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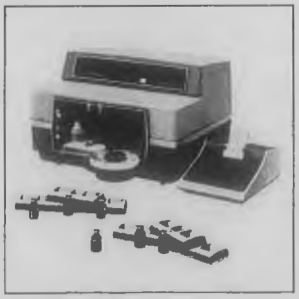

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A Computerised Laboratory Antibiotic Sensitivity System

H. W. Taylor, B.Sc., ANZIMLT, and G. R. Rose, ANZIMLT
Pathology Services and Microbiology Department, Christchurch Hospital.

Received for publication, October 1977

Summary

A computer system for the analysis of data from the sensitivity to antibiotics of micro-organism isolates is described. This system features an on-line data entry and checking section, immediately alerting laboratory staff to unusual sensitivity patterns, and the batch summary information section.

Introduction

Data on antibiotic sensitivity tests performed on micro-organism isolates at the Microbiology Department at Christchurch Hospital have been analysed by computer since June 1974 under the Computerised Laboratory Antibiotic Sensitivity System (CLASS).

This system was originally designed at Christchurch Hospital, to produce summary printouts at certain intervals, initially six monthly. The purpose of these printouts includes:

(a) Provision of data showing the numbers and types of micro-organisms in the hospital over a given period.

(b) Alerting both clinicians and laboratory staff as to the prevalence or emergence of antibiotic resistant strains of micro-organisms.

(c) Acting as a check on the quality of laboratory reports

(d) Providing baseline antibiotic sensitivity patterns to help prescribing policies to be determined in the hospital. Recently the system has been expanded to include an on-line data entry section and is being converted to the Department of Health computer. A function of the new section is to provide an additional check on unusual sensitivity patterns before reports are issued.

Laboratory Method

The disc diffusion technique described by Stokes (1) is used for antibiotic testing on patient specimens from Christchurch and Christchurch Women's Hospital. The laboratory staff have a protocol determining which antibiotics to test depending on the particular micro-organism, the site of the patient from where the specimen was obtained, or the unit within the hospital organisation where the patient occupied a bed. Data recorded on the result form includes where applicable, the organism name, the bacterial count, the antibiotics tested, and the sensitivity result. Antibiotic sensitivities are carried out on known pathogens of significant numbers (10^8—10^{10}/1 for sputum, 10—100 x 10^6/1 for urines) or any growth from sites which would normally be sterile. On occasions sensitivities are performed on each component of heavy growths of mixed organisms. These results will appear in the data processing as either mixed organisms or as the individual organisms that are isolated and identified. The antibiotic sensitivity results are coded as 'R' for resistant, 'S' for sensitive or 'D' for doubtful.

Data Entry

Results on all organisms having antibiotic sensitivity tests carried out are entered into the computer through a visual display unit. The entry system is designed to be used by laboratory personnel as they complete their readings of batches of sensitivity patterns. From each result form the culture site, organism name and sensitivity pattern is entered. The site is entered as a two character code and the organism as a four character code. The sensitivity pattern is a combination of 'R' for resistant or doubtful, 'S' for sensitive or ' ' (blank) for not done. The pattern of antibiotics is determined by the particular culture site, and corresponds to the order on the result form. The system checks each line of data for allowable site code, allowable organism code, and allowable characters in the sensitivity pattern and advises the operator of errors immediately. Each line is echoed on a screen and accepted by the operator if he or she is happy with it. The computer immediately advises the opera-

SENSITIVITY PATTERN FOR ESPE IN UT

FREQ	COTR	SFUR	NFUR	NALI	AMPI	PENI	METH	CARB	GENT	TOBR	AMIK	NEOM	COLI
1	R		R	S	R				S				
1	R	R	R	S	R			R	S	S	S		
1	R	R	R	S	R			R	S	S	S		S
2	S		R	S	R				S				
1	S	R	R	S	S				S				
1	S	R	S	S	R				S				
1	S	S			R				S				
7	S	S	R	S	R				S				
1	S	S	R	S	S				S				
1	S	S	S	S	R				S				
1	S	S	S	S	S								
6	S	S	S	S	S				S				
% TOTAL	87	70	37	95	37			0	95	8	8		4
% DONE	87	80	39	100	37			0	100	100	100		100

Figure 1. The sensitivity patterns for *Enterobacter* species (ESPE) in urinary tract specimens (UT).

SUMMARY OF SENSITIVITY PATTERNS IN UT

	FREQ	COTR	SFUR	NFUR	NALI	AMPI	PENI	METH	CARB	GENT	TOBR	AMIK	NEOM	COLI
ACIN	25	66	61	8	87	58			50	91	85	83		75
CITR	23	56	42	54	86	26			50	94	100	100		100
EAGG	1	100	100	100	100	100				100				
ECLO	1	100	100	100	100	100				100				
ECOL	1075	95	80	97	99	77			30	97	95	94	100	90
ESPE	24	87	80	39	100	37			0	100	100	100		100
KPNE	1	0	0	0	0	0			0	100				100
KSPE	202	65	55	52	74	4			5	94	96	100	100	84
PAER	58	0			0	0			68	85	85	94	100	86
PMTO	1	100	100		100	100					100			
PRMI	240	92	75	7	95	91	100		25	97	100	86	100	50
PRMO	11	90	70	37	100	36				100				
PROA	7	14	14	0	33	28			33	71	33	100		0
PROB	6	0	0	0	33	16			25	40	40	100		0
PRRE	2	50	0	0	100	100				100				
PRVU	22	81	63	0	95	4	0	0	60	100	100	100	0	0
PSEU	11								60	100	100	100	100	100
SAUR	85	71	54	100		0	24	98	100	92	83	33	100	100
SEPI	207	72	45	97	25	75	26	77	66	86	88	75	66	100
SERR	1	100	100	100	100	0				100				
STRR	1	0	0	100	0	100				0				
STRD	148	64	2	97	2	97	50		0	9	14	100	0	0

Figure 2. The summary print out of sensitivity patterns in urinary tract specimens (UT) showing the percent sensitive to each antibiotic.

tor if an unusual or alert pattern is detected. These are organisms which differ from their expected antibiotic sensitivity.

At the conclusion of data entry the operator may call for a display or printout of all the data entered. Alternatively, or additionally a display or printout of all the alert data may be obtained.

Alert Data

When an alert pattern is detected, the patient specimen may be re-examined to enable the operator to confirm the identity of the organism, and recheck the sensitivity test. M.I.C.'s (Minimum Inhibitory Concentration) tests to a particular antibiotic may be activated.

The Batch System

At suitable time intervals, currently six monthly, the batch section of the system is run. The batch stream consists of a suite of programmes to perform a number of functions including the production of period reports, file maintenance, back up, and summarising the data for later investigation. Seven output reports are produced.

Report One

The first report gives the number of occurrences of each observed sensitivity pattern for each site, for each micro-organism (Figure 1).

