most easily done using a mirror over which the transparent based rack may be placed. The presence of antibody can be seen as a compact button of agglutinates which have rapidly settled to the bottom, or in the case of weaker antibodies, a ring pattern similar to that produced in some methods of detecting HCG levels in pregnancy testing[4]. Sera containing no antibodies do not produce a sediment till about 25 minutes after standing.

* *Current address: Howard Institute Salvation Army Hospital, Rhodesia*

FIGURE I.

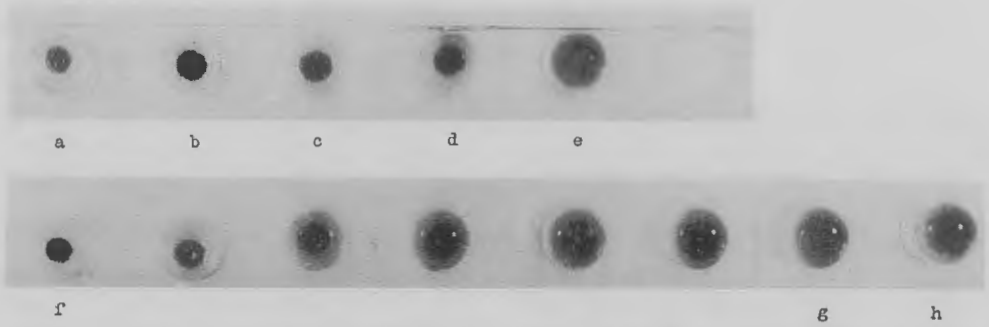

Fig. 1.—Appearance of sedimentation pattern after 10-15 minutes. a, anti-CC^{w}; b, anti-K; c, anti-E; d, anti-c; e, AB serum (negative control); f-g, anti-K serial dilution, endpoint 1:32; h, effect of vibration on negative serum.

Titrations are also possible with this method, however serial dilutions must be made using AB serum as diluent as the induced rouleaux is not dispersable in the lower concentrations of protein that occur in the saline dilutions.

Results

From a series of various antisera supplied by the Otago Blood Transfusion service, all but two produced good reactions with the method, these two, an anti-Jk^{a} and anti-K, also failed to react by their stated optimum methods and were presumed to have deteriorated in transit. The remainder included examples of anti-CD, DE, -e, -K, -k, -Fy^{a}, -S, -Co^{b}. In addition several commercial antisera reacting optimally by various methods all produced good results with their appropriate antigens by the polybrene method.

A series of 100 sera containing no antibodies detectable by traditional means was tested; two of these produced weak positive reactions by the sedimentation technique but were negative when submitted for automated analysis. Coombs tests were done on all the polybrene-containing test tubes, six sera giving positive reactions, all but one appearing to be non-specific. In view of earlier work with polybrene having been abandoned because of the high incidence of false positive reactions (personal communication), no significance was placed on these, except to discount the possibility of using the Coombs test as an ancillary procedure on the same tubes after sedimentation.

Factors Influencing the Results

1. *Cell Suspension.* The final strength of cells in the incubating test tubes is critical; it should be 1 percent for optimum results. Weaker suspensions produce poor sedimentation patterns while heavier suspensions (particularly 3 percent), produce an unacceptably high proportion of false positives. Good reproducibility of cell concentration can most simply be obtained using a single pipette to distribute all three reagents in each batch.

2. *Test Tubes.* The sedimentation pattern depends on a uniformly hemispherical tube bottom[2]; the plastic disposable tubes now in routine use meet this criterion adequately.

3. *Vibration.* Sedimentation must occur in a vibration free area since cells settling uniformly over the bottom of the tube (the negative reaction) may cascade and resemble a weak positive reaction.

4. *Re-suspension.* The ring or button pattern produced by weak antibodies cannot always be reproduced upon resuspension of the once sedimented cells. It appears that dispersion of rouleaux is delayed rather than prevented in such cases, high titre antibodies are unaffected.

5. *Incubation.* Incubation of serum and cells prior to the addition of the polybrene seems to be essential for adequate binding of weak or low titre antibodies. The 30 minute period was sufficient for all post-anti-D gammaglobulin checks done so far, but can easily be extended if preferred. A low ionic strength system could be adapted for this particular purpose[5]. No advantage can be gained by extending the post-polybrene incubation time.

6. *False Positive Reactions.* Though earlier

manual methods using polybrene were apparently discredited on this account, and this does seem to be the case with Coombs testing on polybrene-containing samples, the present version shows considerable improvement in this respect. The two sera showing false reactions in the series mentioned earlier both showed only very weak patterns and were negative by Coombs tests performed on the same tubes though the latter procedure was shown to have a false positive rate of at least 6 percent.

7. *Sedimentation* Time. The time at which the tubes are examined after the addition of the dispersant is critical. If left too long, all tubes will show the typical ring or button of cells. However the much faster rate of sedimentation of even very small agglutinates is sufficient for adequate differentiation. Antibody containing sera produce the expected pattern within 10-15 minutes of standing while the negative sera do not begin to show the pattern till at least 20 minutes after standing.

Discussion

A full evaluation of any antibody detection method is beyond the scope of a smaller laboratory with its low sample turnover. The method is presented in the hope that interested persons better endowed with serum samples may be stimulated to investigate its potential fully.

The method holds promise of considerable savings in time, particularly as a screening procedure. All antisera reacting normally only by ICT that have been tried, produced good visible direct reactions. Much greater simplicity in reading results of batches of sera is possible as these can be rapidly scanned by eye in glass bottomed racks over a mirror.

Positive reacting sera are further examined by traditional methods at present. No false negatives were encountered over a period of six months during which the method was run in parallel with Saline, Albumin, Enzyme and Coombs techniques.

Titres of several antisera showed sensitivity to be at least equal to, and in some cases greater than that of the optimum method. With the Rh antisera tested in this way the pattern could be seen at dilutions several times greater than that shown by the optimum technique.

The question of sensitivity and coverage of the antibody spectrum must be looked at in much greater detail, only twenty different antisera were available for use in the experimentation undertaken, all of which were detected without difficulty.

Acknowledgments

I am indebted to Messrs K. McLoughlin, P. Skidmore, and D. Ford for copies of relevent articles and a supply of antisera.

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Preliminary Evaluation of the BG-8 Blood Grouping Autoanalyser

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Based on a paper read to the NZIMLT Conference, New Plymouth 1974

Introduction

Automated blood grouping was first introduced in 1963[3], although it was not until 1966[6] that the first multi-channel models were in routine use throughout the world. The BG-8 Autoanalyser is a recent addition to the range marketed by Technicon Pty Ltd., Australia.

The Otago Region of the New Zealand Blood Transfusion Services took delivery of their machine in May 1974, and since that date it has been used for routine ABO grouping, Rhesus genotyping and Kell typing of all donations collected.

The Machine and Method of Operation

The BG-8 is an eight-channel machine which is capable of testing 120 samples per hour. Five

channels are used for testing donor cells against specific antisera, and three channels for testing plasma against standard cells.

The donor samples are collected into EDTA and centrifuged to separate the plasma and cells. The specimens are sampled by a double probe, one line of which takes up plasma, the other taking up packed cells.

The plasma is split into three streams to be mixed with A_1; B; and O cells. These standard cells have been pretreated with Bromelin, PVP and albumin. The donor cells are taken to a mixing coil where Bromelin is added, and then split into five streams where they are reacted with the scientific antisera. The antisera is potentiated with a PVP/albumin mixture.

After passing through the reaction coils, saline is added, and the agglutination allowed to settle before being decanted. The decant is then pumped through phasing tubes to a filter paper roll, where they are deposited. Manual reading of the deposits is then performed.

Reagents

1. Antisera

For ABO grouping any commercial grade antisera is satisfactory (titre of 256). The machine samples 0.32 ml per minute, or 19.2 ml per hour. Allowing for time taken to prime the manifold and for "run on" after the last specimen has sampled, the machine uses approximately 22 ml for 120 tests. Whilst this may seem extravagant, it must be realised that the antisera is diluted 1/15 or 1/20 with the PVP/albumin mixture, so the usage of neat serum is between 1.1 and 1.4 ml for 120 tests which is far more economical than most manual methods.