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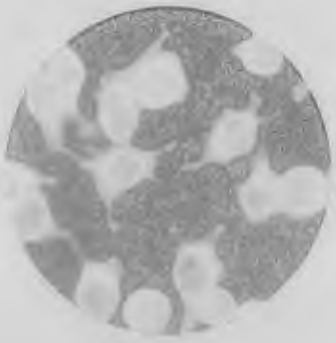

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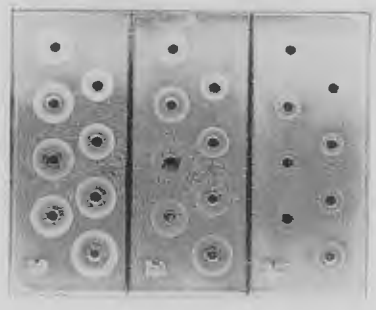

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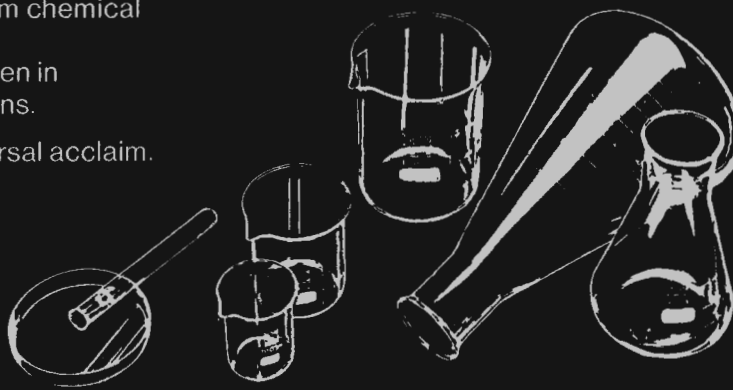

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Summary data are also given. The "% total" is the percentage of sensitive observations for the particular antibiotic to the total number of specimens, where the named micro-organism has been isolated. The "% done" is the percentage of sensitive observations, to the number of sensitive and resistant observations for the particular antibiotic. For example, if we had one hundred isolates of a particular micro-organism from a particular site, and of these fifty were sensitive for a given antibiotic, twenty-five were resistant, and twenty-five were not tested for that antibiotic, the "% total" is fifty percent and the "% done" is sixty-seven percent. The total number of isolates of that micro-organism is also given. This report is primarily used by the laboratory administration. It is used for validating data on the percentage of sensitive organisms to a particular antibiotic and it facilitates such things as the checking that the organism has been tested against appropriate antibiotics.

Report Two

The second report (Figure 2) is a summary for each site giving the observed sensitivity to each antibiotic for each type of micro-organism. The frequency of occurrences of each micro-organism isolate is also given. This report forms the basis of information released to all clinicians showing the patterns of antibiotic resistance and sensitivity in the hospitals. It is hoped that this may assist in the rational selection of an appropriate antibiotic therapy for patients.

Reports Three to Five

The third report is a table of the sensitivity of each antibiotic for each site irrespective of organism. The fourth is a table of the sensitivity of each antibiotic to each organism irrespective of site. The fifth report is a summary of the number of each type of micro-organism identified from each site.

Reports Six-Seven

The final two reports are used for summarising changes in sensitivity over two time periods. Usually the current period is compared with the immediately previous period. Tables are produced showing the number of tests, and the sensitivity of each micro-organism to each antibiotic for each site, and the frequency of each micro-organism. These are summarised for each period and differences are also given.

Sensitivity Summaries to Clinicians

After the period reports are obtained a report is issued to all clinicians. This contains a summary of the relevant data on the sensitivity to each antibiotic to each micro-organism, with a covering letter from the microbiologist outlining any relevant observations such as the increasing resistance of certain organisms to particular antibiotics or the increasing frequency of particular types of micro-organisms.

System Flexibility

The system allows for two hundred different micro-organisms, fifteen sites, thirty antibiotics, and up to fifteen different antibiotics for any one site. Currently there are one hundred and forty different micro-organisms coded in the system and we are collecting data on twelve sites, twenty-two antibiotics and a maximum of thirteen different antibiotics for any given site.

Initial Experience with the System

Initial experience showed that it took untrained operators some twenty seconds to enter and check each record. A six month period in Christchurch involves some 4,300 records of sensitivity data, takes 23 hours for data entry, and uses 10 minutes of computer processing time.

Problems arose during the testing of the online section of the system with the unreliability of the computer and lines. During 11 hours of data entry, data were lost due to computer problems on at least four occasions and due to line problems on at least three occasions. The entry programme was modified to reduce data loss when these problems occur.

Expansion of the System

It was planned at one stage to expand the system to include information from the ward and consultant on each case, and to capture considerably more information on venereal disease, cerebrospinal fluid, and blood culture results. This would best be done by expanding the system to include the computer production of laboratory report forms, while capturing the data required for this system. It was decided to defer this until it became clear what facilities the Nationwide Clinical Laboratory Computer System may contain to obtain this type of information.

Acknowledgments

The authors acknowledge the help of the staff of the Microbiology Department at Christchurch Hospital and Dr J. L. Faoagali for her advice.

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Candida parapsilosis Endocarditis: A Case Report

Jane Fitchett

Microbiology Division, Department of Laboratory Services

Wellington Public Hospital, Wellington

From a paper read to the NZIMLT Conference, Queenstown, 1977

Summary

The importance of fully identifying isolates of yeasts causing infection and testing their antibiotic sensitivity is stressed. This case of *Candida parapsilosis* endocarditis was treated with a combination of amphotericin B and 5-fluorocytosine without removal of the infected valve.

The patient remains well and is expected to continue on 5-fluorocytosine at a dosage of 1 gram 6 hourly for life.

Introduction

Candida parapsilosis endocarditis is a severe and often fatal infection. The case presented was successfully treated using a combination of 5-fluorocytosine and amphotericin B. The laboratory methods used to isolate, identify and test the antibiotic sensitivity of the yeast are outlined.

Case History

In 1967 a 51-year-old male had an aortic homograft valve replacement in Green Lane Hospital, Auckland, for a congenitally abnormal aortic valve. He recovered fully after the operation and was a very fit and well man until February 1977. From then on to April 1977 he began to feel unwell with nothing specific in the way of symptoms. He then developed an infected finger not related to trauma. His doctor treated him with co-trimoxazole for a presumed pulp infection. The infection did not improve and three weeks later on April 28 he was seen at the Wellington Hospital E & A Department for his infected finger. The wound was drained, a swab of the wound taken and cultured and he was started on cloxacillin. He returned home but after dizzy spells, cold sweats, loss of appetite and weight loss he was admitted to Wellington Hospital on May 4 with suspected bacterial endocarditis. The finger wound swab by this time had grown a pure growth of a *C. parapsilosis*. Cloxacillin and Co-trimoxazole were then stopped. Blood cultures were taken on admission.