For Rhesus D and CDE grouping, antiserum can be used at a dilution of 1/10 and there is little difficulty in reading results. Rhesus genotyping does create some problems, and it is essential to find suitable antisera. Details of these are given later.

2. Standard cells

The standard A_1, B and O cells are prepared by diluting washed packed cells in Bromelin/PVP/albumin solution. We have found these cells to retain activity for up to three days providing they are kept in a crushed ice bath whilst in use and in a refrigerator between runs.

Results

For the first six weeks of operation, all donor bloods were grouped by both automated and manual techniques. During this period a total of 1,500 donors were typed for ABO Rh genotype and Kell type. Since that time, all donors, patients and ante-natal cases have been grouped for ABO and CDE typings by automated techniques only.

ABO Groups

During the initial six-week period only one discrepant ABO group was found. This was where a donor of group A_2B whose serum contained anti-A_1 was incorrectly grouped as B. As soon as this discrepant result was obtained the anti-A was changed to one that gave stronger reactions with A_2B cells. Since that one example, no discrepancies have been found in testing more than 8,000 donations.

Rhesus D and CDE

All automated groupings which are typed as D negative on automated technique are manually tested for D^u by indirect Coombs test, irrespective of their reaction with anti-CDE.

In the 8,000 donations tested to date, three examples of r′r and five r″r have been read as negative with anti-CDE. These were detected during the early days of testing, and a change of antiserum has resulted in stronger results with C or E positive, D negative cells. We still find that many anti-CDE sera contain much more anti-D than anti-C or anti-E and care must be taken not to overlook weak positive results in the CDE line, and the choice of antisera is critical.

Rhesus Genotyping

All donations have been tested on the analyser against an anti-C; anti-E; anti-c and anti-C^w with promising results. Again the choice of antiserum is most important as is the serum/PVP/albumin ratio. When using commercial "rapid slide or tube" antisera, it must be remembered that these are already potentiated and it may be necessary to reduce the amount of PVP given in the Technicon instruction booklet. Once suitable antisera have been found the results obtained are quite reliable. The deposits on the filter paper are not "agglutinates" as occurs with ABO antisera, but rather a heavier deposit of cells than the negative results. However the reading of the strip is quite easily mastered.

To date there have been 38 discrepancies between manual and automated genotyping out of a total of almost 8,000 tests. Of these, the automated results have been correct in 25 cases, and the manual in 13. The main cause of false automated results have been with false negatives with anti-E and anti C^w. These have been caused by the difficulty of obtaining suitable antisera from New Zealand sources. Commercial "potentiated" antisera give more reliable results but tend to be much more expensive. At present we have satisfactory antisera, and are no longer encountering the difficulties of early days.

Kell Typing

The same problems are encountered as for Rhesus genotyping, and once a suitable antiserum is found, very reliable results are obtained.

Other Typings

Experimental runs have been performed using a variety of other antisera with encouraging results. To date anti-P_1; anti-Vel; anti-k; anti-Le^a and anti-A_1 have all proved most reliable. Anti-Fy^a, anti-M and anti-N do not appear to work with Bromelin/PVP and it is intended to try a range of antisera using Methyl Cellulose/Ficoll[4] in place of PVP.

Antibody Detection

As the machine has a filter paper readout instead of a bank of colorimeters and recorders, it is obviously not as capable of detecting antibodies in plasma as the low-ionic automated methods. However, our experience has shown that most immune antibodies can be detected if present to a titre of 1:6. Examples of Rhesus, Kell, P_1 and Kidd antibodies have been detected during routine runs, but it would not be advisable to rely solely on the BG-8 for antibody detection. A parallel antibody screen by low-ionic analyser or by indirect Coombs and enzyme methods should always be performed.

Operating Conditions

The BG-8 has been found to be a reliable, trouble-free apparatus. In the six months of operation it has been out of commission for only one day, due to a malfunction of the sampler switch. This was caused by a leakage of saline into the mechanism.

The reagents are easy and quick to prepare and are stable throughout the usage period of two days providing they are kept chilled.

The pump tubes are flow-rated and last approximately 250 hours with no undue stretching. Phasing of the eight channels provides no problems, as it can be performed whilst the specimens are being sampled by addition or subtraction of the transmission tubing immediately prior to the filter readout. As this is after the last pump, no interruption to the run is necessary.

Clotted samples are obvious on the readout, and the solenoid operated wash process ensures that the sample probes are reverse flushed and do not remain blocked for subsequent specimens.

Identification of specimens is relatively simple. A saline blank is placed in every 10th or 20th position as an aid to identification, and the samples are set into blocks corresponding with the sample tray. Donation numbers are stamped onto the filter paper before detaching and reading of results. The Technicon "I-Dee" system of specimen identification can be fitted to the analyser if sufficiently large numbers of samples are being tested to warrant the added expense.

Amendments

It is possible to use the machine for other than ABO and Rhesus groupings. The ART method for syphilis screening[2]; hepatitis B antigen and antibody tests[5], and tetanus antibody screen are some examples of other immunohaematological tests that can easily be fitted into the routine operation.

The use of animal extracts, such as snail albumin gland[1], etc, can be used as a source of antisera, and is said to be very reliable. It is hoped to try these reagents in the future.

Economy

As mentioned previously, the usage rate of antiserum is much less than most manual techniques, and this can result in a considerable saving of cost. The other reagents used are relatively inexpensive and easily obtainable.

The biggest saving is in the number of staff needed to group large numbers of donors. The BG-8 is capable of testing 120 donors for their ABO; D; Kell typings and full Rhesus genotype in approximately two and a third hours, with only one technologist being involved. Manual techniques would take a little longer and involve about four staff members.

Overall, if a laboratory has in excess of 100 groupings a day, the BG-8 is eminently worthwhile, whilst about 60 per day would be the

minimum number at which to consider such an analyser. The machine does mean that a laboratory can cater for an increased workload with no increase in staff. Whilst the original cost of the analyser is moderately expensive it is cheap to maintain and run, and the savings on reagents and staff wages make it an attractive proposition for a large hospital or regional transfusion service.

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An Unusual Case of Macrocytic Anaemia

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Based on a paper delivered to Hamilton Branch Seminar, May 1974

Summary

A sixty-nine-year-old female, under investigation for macrocytic anaemia, was referred to the laboratory for further tests. Upon questioning she admitted to an eleven-year history of laboratory investigation for haematuria. Following a bone marrow examination, evidence of haemolysis was suggested by a macronormoblastic hyperplasia with increased marrow iron stores. A positive sucrose lysis test[1] and the presence of haemosiderin in her urine strongly suggested the diagnosis of paroxysmal nocturnal haemoglobinuria, which was confirmed by a positive Hams test[1].

Introduction

Paroxysmal nocturnal haemoglobinuria is a chronic haemolytic disorder characterised by intermittent haemoglobinuria and persistent haemosiderinuria[2].

There are various theories as to the aetiology of this disorder, which may possibly be due to a somatic mutation when erythropoietic regeneration takes place, giving rise to partial reproduction by a clone of abnormal haemocytoblasts. This theory is supported by the close association of paroxysmal nocturnal haemoglobinuria with aplastic anaemia[3].

There are usually two populations of erythrocytes. Due to an acquired membrane defect one population is approximately twenty-five times more sensitive to complement than normal erythrocytes, the other population is normal. The proportion of each population varies greatly from patient to patient, and with clinical state of the patient, and there may occasionally be more than two affected populations each with varying degrees of sensitivity to complement[3]. All cellular elements, i.e., platelets, leucoctyes and erythrocytes arising from the abnormal precursor cells are affected, often resulting in a pancytopenia.

The affected patient is usually between the third and fourth decade in age, and patients often present with abdominal pain, headaches and nocturnal haemoglobinuria which may be infrequent or even absent. Attacks of haemoglobinuria can be precipitated by infection, administration of drugs, menstruation, transfusion and operation.

Case Report

The following case report covers a period of eleven years, the laboratory results of which are summarised in Table I.