On May 5 his third toe on the right foot and his left great toe developed purpuric pulp lesions and he had a very painful right calf. Blood cultures taken on May 4 grew *C. parapsilosis* as did 4 sets taken on May 5 and 3 sets taken on May 6. Growth occurred in all 3 blood culture media — brain heart infusion broth, trypticase soy broth with 5% glucose and thioglycollate broth after 3-5 days.

At this stage the candida precipitin titre of the patient's serum was 1:16.

On May 7 treatment began using 5-fluorocytosine orally and amphotericin B intravenously.

5-fluorocytosine was started at a dosage of 100mg/kg/day — a total of 8g, 2g given 6 hourly. The amphotericin B dosage was 1mg/kg/day — a total of 80mg/day which was given over 6 hours.

On May 11 he was sent back to Green Lane Hospital, Auckland, where his aortic valve replacement had been done.

Blood cultures taken on May 12 were still growing *C. parapsilosis*.

On May 17 due to renal impairment the 5-fluorocytosine dosage was reduced to 1.5g 6 hourly and the amphotericin B was reduced to 40mg/day. The amphotericin B was stopped on May 18 and 5-fluorocytosine further re-

duced to 1g 6 hourly. Amphotericin B was recommenced on May 25 at a dosage of 40mg/day and then reduced to 20mg/day on May 26.

On May 27 the candida precipitin titre still remained at 1:16 but on June 8 and June 10 the levels dropped to 1:8.

The treatment remained unchanged until June 14 when amphotericin B was increased to 30mg/day.

Amphotericin B treatment was stopped on June 28. 5-fluorocystosine is to be continued at a dosage of 1g 6 hourly.

The patient was well and blood cultures were sterile on discharge from Green Lane Hospital on July 18. Removal of the valve was not thought necessary as antibiotic treatment appeared successful.

Identification of the Isolates of Candida Species

The following tests were used to identify the wound swab and the blood culture isolates:

1. Germ tube test — 2 colonies were inoculated into 0.5ml of pooled human sera. The test was incubated at 37°C for 3 h.
2. Chlamydospore production — the yeast was inoculated onto rice extract tween 80 agar which was examined after incubation at 30°C for 48 h.
3. Uni-Yeast-Tek (Corning medical diagnostic New York) — suspension of the yeast was inoculated into wells on the plate according to instructions provided. The plate was incubated at 30°C for 6 days.

The isolates did not produce germ tubes or chlamydospores, Morphology on rice extract tween 80 agar showed pseudohyphae and blastospores. Arthrospores were absent. The Uni-Yeast-Tek plates showed assimilation of sucrose, maltose and trehalose. The following tests were negative — hydrolysis of urea, assimilation of lactose, raffinose, cellobiose, starch and nitrate.

Antibiotic Sensitivity Testing

The sensitivity of *C. parapsilosis* is totally unpredictable and each isolate must be tested for its level of sensitivity. Minimum inhibitory concentrations (MICs) were done on the wound swab isolate and the blood culture isolate to 5-fluorocytosine and amphotericin B. A sensitive *Candida albicans* isolate was used for the control.

Materials and Methods

5-fluorocytosine is inhibited significantly in the presence of partially degraded biological substances such as commercial peptones, yeast extracts and protein digests (Shadomy 1969)[3]. Therefore the basic medium used was Yeast Nitrogen broth (Difco) supplemented with L. asparagine and dextrose as recommended by Shadomy (1969)[3].

Preparation of standard inoculum: The yeast to be tested and the control *C. albicans* were grown in yeast nitrogen broth (YNB) at 37°C for $3\frac{1}{2}$ hours. The broths were then diluted to a concentration of 1×10^3 yeast cells/cmm with further YNB (calculated using a Neubauer counting chamber).

5-fluorocytosine MIC: 5-fluorocytosine was diluted in YNB in doubling dilutions to cover the range 0.04μg/ml to 10.0μg/ml. One drop of above standard inoculum was added to each dilution and to a growth control tube. The test was incubated at 37°C for 48 h. The MIC was read as the lowest concentration showing reduction of growth. (Results shown in Table I).

Amphotericin B MIC: Amphotericin B was added to drug free Sabouraud dextrose agar in doubling dilutions to cover the range 0.02 μg/ml to 5.0μg/ml. One drop of standard inoculum was spread over each plate and over a growth control plate. The plates were incubated at 37°C for 48 hours. The MIC was read as the lowest concentration showing reduction of growth. (Results shown in Table I).

Table 1

	5-fluorocytosine MIC	Amphotericin B MIC
Test strain	0.08μg/ml	0.6μg/ml
C. albicans Control	0.08μg/ml	.16μg/ml

An MIC of 6.25μg/ml or less is regarded as highly sensitive to 5-fluorocytosine and for amphotericin B a MIC of 1.0 or less is regarded as highly sensitive (Kucers et al, 1975)[1].

Test For Synergism Of Amphotericin B And 5-Fluorocytosine

A chessboard titration was set up using increasing concentrations of amphotericin B 0.07-5.0μg/ml against increasing concentrations of 5-fluorocytosine 0.01-0.64μg/ml in YNB. 1 drop of standard inoculum of *C. parapsilosis* in YNB was added to each tube. The tubes

were incubated at 37°C for 48 hours and then subcultured onto Sabouraud dextrose agar. Synergism was seen between the 2 antibiotics. (Results shown in Figure 1.) A combination of 2 antibiotics is considered to be synergistic when the effect observed with the combination is greater than the sum of the effects observed with the 2 antibiotics independently.

Figure I

µg/ml 5-fluorocytosine \ µg/ml Amphotericin B	0.07	0.15	0.3	0.6	1.3	2.5	5.0
	+	+	+	+	+	+	+	-
0.01	+	+	+	+	+	-	-	-
0.02	+	+	+	+	+	-	-	-
0.04	+	+	+	+	+	-	-	-
0.08	-	-	-	-	-	-	-	-
0.16	-	-	-	-	-	-	-	-
0.30	-	-	-	-	-	-	-	-
0.6	-	-	-	-	-	-	-	-

+ = growth >20 colonies.

- = no growth or <20 colonies

Test For Fungicidal Level Of Patient's Serum

Predose and postdose sera were diluted in YNB in doubling dilutions 1:2 to 1:128. 1 drop of a standard inoculum of *C. parapsilosis* in YNB was added to each dilution. The tests were incubated at 37°C for 48 h and the tubes were then subcultured onto Sabouraud dextrose agar. An adequate level of antibiotic is considered to be present in the serum if a dilution of 1:4 or greater inhibits growth of the test organism. Results: Both predose and postdose sera inhibited growth up to the highest dilution (1:128).

Discussion

Candida species are generally classified as members of the normal flora but may cause serious disseminated infections. They have become important causes of endocarditis in patients with indwelling intravenous catheters, patients undergoing cardiovascular surgery, drug addicts and patients on immunosuppressive drugs. Candida infection of a prosthetic or homograft valve is an uncommon but dangerous complication of cardiac surgery. Record *et al* (1971)[2] reviewed 3 cases of endocarditis due to *Candida* species infection, of which 2 were fatal. The fatal cases were due to infections on prosthetic valves, and the chemotherapy using 5-fluorocytosine failed to eradicate the infection.