The patient, a sixty-nine-year-old female, first presented in 1963 with fever, vomiting, haematuria, bronchopneumonia, and palpable kidneys. After bed rest and laboratory investigation, which included a bone marrow examination, she was discharged. In 1966 she was readmitted with pancytopenia, haematuria and distended abdomen, especially at night when in bed. Recurrent urinary infections and pneumonia were attributed to be the cause of the haematuria, and after bed rest she was again discharged.

TABLE I

Summary of Haematology Results

		Hb	*PCV*	*MCHC*	*WBC*	*Retics*	*Platelets*	*Erythrocytes*
1963	Varied from	8.8—	30—	30—	3,000—	—	70,000	Anisocytosis +
	to	10.8	34	36	5,000		90,000	occasional polychromatic cells
1964		9.9			2,000	3%	130,000	Anisocytosis + occasional polychromatic cells
1965		9.3	30	31	7,000	—	Normal	Anisocytosis + few macrocytes, microcytes, poikilocytes, polychromatic and hypochromic cells
1966		11.7	36	33	4,000	—	125,000	Normal
1968	Varied from	7.4	24—	30—	2,300	7.4—	Normal	Anisocytosis ++,
	to	10.1	32	32	5,000	7.6		macrocytosis +, some macro-ovalocytes, microcytes poikilocytes polychromasia + occasional erythroblasts
1974	Varied from	10.6—	33.0—	32—	3,900—	2.7—	205,000	Macrocytosis ++
	to	12.0	38.0	33	4,200	2.2	255,000	Anisocytosis ++ some macro-ovalocytes poikilocytes. Rouleaux +

TABLE II

Test	*Result*	*Normal Ranges*
Sucrose Lysis	Haemolysis present	No haemolysis
Ham's Test	Positive (16.5 percent lysis)	No lysis
NAP Score	54	14—100
Haptoglobin	54	40—180 mg/100 ml
Bilirubin	1.1	0.1—1.0 mg/100 ml
LDH	1,200	50—225 U/litre
Iron	125	80—180 ug/100 ml
Iron Binding Capacity	270	250—450 ug/100 ml
Folic Acid	12.5	4.0—21.0 ng
B12	435	165—900 pg

The patient was again admitted to hospital in 1968 with recurrent early morning haematuria which cleared later in the day. Occasional early morning urine specimens were dark coloured suggesting haemoglobinuria.

Cystoscopy did not reveal any cause of the haematuria which was again attributed to renal infection. After discharge from hospital she had no further laboratory investigation until 1974 when a routine haematological examination revealed a pancytopenia and macrocytic anaemia. As a result of this a bone marrow examination was carried out and further tests were performed to elucidate the cause of the pancytopenia. These are summarised in Tables II and III.

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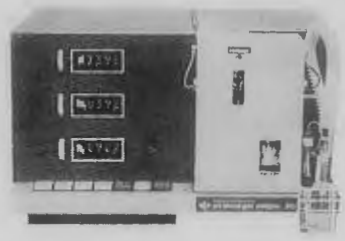

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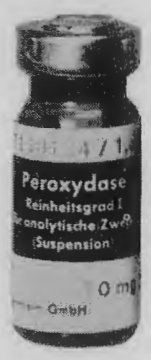

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TABLE III

Bone Marrow Examination Results Summary

Myeloid/Erythroid Ratio:	1.1:1 (normal 2.5—6.0:1)
Erythron:	Macronormoblastic hyperplasia
Leucon:	Slight granulocytic hyperplasia
Thrombon:	Megakaryocytes slightly reduced
Iron Stores:	Increased

Discussion

In spite of this patient having exhibited classical symptoms of PNH for many years, the tests for establishing the diagnosis were never performed. Even though PNH is a rare disorder we feel it is advisable to carry out a sucrose lysis test and urine haemosiderin[1] on all patients with pancytopenia and macrocytic anaemia, where there is no obvious underlying disorder.

In our laboratory these two tests are routinely performed on all obscure anaemias, and as this is our second case of PNH diagnosed in the last four years, we consider the occasional positive result adequately compensates for all the negatives that are reported.

Acknowledgments

I would like to thank Dr R. S. Stephens and Dr W. A. Symmans and Mr C. S. Shepherd for their assistance and encouragement.

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Syphilis Reference and Quality Control

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Introduction

The Department of Serology, Auckland Hospital, recently renamed Department of Immunology, has been doing reference work for various tests, including syphilis, for many years. It was designated Treponema Reference Laboratory for New Zealand by the Health Department in 1968. Evaluation of early results were reported previously (Fischman *et al.*, 1971)[3]. This paper discusses further data on confirmatory tests, as well as quality control.

Historical

While Quality Control—both internal and external—has been widely used in most departments in recent years, historically it was attempted first in syphilis serology. Under the auspices of the League of Nations several conferences were held between 1923 and 1930 in an attempt to bring order into the confusion caused by the proliferation of new tests, countless variants of each test, and the enormous discrepancies in results. In 1934 and 1937 the United States Public Health Department conducted a survey by sending hundreds of sera to author-serologists and serological laboratories to compare performances. Altogether 12 Wassermann variants and 11 flocculation tests were being used. Examples of discrepancies found were that sensitivity of the Kolmer WR varied from 32 percent to 72 percent and the Kahn Test from 49 percent to 87 percent. A comment by one of the author-serologists was, "A good technique does not *per se* insure the proper performance of a test: the gross inadequacy of the tests as now performed in many laboratories is only too apparent" (Eagle, 1937)[2]. This was before the introduction of the treponemal tests. Since then the number of reagin tests in use have been greatly reduced, and more uniformly standard methods are available both for reagin and treponemal tests. But the need for quality control remains.

The first contribution of the World Health Organisation (WHO) was in the early 60's by preparing an International Standard Reference Serum for syphilis. This was distributed by the Statens Seruminstitut, Copenhagen, in 2 ml freeze-dried lots. It was used by receiving laboratories, including ourselves, to prepare

larger volumes of standard sera of similar sensitivities in various tests, and was useful to check Wassermann and other antigens prepared in this laboratory and for distribution to other laboratories requiring controls.

External Quality Control

The CDC, Atlanta, has been authorised to run this, under the name Proficiency Survey, for the WHO, designed for National Laboratories. This is a tough control, consisting of sending 10 sera at frequent intervals, including duplicate samples, and grading to a maximum of 100. Ten marks are given for each correct result, five marks are lost for a weakly reactive-reactive disagreement, and 10 marks for complete disagreement on one serum. A score of 90 is accepted as minimally satisfactory. This laboratory has scored 95 initially, while more recently consistent 100 scoring has been achieved. (Performed by David Bree and Carol Wright.)

For laboratories other than reference laboratories, several schemes are available, run by overseas societies of pathologists. The importance of quality controls for the smaller laboratory in New Zealand has been emphasised by Bolitho (1971)[1].

Regional Quality Control

Thirty-six laboratories participated recently in the Quality Control scheme conducted by the Auckland Laboratory, including 24 public hospital and 12 private laboratories, twice as many than in the previous year. Table I indicates the number of tests performed in each centre from the following list: WR, VDRL, RPR, RPCF, TPHA and FTA. The results were graded as follows: A, satisfactory; B, acceptable, some improvement desirable; C, unsatisfactory. The distribution of gradings for the qualitative results is shown in Table II. C grade was given to 15 percent of all tests (11); this involves 22.2 percent of all laboratories (8).

Only 25 percent of the laboratories (9) performed quantitative evaluation of one or more tests. This is unsatisfactory. At least one test should be quantitated on each serum for routine purposes. All round, the majority performed reasonably well. The poorest results were with the RPCF. However the performance of the other two treponemal tests also need to be improved. With the increasing use of TPHA it should be considered that while one can easily believe this to be a simple test, and be complacent about it, only one-third of the results were rated A. Thus greater care is required, and confirmation by FTA-ABS is desirable, also with impeccable technique.

Of the five laboratories sending in results of FTA-ABS, less than half achieved A grading. Two of the five laboratories have been reporting FTA direct to the requesting source, without having it checked by the Reference Laboratory. (A Health Department subcommittee recommended that the Reference Laboratory alone should perform FTA for final reporting but this directive has been disregarded by some laboratories.) Two others, after finding that they have reported incorrect results, have resumed sending sera to us.

Table I: The number of techniques performed by each laboratory

No. of tests	Laboratories	
	No.	*Percent*
1	18	50
2	8	22.2
3	4	11.1
4	4	11.1
5	1	2.8
6	1	2.8

Table II: Distribution of gradings given for all tests performed

Test	Grade			
	A	*B*	*C*	*Total*
VDRL	18	7	2	27
RPR	10	5	0	15
WR	7	3	4	14
RPCF	1	2	3	6
TPHA	2	3	1	6
FTA	2	2	1	5

Confirmatory Testing

In addition to the 10 Auckland public hospitals 34 laboratories sent sera for confirmation, or full testing, including reagin and treponemal tests. Approximately 1,000 positive FTA-ABS reactions were obtained in a 12-month period. These included 300 Europeans and 70 Maoris, confirming syphilis. The remainder were mainly Pacific Islanders, with a small group of unknown race. These figures are similar to the

ones obtained in previous years. The incidence of positives in Maoris is about twice as high as in Europeans if considered in relation to the proportion of the two races in the general population. The TPHA was performed when requested or if it was considered useful in addition to FTA-ABS.

Syphilis in the immigrant Pacific Islanders appears to be still rare. While no single test has been found yet to differentiate syphilis from yaws, in the New Zealand situation, with mostly residual yaws antibodies and active yaws a rarity, a pattern of tests, including crude antigen WR, VDRL, TPHA and RPL (Reiter Latex), done quantitatively, helps to decide whether yaws or syphilis is more likely to be present (Fischman *et al.*, 1971; 1973)[5,6].

It was found that for the purpose of screening Pacific Islanders in New Zealand to detect yaws antibodies, the treponemal tests: Reiter, Latex and/or TPHA are more sensitive and/or convenient than the reagin tests or the microscopical treponemal tests FTA or TPI (Fischman et alii 1974)[4]. This does not, however, apply to the European-Maori population when one looks for syphilis. A recent circular of the WHO Reference Laboratory, CDC, Atlanta, emphasises that TPHA should not be used as a routine screening test. This should be done with reagin tests.

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5. Fischman, A., and Mundt, H. (1971), *Brit. J. ven. Dis.*, **47**, 91.
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Technical Communication

A Problem Encountered with an Antibody of the Kidd Blood Group System

A 46-year-old male Caucasian patient was admitted to Dunedin Public Hospital to undergo open heart by-pass surgery.

As is routine at this laboratory the following immunohaematological tests had been performed on this patient prior to admission:

a. Full ABO and Rhesus group including genotype.
b. Kell and Duffy typing.
c. Cold agglutinin titres.
d. Screening for atypical antibodies by the following techniques:
   i. Automated low ionic strength. The patient's serum tested through one channel against a pool of Rhesus positive cells and through the second channel against a Rhesus negative Kell positive cell. These cells selected for other common antigens.
   ii. Enzyme (ficin) pre-treated cells (a) Rhesus positive (R_1R_2) and (b) Rhesus negative.
   iii. Testing the patient's serum against a panel of cells containing the common antigens by saline techniques at 15°C, room temperature, one stage enzyme (Low's Papain) and Albumin Coombs Techniques at 37°.
   iv. Screening for leucocyte antibodies.
   v. Test for Hepatitis Associated Antigen.

The patient was found to be Group A, Rhesus Negative ($\bar{c}de/\bar{c}de$), Kell Negative, Duffy A Positive.

His serum was found to contain Rhesus antibodies (Anti-CD) active by saline, enzyme and indirect antiglobulin techniques.

On admission to the hospital a further sample was collected and repeat ABO and Rhesus group was performed together with antibody screens (automated and ficin techniques), the results being consistent with those found earlier.

For the compatibility testing, samples were collected into ACD from 14 A Rhesus negative donors and the cross-match performed by the following techniques:— Saline 15°C and room temperature, one stage enzyme (Lows Papain) and Albumin Coombs at 37°C.

All 14 donors were found to be compatible by all methods.

The patient underwent surgery for which six units of blood collected the day previous were taken to prime the by-pass machine and a further four units, freshly bled, were used

during the operation.

Following the operation routine haematological investigations were performed and it was noticed that there was a gradual decrease in Hb from 15.2 gm percent pre-operatively to 9.0 gm percent and a corresponding drop in PCV from 43 percent-33 percent. The total white cell count increased from 12,000-21,000/cu mm. The blood film showed the presence of spherocytes, erythroblasts and Howell-Jolly bodies. A reticulocytosis reaching a maximum of 10.5 percent was also seen.

A fresh specimen was obtained to crossmatch blood for a further transfusion, and incompatibility was found when a moderately strong reaction appeared by indirect Coombs technique in three out of four units being crossmatched, the one compatible unit being one crossmatched originally but not used. The antibody was identified as anti-Jk^a and the patient typing as Jk(a=). Four group A rhesus negative Jk(a=) units were then selected all being compatible by all techniques.

Of the 10 units originally transfused during surgery six were found to be Jk(a+).

It was considered therefore that the original samples contained a low titre antibody which was undetectable by routine methods. Transfusion of six units of Jk(a+) blood had stimulated this latent antibody causing a mild haemolytic episode.

The patient made an uneventful recovery, requiring no further transfusions and prior to his discharge from hospital a further antibody check was performed with the result that the anti-Jk^a was only weakly detectable.

The fact that the antibody was not detectable immediately prior to transfusion but appeared strongly soon after stimulation and had almost disappeared within a further fortnight is further proof of the transient nature of some antibodies. Anti-Jk^a is a notable example of this and has been well documented.[1] Other antibodies noted for this phenomenon include anti-Vel and some examples of anti-Duffy.

From the above experience any patients that show the presence of spherocytes, with or without a decrease in haemoglobin, post-transfusion should be investigated promptly for the presence of atypical antibodies.

A. E. Knight,
Immunohaematology,
Dunedin Public Hospital.

March, 1975

REFERENCE

1. Issitt, P. D., Applied Blood Group Serology (1970).

What's New?

HAMILTON INTRODUCES NEW HI-PRESSURE SYRINGE

A new 50μl syringe, with a special finger grip, designed for high pressure liquid chromatography has been designed by Hamilton Company. It is both gas tight and liquid tight and will withstand working pressures up to 6000psi.

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Further information is available from sole New Zealand agents, Watson-Victor Ltd., at Auckland, Wellington, Christchurch and Dunedin.

Hamilton Company Hi-Pressure Syringe

Abstracts

Contributors: D. G. Bolitho, L. M. Milligan, Beverly Stubbs and A. G. Wilson

CLINICAL BIOCHEMISTRY

S.I. Units. How Systematic and how International are they? McLauchlan, D. M., Raggott, P. R. and Zilva, Joan, F. (1975). *Ann. clin. Biochem.* **12,** 1.

The authors discuss the difficulties which have arisen in expressing SI units. The confusion is particularly rife in reporting results of thyroxine and lipid assays. —A.G.W.

The Lag Factor in Continuous Flow Analysis. Walker, W. H. C. and Andrew Kalina, R. (1974), *Clin. Chim. Acta.* **57,** 181.

The major determinants of lag factor in continuous flow analysis are shown to be the volume of the segmented stream, the ratio of flow rates of air and liquid, the time interval between bubbles, and a minor portion derives from the unsegmented stream in the flow cell.

The authors have confirmed previous work in this field. —A.G.W.

Blood in Faeces. A Comparison of the Sensitivity and Reproducibility of Five Chemical Methods. Christensen, F., Anker, N. and Mondrup, M. (1974), *Clin. Chim. Acta.* **57,** 23.

As the benzidine test is no longer in use because of its carcinogenic properties the authors have tested five different methods for reproducibility and sensitivity. They conclude that Hemoccult method provided an effective substitute for the benzidine test and more sensitive and reproducible than the other tablet methods, i.e., Hematest and Okokult.

—A.G.W.

The Derivation of Biochemical Normal Ranges from a Hospital Outpatient Population. Little, A. J., William, R. B., Parker, S. D. and Payne, R. B. (1974), *Clin. Chim. Acta.* **57,** 91.