Kucers *et al* (1975)[1] report that naturally occurring strains of *Candida* species resistant to 5-fluorocytosine have been encountered. Resistance is thought to be due to a change in the plasma membrane which prevents penetration of the drug into the cell. Stieritz *et al* (1973)[4] found that development of resistance to amphotericin B does not commonly develop *in vivo* or if it does it requires treatment for more than 13 days.

It is reported by Kucers *et al* (1975)[1] the synergistic effect of 5-fluorocytosine and amphotericin B against some yeasts is thought to result from the action of amphotericin B on the plasma membrane which allows the penetration of greater amounts of 5-fluorocytosine.

The candida precipitin titre represents the highest dilution of serum producing a band after reaction with a cytoplasmic or cell wall antigen purified from a *Candida albicans* strain which has been the cause of systemic candidiasis.

A rising titre of precipitin in serum is believed to be a reliable indicator of the presence of visceral candidiasis but in this case the patient's serum showed candida precipitins were only marginally elevated.

Acknowledgments

I wish to thank Miss Shirley Gainsford, Grade Technologist, for her helpful advice in the preparation of this paper, Doctor M. Humble, Microbiology Registrar, for encouraging the laboratory work involved and Mrs Carol Crawford for typing the manuscript.

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Addendum. One of the referees asked if minimum fungicidal concentrations had been determined. The author indicated that they were

the internationally recognised standard for analytical reagents

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not reported in the paper as it was decided that only minimum inhibitory concentrations were of interest. However, the minimum fungicidal concentrations were determined. The MFC of Amphotericin B for the test strain was greater than 5μg/ml, that is greater than the range covered and the MFC of 5-fluorocytosine was 0.16μg/ml.

An *in vitro* Comparison of the Aminoglycosides Gentamicin, Tobramycin and Amikacin by the Disc Diffusion Method

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Introduction

Over recent years there has been a significant increase in the numbers of strains of bacteria resistant to commonly used antibiotics. Gram negative bacteria have become a major cause of nosocomial infections and in particular *Pseudomonas aeruginosa* with its multiple resistance to the commonly used antibiotics has become increasingly prevalent, especially in the compromised host. Two new Aminoglycosides Tobramycin and Amikacin were introduced to Palmerston North Hospital this year (1977), but have only been used once each in clinical situations. This study was set up to find the selection of strains that has occurred up to now and to compare these results with the newer virtually unused aminoglycosides Tobramycin and Amikacin.

Materials and Methods

Isolates. One hundred consecutive *Pseudomonas aeruginosa* strains were isolated from urine, pus swabs and sputum from hospitalised patients and from specimens referred into the hospital over the period April to November 1977.

Bacteriology. Gram negative bacilli that were motile, catalase positive oxidatively attacked glucose, citrate and urease positive and did not decarboxylate ornithine or lysine were considered *Pseudomonas aeruginosa.* Occasionally further identification according to King (1972)[4] was required.

Sensitivity Testing. Sensitivity or resistance to the aminoglycoside was performed by the CDS method of Bell (1975)[1]. A straight wire inoculum of the bacterial colony was emulsified in 2.5ml of saline to give a concentration of approximately 10^6-10^7 viable cells/ml. Sensitivity Test Agar plates (BBL) were flooded with the test suspension and the excess fluid removed. The plates were allowed to dry at room temperature for 15-45 minutes. 10mcg concentration discs of Gentamicin, Tobramycin and Amikacin were placed on the plates and incubated overnight at 36.5°C. The zones of inhibition were read to the nearest 0.5mm.

Results

The results of the sensitivity tests are shown in Figure 1. Tobramycin gave consistently larger zone sizes than Gentamicin or Amikacin. In general Gentamicin and Amikacin gave similar zones. Tobramycin gave larger zones than Amikacin with all the isolates tested. In only two cases did Gentamicin give larger zones than Tobramycin and in one case the same diameter of inhibition. The average zone diameters were Tobramycin 25mm, Gentamicin 19mm and Amikacin 18.5mm. Taking a zone size of 14mm or more as meaning susceptible for Tobramycin and 13mm or more as meaning susceptible for Gentamicin and Amikacin[2], 11% of *Pseudomonas aeruginosa* strains were resistant to Amikacin, 9% to Gentamicin and 1% to Tobramycin. Moreover, another 10% of strains had zone diameters less than 2mm from the cut-off point, for Amikacin, 8% for Gentamicin and 1% for Tobramycin. Zone sizes for Gentamicin ranged from 9 to 31mm, Tobramycin 11 to 34mm and Amikacin 9 to 31.5mm.

Discussion

This study shows a surprisingly high percentage of strains of *Ps. aeruginosa* resistant to Amikacin. The most likely explanation for this

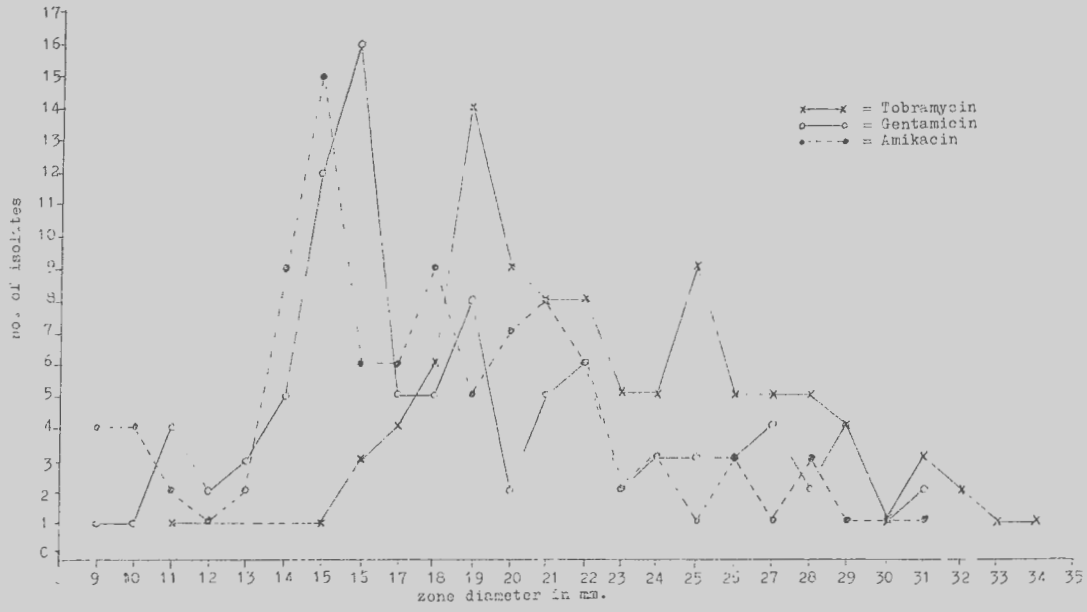

Figure 1. Comparison of Aminoglycoside Sensitivities

is cross resistance between Gentamicin and Amikacin. This lack of cross resistance between Gentamicin and Tobramycin is in agreement with the work of Uwaydah (1975)[7]. The findings in this study are in agreement with other workers who have found *Ps. aeruginosa* more sensitive to Tobramycin than Gentamicin, Britt et al (1972)[3], Lockwood and Lawson (1973)[5], Traub and Raymond (1972)[6], and Waterworth (1972)[8]. Since serum levels for Gentamicin and Tobramycin are similar, Uwaydah *et al* (1975)[7], the use of Tobramycin from an *in vitro* point of view merits serious consideration.

Summary

The disc diffusion method in this study has shown that Tobramycin has greater *in vitro* activity against *Ps. aeruginosa* strains isolated in this hospital. 11% of strains were resistant to Amikacin, 9% to Gentamicin and 1% to Tobramycin. Cross resistance between Gentamicin and Amikacin occurred in most of the resistant strains tested.

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An Enzymatic Test of Placental Function

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Received for publication, October 1977

Introduction

Cystyl aminopeptidase (CAS, E.C., 3.4.1.10) activity was first discovered by Fekete[8] in 1930, when it was observed that an extract from the posterior pituitary, consisting predominantly of oxytocin, lost its oxytocic properties when incubated with plasma from pregnant women. The same preparation incubated with plasma from non-pregnant women did not have this property.

More recently CAS activity has been used as an index of placental function.

Source

CAS has been shown to be secreted into the intervillous spaces by the syncytiotrophoblast, using immunohistochemical techniques.[17] Whilst non-pregnancy plasma has some aminopeptidase activity, this is due to leucine aminopeptidase. No CAS activity has been detected in non-pregnancy plasma. Levels of CAS activity continue to rise after ligation of the umbilical cord indicating that it is placental in origin.

Structure

CAS is a glycoprotein with a molecular weight of 300,000 and has a high content of carbohydrate residues.[21] Two isoenzymes of CAS exist in the plasma and can be separated using polyacrylamide gel electrophoresis. Both isoenzymes differ in pH optimum and substrate affinity.

Function

The role of CAS is poorly defined. It will attack oxytocin, cleaving the amide bond between the N-terminal cystine and adjacent tyrosine, thereby rendering oxytocin inactive.[20] Hence its synonym oxytocinase. However, *in vivo* it does not appear to inactivate circulatory oxytocin. No variation in the levels of CAS activity are found at the onset or during labour. A tissue bound CAS isoenzyme has been described, located in placental tissue; this isoenzyme has been shown to inactivate circulatory oxytocin.[3]

Assay

CAS activity may be assayed using a wide variety of substrates. The substrates commonly used are shown in Table 1. The hydrolysis product β-naphthylamine is a known carcinogen and should not be introduced into hospital laboratories.[4] One further problem is the reactivity with both CAS isoenzymes; of the five commonly used substrates, only S-benzyl-L-cystine-4′-nitroanilide appears to react equally with both isoenzymes.

Substrate	Carcinogen	Cas—1	Cas—2
L—Cystine—2—Naphthylamine	Yes	Yes	Partial
L—Cystine—4—Nitroaniline	No	Yes	Partial
L—Cystine—Di—2′—Naphthylamide	Yes	Yes	Partial
S—Benzyl—Cysteine—2′—Naphthylamide	Yes	Yes	Partial
S—Benzyl—L—Cysteine—4′—Nitroanilide	No	Yes	Yes

Table I: Commonly used cystyl-aminopeptidase substrates, indicating carcinogenicity of the reaction product and the relative reactivity of the isoenzymes with the respective substrates.

There are a number of methods for the assay of CAS in the literature, however, two have been found to work particularly well in the author's laboratory. The reaction rate method of Tovey et al[19] and the end-point method of Durham.[7] When compared against each other a correlation coefficient of 0.962 was obtained. An important point regarding CAS activity is the change in optimum assay pH during the course of pregnancy. Klimek has shown that pH optimum of CAS changes from pH6.0 in the first trimester to pH7.8 in the third.[11] It is thought that this may be due to a change in the ratio of the isoenzymes.

Chard *et al* have developed a method which measures the destruction of ^{131}I labelled oxytocin by measuring its ability to bind to specified oxytocin antibody.[6] It is doubtful whether this particular technique would replace the more conventional and technically easier spectrophotometric techniques.

Clinical Significance

Normal Pregnancy

CAS activity can be first measured at about the fifth week of pregnancy and increases in activity towards term (Figure 1). It is recommended that the CAS activity in an individual should be followed as these tend to follow a trend either above or below the population mean. There is a secondary relationship between CAS activity and placental weight. CAS activity decreases by 50% in 72 hours postpartum but may have a secondary peak.[9]

Prolonged Pregnancy

In pregnancies which go beyond 40 weeks gestation, CAS activity may continue to rise or fall.[12, 16]

Multiple Pregnancies

Levels of CAS activity are usually above the upper limit of normal (Figure 2). This relates to placental mass.

Abnormal Pregnancies

Threatened Abortion

In threatened abortion which is due to placental dysfunction CAS activity falls rapidly (Figure 3). Abortion may occur with normal CAS activity when the placenta is healthy. This reflects the difference between placental function and foetal function testing.

Foetal Death

In cases of foetal death where the cause is not directly related to placental dysfunction, Tovey reports CAS activity remaining normal for up to five weeks after the estimated time of death.[18] If placental dysfunction was the cause of foetal death, then CAS activity falls rapidly prior to foetal death.[1, 18]

Diabetes Mellitus

CAS activity rises more rapidly in patients with diabetes mellitus, this may reflect the rapid increase in placental mass.[5] CAS activity is unaffected by high plasma glucose concentration.[14]

Intrauterine Growth Retardation

When intrauterine growth retardation is secondary to placental insufficiency, serial CAS activity assays are low. Petrucco *et al* concluded that serial CAS activity assays after 28 weeks gestation were superior to serial urinary oestrogens in the prediction of intrauterine growth retardation.[15] Once again if placental mass is low, CAS activity will, in general, be low.

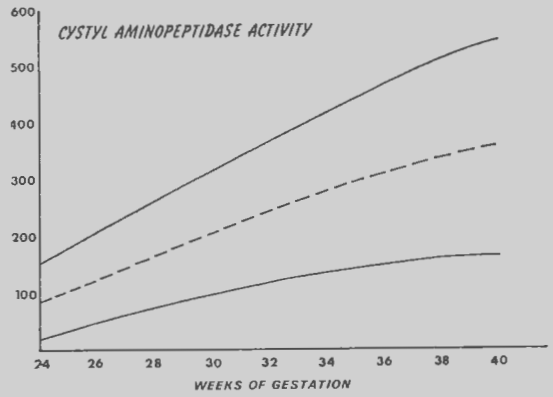

Figure 1: Normal range of cystyl aminopeptidase activity during pregnancy. — — — — — — = mean. ———— = ± 2 standard deviations.