A number of biochemical tests were performed on serum from 290 outpatients attending hospital for haematological investigations as well as 117 blood donors. The 95 percent limits of the derived normal ranges showed good agreement for albumin, alkaline phosphatase, bilirubin, calcium, phosphate and protein. The authors also conclude that haematology outpatients are a convenient source of samples for normal ranges for electrolytes, urea, and creatinine.

—A.G.W.

The Difference between the Glucose Concentrations in Plasma and Whole Blood, Holtkamp, H. C., Verhoef, N. J. and Leijnee, B. (1975), *Clin. Chim. Acta.* **59,** 41.

The authors have studied the differences that are found between the glucose content of whole blood and plasma. In whole blood diluted with water the glucose concentration is 13 percent higher than in whole blood prediluted with saline. They recommend that plasma be used for glucose determination unless there is good correlation between whole blood glucose and plasma glucose. A satisfactory method is the ferricyanide method in combination with a protein-free filtrate. —A.G.W.

Lactosuria. A New Metabolic Feature of Severe Cerebrocranial Trauma. Vitek, V., Vitek, K. and Adams Conley, R. (1975), *Clin. Chim. Acta.* **58.** 109.

Under controlled dietary conditions the urinary lactose was measured in 15 healthy persons and 15 patients with cerebrocranial trauma, and 15 patients with extracranial trauma.

They found a marked increase in the patients from the head injury group the highest levels of lactose was associated with comatose subjects. Lower levels were found in drowsy or disoriented subjects.

—A.G.W.

Calculations and Correction Factors used in Determination of Blood pH and Blood Gases. Burrett, R. W. and Noonan, D. C. (1974), *Clin. Chem.* **20,** 1499.

This review article discusses and explains all the parameters measured and derived from blood gas analysis. Recent information regarding temperature correction is included. —A.G.W.

Evaluation of a New Device for Rapidly Separating Serum or Plasma from Blood. Mathies, J. C. (1974), *Clin. Chem.* **20,** 1573.

The "Sure Sep" serum plasma separator of General Diagnostics has been evaluated for 34 analyses of 750 paired blood samples. It was found to be satisfactory in all the analyses tested.

—A.G.W.

The Hoesch Test. Bedside Screening for Urinary Porphobilinogen in Patients with Suspected Porphyria. Canon, J. with T. K. and Redeker, A. G. (1974). *Clin. Chem.* **20,** 1438.

The little known Hoesch test for porphobilinogen was examined for sensitivity, specificity and utility as compared to the Watson-Schwartz test. It was found to be equally sensitive and because the Hoesch test is without false positive reaction secondary to urobilinogen and is simpler to perform, the authors conclude it should replace the Watson-Schwartz test.

—A.G.W.

Rocket Immunoselection for Detection of Heavy Chain Diseases. Gale, D. J. J., Versey, J. M. B. and Hobbs, J. R. (1974), *Clin. Chem.* **20,** 1292.

A method is described that allows heavy chain disease proteins to be simply detected. The sample is electrophoresed in an agarose plate containing

Kappa and lambda antisera in the lower section and the relevant heavy chain antisera in the upper section. The light chains and immunoglobulins are precipitated in the lower region. Heavy chains migrate to the upper zone where they precipitate as a separate rocket.

The method is quick, sensitive, and requires little antisera. —A.G.W.

HAEMATOLOGY AND IMMUNO-HAEMATOLOGY

HL-A Antigens: Association with Disease (1974), Vlroutui, O. and Rose, N. *Immunogenetics* 1, 4.

The authors give a brief but accurate summary of the known facts concerning the genetics of the HL-A system and a summary of their research, results and conclusions reached. —L.M.M.

Microcapillary Agglutination for the Detection of Leucocyte Antibodies: Evaluation of the Method and Clinical Significance in Transfusion Reactions. McCullough, J., Burke, M., Wood, N., Carter, S., Weilblem, B. and Yunis, E. (1973), *Trans.* **14**, 5.

The present study is an evaluation of the microcapillary agglutination (MCA) and compares results obtained using this method, with those obtained by lymphocytoxicity (LC) in the study of specific HLA antibodies. In addition, the clinical significance of MCA was investigated in a number of patients with non-haemolytic transfusion reactions.

—L.M.M.

Incidence of Bombay (Oh) Phenotypes and Weaker Variants of A and B Antigen in Bombay (India) (1974), Bhata, H. and Sathe, M. *Vox Sang*—**27**, 524.

A study of a large number of Indians in Bombay was carried out to determine the incidence of Oh and weak A and B variants. The Oh phenotype was observed in a number of random persons, who were found to come from the South-West districts.

—L.M.M.

Effects of *In Vitro* Storage on Red Blood Cell Agglutinogens. Greendyke, R. M. and Banzhof, J. (1974), *Trans.* **14**, 4.

Quantitative haemagglutination studies were conducted with an electronic particle counter to assess the comparative efficiency of several red blood cell storage media, in the preservation of red cell agglutinability—including studies with added adenine, inosine and adenosine. The methods and results are discussed. —L.M.M.

Kidd Blood Group Antigens of Leucocytes and Platelets. Marsh, W., Oyen, R. and Nichols, M. (1974), *Trans.* **14**, 4.

Neutrophil leucocytes have a strong absorptive capacity for Anti Jk^a Jk^b, made by Jk (a-b-) persons, but do not absorb anti Jk^a or Jk^b. The Jk^a Jk^b antigen does not appear to be present on lymphocytes or platelets. The results indicate that Jk^a Jk^b activity is not a cross reaction between Jk^a and Jk^b, but is a distinct and separate specificity.

—L.M.M.

Cell Electrophoresis for the Detection of Platelet Antibodies. van Boxtel, C., van der Weerdt, C. and Engelfriet, C. (1973), *Vox Sang*—**27**, 489.

The method for the detection of platelet antibodies using electrophoresis is discussed. A description of the equipment, reagents, methods and results are set out clearly. —L.M.M.

Anti I (A+B): An Autoantibody Detecting an Antigenic Determinant of I and a Common Part to A and B. Ropars, C., Salrion, C. and Daniel, C. (1974), *Vox Sang*—**27**, 515.

Some cold autoantibodies found in acquired haemolytic anaemias react with both the A, B or H and the I blood group antigens. The agglutinating activity of such an autoantibody of the IgM type was studied by a quantitative method in an AB patient. The conclusion reached is that the antibody recognised a determinant which comprised I and some part shared by A and B. The antibody was named: Anti I (A+B). —L.M.M.

Erythrocytosis in Hepatocellular Carcinoma. Tso, S. C. and Hua A. S. P. (1974), *Brit. J. Haem.* **28**, 497.

Red cells were removed by phlebotomy in nine patients having erythrocytosis associated with hepatocellular carcinoma. This resulted in an increase in the plasma levels of erythropoietin in seven. It suggests that, in the absence of systemic hypoxia, erythrocytosis develops in hepatocellular carcinomas as a response to hypoxia in the liver. —B.J.S.

Classical Factor X Deficiency. Girokimi, A., Coser, P., Brunetti, A. and Prinoth, O. (1975), *Acta haemat.* **53**, 118.

A case of classical factor X deficiency is reported. The propositus is a 28-year-old male who presented with easy bruising, epistaxis, haematomas, haematuria and occasional haemarthrosis since early childhood. The severely prolonged prothrombin time was corrected by normal serum, but not by normal plasma.

—B.J.S.

Impaired Erythropoietin Response in Anaemic Premature Infants. Buchanan, G. R. and Schwartz A. D. (1974), *Blood*, **44**, 347.

Serum erythropoietin levels (ESF) were measured in a group of normal premature infants with physiological anaemia. No ESF was detected in these infants, while a group of older anaemic children with similar haematocrit values had elevated levels.

—B.J.S.

Granulopoiesis Following Chemotherapy. Greenberg, P., Box, I., Mara, B. and Schrien, S. (1974), *Blood*, **44**, 375.

Clonal proliferation of marrow granulocytic progenitor cells in vitro and the daily output of granulocytic colony stimulating factor were determined in two patients with acute myeloid leukaemia and one with malignant lymphoma. During and immediately following therapy, a marked decrease of granulocytic colony-forming capacity occurred. —B.J.S.