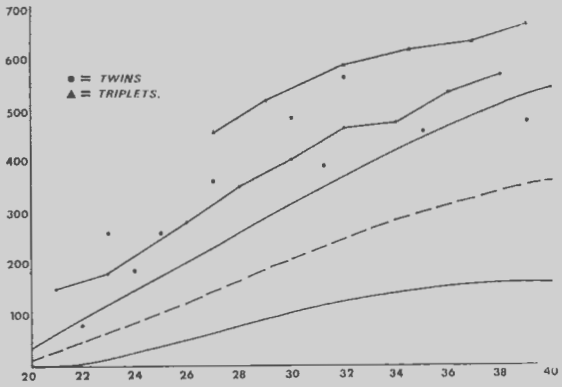

Figure 2: Cystyl aminopeptidase activity in multiple pregnancies in relation to the normal range.

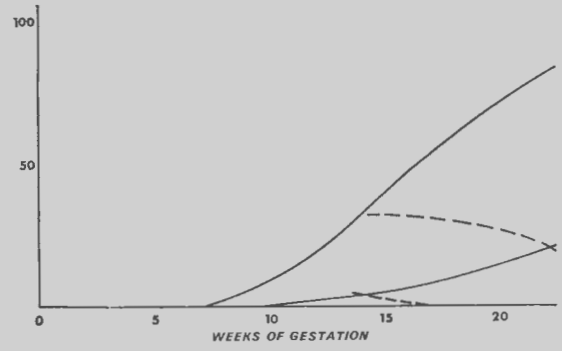

Figure 3: Cystyl aminopeptidase activity in threatened abortion — — — — — — = range in which CAS activity of eventual abortions fell. ———— = normal range ± 2 standard deviations.

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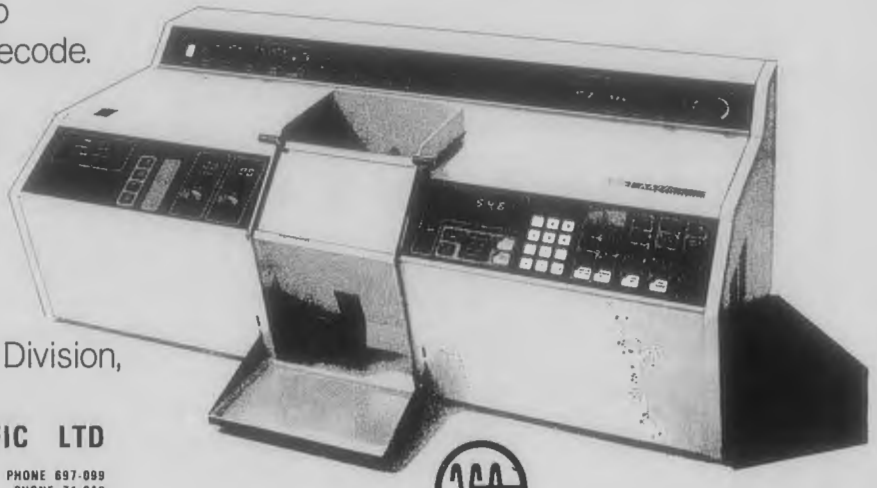

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Hypertension and Toxaemia

In pregnancies complicated by hypertension where the foetus growth is retarded, CAS activity is usually low. In mild toxaemia little change from the normal CAS activity trend is seen. In patients suffering from moderate or severe toxaemia low CAS activity is usually seen, although the differences are not always significant in individual patients.[10] Intrauterine foetal death usually results in patients with toxaemia who have decreasing CAS activity.[2] Tovey, has reported that patients with toxaemia whose CAS activity failed to rise, all had infants with some degree of dysmaturity.[18]

Foetal Abnormalities

In the author's laboratory plasma CAS activity was found to be normal in patients who subsequently delivered infants suffering from spina bifida, anencephaly, Trisomy 17 and 21, Potters syndrome, and prune belly (Triad) syndrome. These findings help to confirm that the foetus is not the origin of CAS during pregnancy.

Comparison With Human Placental Lactogen and Urinary Oestrogens

When compared with human placental lactogen (HPL) a correlation coefficient of 0.758 is obtained. However, there are some differences in the results. HPL results are far more reliable in predicting threatened abortion, foetal distress and neonatal asphyxia.[13]

In the author's laboratory a poor correlation has been found between CAS activity and urinary oestrogen assays in normal pregnancies. This probably reflects that the two methods are measuring quite different parameters. A better correlation between the two assays is found when the urinary oestrogens are sub-normal. Neither test can substitute for each other.

Conclusion

As with HPL the advantages of CAS activity determinations are in the speed by which results can be produced.

CAS activity appears to be reliable in predicting placental dysfunction and also relates to placental mass, thereby giving some indication of foetal growth. In order to obtain an adequate picture with respect to the condition of the foetus and placenta it is imperative that urinary oestrogens complement CAS activity measurements.

The existence of two isoenzymes warrants investigation as one predominates in the first trimester and differs in its reaction kinetics to the other CAS isoenzyme predominating the third trimester.

As with other tests of foetal placental function the trend must be studied using serial assays. Normal CAS activity does not necessarily mean a normal foetus and may give a false sense of security in some at risk patients. It is imperative, therefore, that CAS activity is measured in conjunction with urinary oestrogens.

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Serial Bone Marrow Culture in Ten Patients with Acute Non-lymphoblastic Leukaemia

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Summary

A semi-solid agar culture system has been utilised to study the colony-forming ability of ten patients with acute non-lymphoblastic leukaemia. Marrow aspirates have been cultured serially over the remission-induction period until the death of the patient occurred or a partial or complete remission was achieved.

Seven of the ten patients have been studied from the time of initial diagnosis, one patient from the time of clinical relapse and the remaining two being followed from the first remission-induction course of chemotherapy.

At the initial culture, only one patient showed any ability to produce colonies and in this case the number was reduced. The remaining nine patients either produced a variable number of clusters or showed no cluster or colony growth.

Two patients achieved a stable remission lasting more than seven months, three patients showed improvement of haematologic elements but relapsed within several months and the remainder showed no recovery at all in haematologic parameters.

The repeated observation of granulocyte colony-forming ability appears to distinguish a patient whose remission will be lasting from those patients who show infrequent or no colony-forming ability and do not obtain a stable remission.

Introduction

In normal bone marrow the committed progenitor cells of the granulocyte series and in part the macrophage-monocytic series proliferate in semi-solid agar in the presence of colony stimulating factor (CSF) to form aggregates of cells.[7] Aggregates containing more than 40 cells are called colonies and those aggregates containing 5-40 cells are called clusters. The myeloid progenitor cells which form colonies *in vitro* have been called colony forming units (CFU-C)[5]. The majority of colonies are composed of granulocytes, 90% of the cells being either band forms or mature neutrophils, after seven days incubation. A small number of monocytes are found in these aggregates. The clusters and colonies are of the same cell type; the cluster forming cells being derived from the myeloid progenitor cell compartment.[8]

The most generally accepted explanation for the absence of CFU-C at the time of diagnosis of acute non-lymphoblastic leukaemia is that the leukaemic cells exert an inhibitory effect either directly on the CFU-C population or at a more primitive cell level.[9, 2]

At the time of remission colony formation returns, often to a higher level than normal. At the time of relapse the colony numbers fall to low or zero values, the fall in CFU-C numbers usually preceding clinical relapse by several weeks to a month.

Materials and Methods

The patients in this study are those from whom we were able to obtain serial marrow aspirates from the time of patient admission to either remission or death.

The culture medium used throughout this study is a modified Eagles medium enriched with 20% serum, the contents of the medium being shown in Table 1.

Bone marrow samples were collected into sterile, capped bottles containing 2ml of 6% dextran in saline with 1% sodium heparin (preservative-free, sigma) being added so that the final concentration of heparin plus bone marrow did not exceed 25 u/ml. The aspirates were left to stand at room temperature for the cells to settle. The nucleated cells plus plasma were removed and cultured as previously described, [6, 3] with minor modifications. Briefly, the nucleated marrow cells were cultured in agar—Eagles modified medium at a concentration of 200,000 cells/ml. These overlays are stimulated by previously prepared underlays consisting of agar-Eagles modified medium containing normal peripheral blood leucocytes at

a concentration of 1,000,000 cells/ml. In the preparation of the overlays, 0.6% agar is mixed with equal volumes of Eagles medium to give a final agar concentration of 0.3%. In the preparation of the underlays, 1% agar is mixed with equal volumes of Eagles medium to give a final agar concentration of 0.5%. The cultures are incubated in a water-jacketed National incubator in an atmosphere of 10% CO_2 in air which is fully humidified. The plates are scored at 7 and 12 days using a stereo dissecting microscope at x40 magnification.

Table I

Contents of medium for agar culture	
" Gibco " Dulbeccor Modified Eagles Medium	10g (1PK)
Deionized distilled water	215ml
L-Asparagine 6.6mg/ml (Final conc. 20μg/ml)	3.0ml
Deae Dextran 50μg/ml (Final conc. 75μg/ml)	1.5ml
Sodium bicarbonate 2.8%	175ml
Millipore filter (0.22μ membrane)	
* Horse serum	63ml
Foetal calf serum	187.5ml
Store at 4°C up to one week.	

* Not heat inactivated — (Commonwealth Serum Laboratories)
Foetal Calf Serum - (Laboratory Services, Auckland)

Results

The initial bone marrow data on the 10 patients is presented in Table II. The data on Patient TH was obtained at the time of relapse, SH and OM being at the end of their first block of chemotherapy. The data on the remaining patients was obtained at the time of initial diagnosis.

Eight of the patients had hypercellular marrows, with the majority of cells being blasts (see Table II). With the exception of one patient, colony formation in agar culture was absent, although cluster formation was variable. Patient RL showed colony forming ability although the number of colonies was very much reduced when compared to a normal marrow.

As a control group, aspirates from 38 hospital patients with non-leukaemic marrows were cultured. They demonstrated satisfactory colony forming ability with normal cluster to colony ratios. The results from this control group are shown in Table III, with the range of results from the leukaemic patients included. The

Table II

Pretreatment data of 10 patients with acute non-lymphoblastic leukaemia

Patient	Age/Sex	Initial Marrow Blast %	Marrow Cellularity	Culture Pattern Colonies	Clusters
B.W.	M/42	40	Normocellular	0	578
H.S.	M/47	85	Hypercellular	0	0
R.O.	M/55	90	Hypercellular	0	0
O.M.	M/53	Erythro-leukaemia	Hypercellular	0	0
G.P.	F/56	90	Hypercellular	0	26
M.K.	M/69	95	Hypercellular	0	0
H.B.	M/65	30	Normocellular	0	0
R.L.	M/47	50	Hypercellular	8	99
S H.	M/72	90	Hypercellular	0	0
T.H.	M/27	80	Hypercellular	0	0

Table III

Control Group of 38 Patients and Initial Data on Patients with Acute Non-lymphoblastic Leukaemia

	Colonies	Clusters	Cluster Ratio Colony
Control Group	7 - 254 (Mean 45.4)	32 - 550 (Mean 167.5)	1.1 - 15.6 (Mean 5.0)
Acute non-lymphoblastic leukaemia group	0 - 8	0 - 578	

Table IV

Days to Recovery of Colony Formation and Haemopoietic Elements

Patient	Days to return of colonies	Days to normal blood and marrow elements	Days of Haematologic remission
H.B.	42	50	17
R.O.	58	76	128
B.W.	40	86	117
G.P.	99	58	7 months
O.M.	46	71	7 months
	Mean = 57 days	Mean = 68 days.	

marrow aspirates from the 10 patients were cultured serially throughout the remission induction period, usually at 1-2 week intervals. While receiving intensive chemotherapy, patients HS, MK, RL, SL, and TH showed no return of colonies, no haematological improvement and died without achieving remission.

Data of patients showing a return of colonies in agar culture during the remission induction period is shown in Table IV. The results demonstrate that the time taken for return of colony forming ability following the commencement of chemotherapy is variable and does not

Table V

CULTURE RESULTS OF PATIENTS O.M., H.B. H.S. DURING REMISSION INDUCTION

Patient	Days from commencement of Chemotherapy	Colonies	Clusters	Clusters / Colonies	Marrow Status
O. M	3	0	0		Toxic changes
	13	0	3		Hypocellular, blasts increased
	28	0	0		Hypocellular, blasts increased
	46	116	912	7.8	Hypercellular, blasts 3 %
	60	1	771	771	Normocellular, blasts 5 %
	65	55	520	9.4	Normocellular, blasts 3 %
	91	225	134	0.6	Hypercellular, blasts 5 %
	126	214	36	0.16	Hypercellular, blasts 3 %
H. B.	5	0	0		Hypercellular, blasts increased
	16	0	0		Hypocellular, blasts increased
	29	0	0		Hypocellular
	42	20	420	21	Normocellular, blasts 2 %
	47	0	506		
	55	61	318	5.2	Normocellular, blasts 2 %
	88	0	0		Mildly hypocellular, blasts normal
	136	0	56		Mildly hypocellular, blasts normal
	163	0	80		Mildly hypocellular, blasts normal
H. S.	9	0	0		Hypercellular, blasts increased
	20	0	4		
	27	0	9		Hypercellular, blasts increased
	32	4	6		Normocellular, blasts mildly increased
	43	0	0		Moderately cellular, blasts increased

Aspirates cultured 1 or 2 weeks apart.

appear to be related to the length of remission, although the number of patients studied is small.