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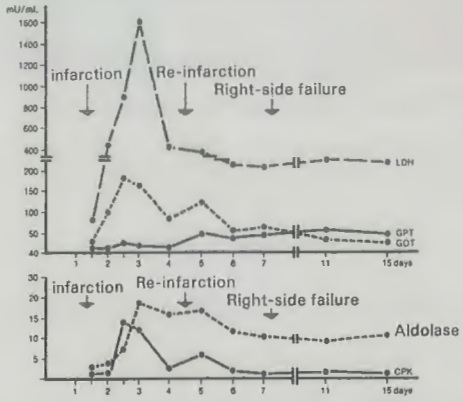

Schmidt, E and Schmidt, F.W: Guide to Practical Enzyme Diagnosis Mannheim, Boehringer Mannheim, GmbH, 1967

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Blood Viscosity in Waldenström's Macroglobulinaemia. Schiffer, L., Markor, A., Winkelstein, A., Nelson, Janet, and Mikulla, J. (1974), *Blood,* 44, 87.

Erythrocytes and plasma proteins contribute to the viscosity of blood. Blood viscosity was measured in 16 persons with macroglobulinaemia with a cone plate viscometer. In each person significant correlation was found between haematocrit and the logarithm of blood viscosity. —B.J.S.

Two Cases of G6PD Deficiency. Sonnet, J., Lievens, M., Verpoorten, C., Kriekernans, J. and Eeckels, R. (1974), *British Journal of Haematology,* 28, 299.

G6PD deficiency is found chiefly in Negroes originating from West Africa and in some other racial groups living in tropical and subtropical areas. This paper presents the cases of two unrelated children of Western European ancestry, originating from the North Flemish part of Belgium. Characterisation of the enzyme of the first case revealed that it was a variant which had not been previously reported. —B.J.S.

Cytochemical Estimation of Dihydrofolate Reductase. Tzartzatou, F., Hayhoe, F. G. (1974), *British Journal of Haematology,* 28, 199.

The enzyme dihydrofolate reductase plays an important role in the conversion of dietary folate to the active coenzymatic form. This enzyme may be an important target for the action of folic acid antagonists. A method has been devised for the cytochemical demonstration of dihydrofolate reductase activity using dihydrofolate as the substrate. —B.J.S.

MICROBIOLOGY

Sensitivity of *Citrobacter freundii* and *Citrobacter koseri* to Cephalosporins and Penicillins. Holmes, B., King, A., Phillips, I. and Lapage, S. P. (1974), *J. clin. Path.* 27, 729.

It was discovered that 79 percent of 99 strains of *Citrobacter freundii* examined were resistant to cephalosporins and sensitive to carbenicillin. Ninety-six percent of 45 strains of *Citrobacter koseri* were sensitive to cephalosporin and resistant to carbenicillin.

The authors suggest that tests of susceptibility to these antibiotics may well be a useful differentiating characteristic. This paper also gives a good description of other differentiating characteristics of the two species. —D.G.B.

Tests of Disinfection by Heat in a Bedpan Washing Machine. Ayliffe, G. A., Collins, B. J. and Deverill, C. E. A. (1974), *J. clin. Path.* 27, 760.

Ingenious tests of sterilising efficiency using suspensions of organisms sealed in plastic capillary tubing and attached to the surface of the pans are described. The use of these tests showed that with the particular bedpan washer disinfector used the efficiency of the disinfecting process was adequate for metal pans but not for polypropylene pans. The authors also discuss factors leading to the choice of disposable bedpan systems or disinfectors. —D.G.B.

Aerosol Production Associated with Clinical Laboratory Procedures. Stern, E. L., Johnston, J. W., Vesley, D., Halbert, M. M., Williams, L. E. and Blume, P. (1974), *Amer. J. clin. Path.* 62, 591.

This paper describes experiments using a T3 coliphage and a radio isotope. The experiments were designed to simulate viral rather than bacterial contamination. Several common items of laboratory equipment were used and simulations of actual use were made, i.e., opening centrifuges while running down, etc. Surprisingly the amount of aerosol generation from centrifuges, Vortex mixers and when opening containers was found to be considerably less than that described by other authors working with bacterial indicators of contamination. Surface contamination of the interior of centrifuges etc. was however considerable. The authors discuss fully the implications of their findings. —D.G.B.

Identifying Yeasts. English, M. P. (1974), *Med. Lab. Technol.* 31, 327.

Two useful keys for the identification of yeasts by simplified morphology, carbohydrate assimilation and fermentation tests are presented. These keys may be useful to laboratories which have not yet commenced the identification of these organisms. —D.G.B.

***Haemophilus parainfluenzae* endocarditis.** Dahlgren, J., Tally, F. P., Brothers, G. and Ruskin, J. (1974), *Amer. J. clin. Path.* 62, 607.

This interesting paper describes three patients with *Haemophilus parainfluenzae* endocarditis who were seen in one hospital in a six-month period. Finding such a remarkable number of cases in such a short time suggests that the organism may be more prevalent than is thought. The patients did not present with the normal symptoms of endocarditis, showing instead fairly normal laboratory and physical findings with the exception of a severe fever. Interestingly, two of the three cases had had their teeth scaled shortly before developing the condition.

The authors stress the importance of using a culture medium which will grow fastidious organisms and of subculture on to chocolate agar. —D.G.B.

Kurthia, An Unusual Isolate. Faoagali, J. L. (1974), *Amer. J. clin. Path.* 62, 604.

This paper describes the isolation in an isolate in New Zealand of a Kurthia species from an eye swab. The organism appears to have had no pathological role. —D.G.B.

Evaluation of the Bag CO_2 Generating Tablet Method for Isolation of *Neisseria gonorrhoeae*. Holston, J. L., Houstie, T. S. and Martin, J. E. (1974), *Amer. J. clin. Path.* 62, 558.

The authors compared the results by the use of candle jars to transmit the Thayer-Martin plates from venereal disease clinics to central laboratories and a technique of enclosing the plates in plastic bags and using carbon dioxide generating tablets supplied by the Ames Company. Several thousand specimens were examined from both sexes.

The authors conclude that the plastic bag CO_2

tablet method is as effective as the candle jar method and of course the plastic bags are much more easily transmitted by post. Presumably Ames will be marketing their CO_2 tablets in New Zealand in due course and when this occurs the method may be of value to those laboratories dealing with mailed-in VD specimens. —D.G.B.

Pristinamycin: In vitro Activity Against *Stapylococcus aureus* and Evaluation of Proposals for Interpreting Diffusion Susceptibility Data According to Kirby Bauer Technique. Marwan Uwaybah. (1974), *Amer. J. clin. Path.* **62,** 553.

This paper describes the in vitro activity of a new antibiotic, pristinamycin against *Staphylococcus aureus* and establishes zone sizes for the evaluation of susceptibility by the Kirby-Bauer technique. Pristinamycin is effective against gram positive organisms and has been used in France for the past ten years. —D.G.B.

Leprosy. Kirchheimer, W. F. (1974), *Amer. J. Med. Technol.* **40,** 474.

A useful short review of current knowledge of this disease is presented. —D.G.B.

A New *E. coli* 'O' Group 0158 Associated with an Outbreak of Infantile Enteritis. Rowe, E., Gross., R. J., Lindop, R. and Baird, R. B. (1974). *J. clin. Path.* **27,** 832.

An outbreak of acute diarrhoea in a neonatal unit is described. This outbreak was found to be due to an *E. coli* with the flagellar antigen 'H' 23 and with a new 'O' antigen (initially unidentifiable) which has been accepted into the International Scheme as 'O' 158. —D.G.B.

Surface Viable Counts with Nichrome Wire Loops. Omar Khairat. (1974), *J. clin. Path.* **27,** 834.

A method of performing surface viable counts using nichrome wire loops with an external diameter of 5mm is described. The author claims that this method gives results which compare favourably with the Miles and Mizra dropping method. —D.G.B.

The Isolation and Identification of *Vibrio cholerae.* Furniss, A. L. and Donovan, T. J. (1974), *J. clin. Path.* **27,** 764.

This technical method describes an adequate method for the isolation and identification of *Vibrio cholerae.* It does not differ substantially from that recommended by the National Health Institute for use in this country. —D.G.B.

Blood Culture Medium. Coetzee, E. F. C. and Johnston, R. S. A. (1974), *Med. Lab. Technol.* **31,** 299.

The authors describe a modification to the biphasic blood culture medium which is fairly widely used. This modification is the incorporation of 2-3-5-triphenyl tetrazolium chloride (TTC) at a concentration of 0.05 percent, the TTC acting as an indicator of microbial growth. There have been reports of microbial inhibition by TTC. The authors claim and present some evidence to support their claim that in the case of this medium there is no apparent inhibition. A disadvantage of the medium and one which may severely limit its use in circumstances where blood culture bottles are issued to wards is their necessity for continuous storage in the dark if the TTC is not to be reduced. —D.G.B.

Isolation of Aerobic Sporing Bacilli from the Tips of Indwelling Intravascular Catheters. Freeman, R. and King, B. (1975), *J. clin. Path.* **28,** 146.

The authors conclude that aerobic sporing bacilli from intravenous catheters and infusion sets can be regarded as contaminants in early and very few instances are the present day pathogenic role. —D.G.B.

Strains of *Pseudomonas putrefaciens* from Clinical Material. Holmes, B., Lapage, S. P. and Malnick, H. (1975), *J. clin. Path.* **28,** 149.

This paper describes eight strains of *Pseudomonas putrefaciens* including two isolated from patients with otitis media and otitis external in which the organism was thought to play a pathogenic role. The biochemical characteristics and identifying features of this strain are well described in this paper.

It may be of interest to note that in this abstractor's own laboratory *Ps. putrefaciens* has recently been isolated from a blood culture and appeared to be responsible for a febrile illness in an elderly debilitated man. —D.G.B.

Book Reviews

Biochemistry. A Case Orientated Approach. Rex Montgomery, Ph.D., D.Sc., Robert L. Dryer, Ph.D., Thomas W. Conway, Ph.D., and Arthur A. Spector, M.D. Published by C. V. Moseby Company, Saint Louis, 1974. Price $NZ10.95. 637 pages with numerous illustrations. Supplied by N. M. Peryer Ltd., Christchurch.

Textbooks unless adding new material, new concepts or a new treatment of the subject matter, require some justification. The authors of this book have tried to introduce a new dimension into the teaching of elementary biochemistry by referring to abnormal biochemical processes. My assumption was that these references would be an integral part of the text but in fact the examples along with additional questions and problems are collected at the end of each chapter. They could be read separately and undoubtedly make interesting reading. A wide range of chemical pathology is covered in these illustrative cases some of the examples being rather esoteric. Acanthocytosis, 5 methyl tetrahydrofolate methyl transferase deficiency, arotic aciduria and tetracycline induced hypoplasia of the teeth, to mention a few.

Apart from this novel approach the basic material is of necessity presented in a conventional manner. The specific aim of the book is to present an introduction to biochemistry for health science students. There are many other books which have the same aim and I compared this one at random with two similar texts namely Mazur and Harrow's Biochemistry and Conn and Stumpf's Outline of Biochemistry. There was a great degree of similarity in the treatment and illustrations as one would expect. One unusual feature in the book being reviewed is that it opens with a chapter on nutrition. The authors believed that this was a good topic of general interest to awaken enthusiasm. I felt that this might be dampened by the need to wrestle with the constitution of the vitamins at the end of the chapter. Most books start logically with the cell organisation which does not rate a chapter in this book. This is a minor criticism and in general the subjects are thoroughly treated and well illustrated.

While the preface states that the intent is not to describe clinical practice and management I think the case material might prove of more than passing interest to the clinical biochemical or chemical pathologist. I suspect that most students would find the basic text sufficient to occupy their time. (The total text is presented in a 16-week period at the rate of one chapter a week at the University of Iowa.)

This paper covered edition seems reasonably priced for its size and quality. R.D.A.

Intrinsic Mutagenesis. A Genetic Approach to Ageing. Sir McFarlane Burnet. 1974. Published by the Medical and Technical Publishing Co. Ltd., Lancaster. 244 pages. Supplied by N. M. Peryer, Ltd., Christchurch. Price $NZ13.20.

Two years ago, the author wrote a reflective and discursive book on auto-immunity which he regarded as a manifestation of Darwinian selection. This current work is written in a similar vein and the idea of intrinsic mutagenesis is a logical extension of the same concept. In the author's view, animals in the natural course of their life have a period of growth up to maturity, fulfil a reproductive function then decline into senescence and die in predetermined fashion. The manner of their going is by a progressive acceleration of somatic mutation. This comes about by the accumulation of metabolic errors in the course of cell division with particular emphasis on the DNA enzymes for replication and repair. The concept of a biological clock which inevitably runs down has been remarked on by others and the change in one's appearance suggests slight imperfections in reproduction resulting in a caricature of one's younger self. Experimental evidence has been produced for the finite capacity of cell division. Hayflick showed that for normal non-malignant diploid cells, 50 ∓ 14 generations is the limit. In progeria and Werner's syndrome, both of which are characterised by premature ageing, it is of significance that fibroblast culture show a limit of 11 and six cell generations only in each respective case. The end result of natural random mutation which is essential for adaptation would appear

to be an aberrant cell line. In general all forms of malignant disease become more frequent with age. If you live long enough you will probably die of cancer—almost a natural process! If this view were accepted it would have a radical effect on the direction of cancer research.

Certainly there are many objections to this thesis. Early manifestation of malignancy and the association of viruses with tumour growth have to be considered. In the first case it is suggested that malignant diseases of early childhood such as acute leukaemia may be associated with an immunological deficiency resulting in a faulty surveillance of aberrant cells. Supporting evidence is found in the frequency with which people on immunosuppressive drugs develop lymphoreticular malignancies. The author regards viruses as agents which accelerate the mutagenic process like carcinogens. In the specific case of Burkitt's Lymphoma it is known that the EBV virus is widespread in the population of the region where the disease occurs but so also is malaria. This could cause an increase in somatic mutation increasing the chance of malignant change by viral infection. The last part of the book consists of three chapters on age-associated conditions under the headings of Human Congenital Anomalies, Normal and Pathological Ageing in the Post-mitotic Cells of the Brain, and Social Implications of the Biological Approach to Ageing.

It is a pleasant change to read a book on a large topic of general interest. The author argues his case very persuasively and I believe Intrinsic Mutagenesis, A Genetic Approach to Ageing, can be read for both profit and pleasure. R.D.A.

Practical Human Cytogenetics, Angela I. Taylor. Baillière Tindall. 174 pages. Price £UK3.00.

This little book, another laboratory monograph, continues in the vein of its predecessors by presenting a block of information in an accessible and readable way that larger texts cannot achieve.

Dr Taylor has acknowledged teaching experience and her approach to the book makes this evident. The introduction and techniques are kept comparatively short in favour of a surprisingly broad coverage of clinical conditions arising from chromosome abnormality. Some knowledge of cytogenetics is assumed and the material presented is of sufficient depth to be challenging but not in such detail as to detract from its readability.

Care has been taken to view each group of abnormalities from the phenotypic appearance and the frequency of these findings is given for each syndrome or condition. For many, a specimen case report is given. Good use is made of photographs and figures and diagrams have been kept simple. A tight list of major references is given in the appendix.

The value of banding techniques is introduced but in a text of this size it is wisely kept short.

The final few chapters remind the reader that chromosomal abnormalities do not always fall into clear-cut syndromes but that a variety of complexities continue to be found. Antenatal chromosome studies are briefly introduced as is the value of population studies.

A very useful book, ideally suited to the general practitioner and, in particular, to the cytogenetic technician who may be inclined to become so involved in techniques as to forget the patient. H.E.H.

Protein Nutrition. Henry Brown, A.B., M.D., 1974. Published by Charles C. Thomas, Springfield, Illinois. Price $NZ16.50. 240 pages.

This book has taken a long time to appear and must be considered outdated to some extent as it is derived from a conference held under the auspices of the Harvard Medical School in 1971 and there is little evidence of updating the reference sections.

There are eight essays by different authors on an alleged theme of protein nutrition, directed primarily at the physician interested in the subject. However, much of the material does not fall into that category. The first two chapters, for example, which comprise one-third of the total text, consist of a detailed discussion of the theory and practice of *in vitro peptide synthesis.* Chapter 1 deals at great length with peptide synthesis in solution and solid phase with detailed methodology even to including line drawings of manual and automated apparatus. The text is liberally decorated with formulae of peptides and reagents, many of which are unnecessary and space wasting. Most of Chapter 2 is also taken up with detailed methodology of the preparation and

separation of the α and β chains of rat haemoglobin and the subsequent solid phase synthesis of a separated decapeptide. It is difficult to relate this data directly, if at all, to clinical aspects of protein nutrition. Another lengthy chapter considers the contribution of intestinal microflora of the rat to the basal metabolism of the host, their role in choline metabolism, and their beneficial and harmful effects in amino acid and protein utilisation. In contrast to the space devoted to the above topics, the important problem of protein metabolism in liver disease is superficially dealt with in six pages! Other topics of more direct interest to the clinician include dietary requirements of amino acids in man and their homeostatic control in blood plasma, the role of insulin in regulation of amino acid nitrogen metabolism, protein metabolism in trauma and sepsis and its relationship to carbohydrate and fat metabolism, and a description of parenteral protein nutrition including intravenous hyperalimentation. All these topics are covered in a somewhat simplistic way which allows for easy reading but makes them far from comprehensive. They do, however, provide some good source material.

The presentation of the book is luxurious. The text is easy to read, but the figures are not up to the same standard; they are inconsistent in style with some being excessively large and rather crudely drawn.

While there is much interesting reading here, the book does not fully live up to its title or aims due to the imbalance of its content and, therefore, detracts somewhat from its overall usefulness, particularly when considering its cost. C.W.

Disseminated, Intravascular Coagulation in Man. John D. Minna, Stanley J. Robbay, Robert W. Colman. Published by Charles C. Thomas, Springfield, Illinois, U.S.A. 1974. 200 pages. Price $US18.50.

The authors of this slim volume of 177 text pages set out to collect and analyse data from patients with the syndrome of Disseminated Intravascular Coagulation (DIC) and to present this information in a form useful to both clinical and laboratory staff. Their presentation is based on a prospective study of patients who showed clinical features suggestive of DIC (91 patients of whom 45 were considered to have DIC following laboratory study), together with a smaller group of 15 patients collected retrospectively.

A major problem with DIC is that it involves the complex and incompletely understood coagulation system, and the pathological processes in DIC are somewhat variable depending on the aetiology. This book deals in a straightforward manner with the pathophysiology of the coagulation, fibrinolytic and kinin systems, which are inter-related in DIC. The laboratory diagnosis is also dealt with clearly and the authors commendably limited their studies to procedures which could be reasonably performed in most hospital laboratories. This is essentially a practical discussion and while assuming a basic knowledge of coagulation tests, covers the tests useful in screening for and confirming the presence of DIC. In particular they provide a good general discussion of the different tests available for FDP's with reference to the sensitivity of detection of early and late degradation products of fibrin and fibrinogen. On the basis of their studies criteria for the diagnosis of DIC are delineated based on the prothrombin time, platelet count and fibrinogen level. Unfortunately these are of rather more limited value than might at first appear as an additional and important component, which cannot be measured, is the change in these values shown by the patient during the onset of DIC. It is at this point that the extra component of clinical judgment becomes important in many patients with DIC and this is certainly implied by the authors.

This book deals extensively with the clinical aspects of the DIC syndrome largely by means of presenting case histories to exemplify points categorised in the text. This is probably one of the book's more valuable points. Any individual clinician or laboratory worker will see few florid cases of this syndrome and the extensive presentation of case histories with the pertinent laboratory findings from this series of patients is of considerable value.

Some aspects of this book are not handled quite as well. Much of the accumulated information from the study is presented in the form of tables and these are sometimes difficult to follow and in one case ambiguous. Also, although this is a relatively large series of

patients in total numbers, DIC is a heterogeneous syndrome where different mechanisms appear to operate with different aetiologies. The attempt to draw conclusions about the results of therapy with heparin go further than the data permit, when the individual patients in the heparin-treated and untreated groups are considered. This aspect obviously has more relevance to those involved in treating patients with a significant degree of DIC and in order to clearly understand what the author's results are based upon requires a laborious study of the details of the individual cases presented. This is an important point and unfortunately detracts from a volume which is otherwise a very useful contribution to a difficult clinical problem. J.M.F.

Correspondence

Sir,—Further to the communication by M. W. Dixon on the Westergren E.S.R. using Dispettes which appeared in Volume 29, No. 1, of our Journal.

Perhaps the following information may help not only solve the problem of verticality in the simplest and cheapest possible manner, but also overcome the objections to, and expense of, Oxford pipettes and specially constructed racks. The system should pose no problems for your staff nor create any difficulty with technical accuracy and reproducibility. It is realised that in the environment quoted in M. W. Dixon's note, volume of blood available could well be an important criterion. However, we too cater for babes and newborns.

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I. C. KING, ANZIMLT, AAIMT,
Queensland Medical Laboratory,
Ipswich, Queensland, 4305,
Australia.

May, 1975.

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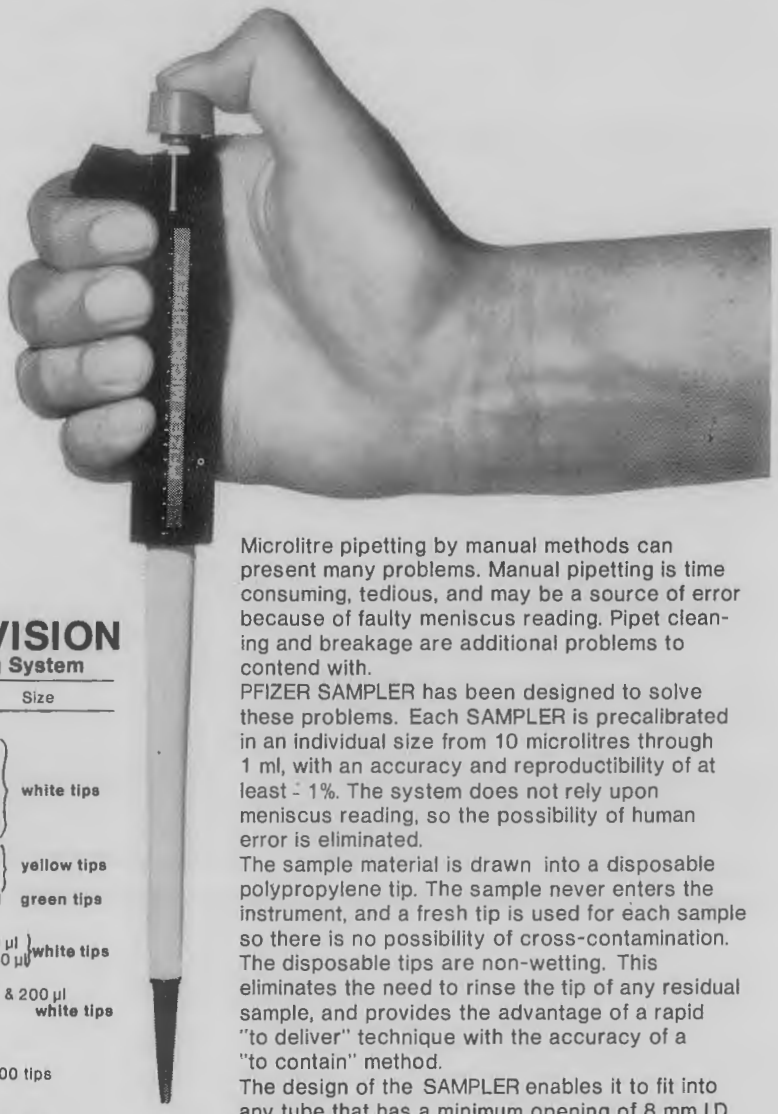

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