Patients HB, RO, BW, in Table IV showed a return of colony formation following chemotherapy and showed haematological recovery of peripheral blood and bone marrow. However, the colony forming cells did not persist for more than 21 days and these patients relapsed within a relatively short space of time. Patients GP and OM in Table IV achieved a stable remission and are still in remission at this point, the length of remission being greater than 6 months. The mean number of days from the commencement of remission-induction therapy to the appearance of colonies in culture was 57 days, for the five patients. The mean number of days to normal marrow and blood elements was 68 days.

Patient OM showed colonies in culture on three consecutive occasions and is presented in Table V. The data on patients OM, HB and HS is presented in detail to demonstrate several different patterns of results which we have obtained. Patient HB is presented as an example of the return of colony formation being intermittent and temporary. Patient HS showed complete absence of colony formation during the remission-induction period with no haematologic remission being achieved.

No direct correlation between the numbers of colonies or clusters in culture and the absolute number of neutrophils or platelets in the peripheral blood was demonstrated. However, in studying the patients individually the recovery to normal of the neutrophil and platelet counts generally lagged behind the recovery of colony formation in culture in varying degrees.

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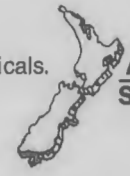

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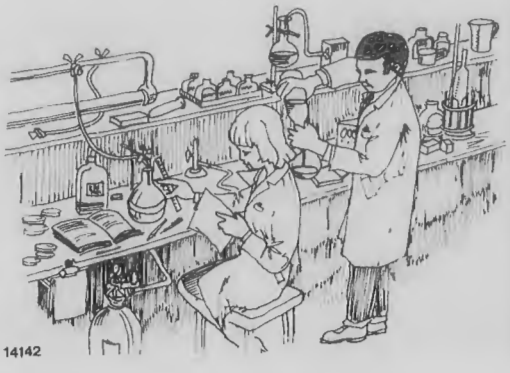

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Discussion

The myeloid progenitor cells which form colonies *in vitro* have been called colony forming units—culture (CFU-C)[8]. In adult acute myeloid leukaemia, abnormalities of CFU-C proliferation frequency and differentiation occur.[4] The leukaemic origin of clusters in culture in marrow of leukaemic patients has been demonstrated by abnormal morphology, buoyant density and cytogenetic analysis.[4]

At the time of diagnosis, colony formation in culture is infrequent but cluster formation is variable, with up to 16,000 clusters per plate being observed in one of our patients (unpublished data), the majority of these clusters containing 15 cells or less.

During the remission induction period, other workers have shown that a demonstration of response to chemotherapy in the form of a return of colony formation in agar may occur at a time when the bone marrow morphology does not necessarily show the persistence of leukaemia or a regeneration of normal elements.[8]

The data presented in this paper shows that in our patients a stable remission was not achieved unless a return of colony forming ability was serially demonstrated during the remission induction In the absence of a return of colony formation with serial culture for a period of up to 8-10 weeks following the commencement of chemotherapy, bone marrow hypoplasia persisted and the patients died. The *in vitro*-culture technique appears to be predictive of final clinical outcome when serial cultures are carried out over a period of the initial 4-10 weeks of remission induction.

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Technical Communications

A Simple 7 Parameter Haematology Quality Control Blood

With the increasing use of automated cell counters in Haematology there is also a need for standardisation and quality control.

There are many commercial "7 parameter" blood standards on the market and while these may be of a good quality with good reproducibility they are also expensive.

We have used the following method for some considerable time as a secondary standard to one of the commercial standards and have found it to be most reliable.

Principle

A stabilised suspension of erythrocytes and white blood cells is made such that when used for either electronic or manual cell counting the product will give reproducible results for *at least* 30 days, and in practice up to 45 days, of the following parameters.

(a) Red blood cells	/10^{12}/litre
(b) White blood cells	/10^{9}/litre
(c) Haemoglobin	/gm/decilitre
(d) Haematocrit packed cell volume	%
(e) Mean corpuscular haemoglobin	/picrograms
(f) Mean cell volume	/femtolitre
(g) Mean corpuscular haemoglobin concentration	/g litre

Preparation

I *WBC Suspension Preparation*

(a) Take 100 ml of Bank blood, not more than 21 days old, and wash 5 times with normal saline to remove proteins.

(b) To the packed cells add normal saline and *mix thoroughly* so that the cells are not clumped or aggregated. Make up the volume to 100 ml.

(c) To 600 ml of 10% neutral formalin[1] add *slowly* the 100 ml of suspended RBCs and allow the whole to mix on a mag-

netic stirrer for 24 hours.

(d) Centrifuge the suspension and wash this 3 times with normal saline to remove the formalin. Be sure the cell aggregates are well separated.

(e) Resuspend the cells with an equal volume of isotonic diluent as used in an automated cell counter.
This now has produced a stabilised WBC suspension, of formalised RBCs which will not lyse when a lysing reagent is added.

II

(a) Take 1 litre (or quantity required) of outdated Bank blood and filter this through several (at least 6) layers of 4in x 4in gauze—Johnson and Johnson or similar. This is to remove any small clots.

(b) Mix thoroughly but gently on a magnetic stirrer for 30-45 minutes.
This has now produced a RBC suspension which will give consistent and reproducible results for the 6 parameters other than WBCs.

III To produce the final product, which is a combination of I and II above, proceed as follows.

(a) Determine the cell count of the stabilised WBC suspension as produced in I (e) above by making a 1:2 dilution of the suspension in isotonic diluent and reading this on the cell counter on the RBC parameter.
e.g. If the cell count is $1.8/10^{12}$ litre then the original suspension has a count of $3.6/10^{12}$ litre.

(b) To obtain a WBC of approximately $10{,}000/10^{9}$ litre use the following formula.
To a litre of the RBC suspension obtained in II add the following amount of the original WBC suspension prepared.

$$\frac{10}{\text{cell count obtained}} = \text{ml of WBC suspension to add}$$

$$\text{e.g. } \frac{10}{3.6/10^{12}\text{ litre}} = 2.77 \text{ ml of WBC suspension to add per litre of RBC suspension.}$$

(c) Add the required amount of WBC suspension as determined above to the litre of RBC suspension and mix on a magnetic stirrer slowly for 1 hour to allow for even distribution of the cells.

IV This final product can now be dispensed into suitable sterile containers for use as a blood standard as required.
We have found it very reliable as a secondary standard, and it can well be used as a primary standard if well standardised against one of the commercial control products.

I 10% neutral formalin (2 litres)

(a) 1.7 litres of distilled H_2O

(b) .19 litres of reagent grade formaldehyde 37% (HCHO)

(c) 7.64 gm of sodium phosphate monobasic ($NaH_2PO_4H_2O$)

(d) 23.275 gm of sodium phosphate dibasic ($Na_2HPO_47H_2O$)

October, 1977

C. S. Shepherd
Haematology Department
Hamilton Medical Laboratory

Faecal Tryptic Activity — A Source of Error

A term female neonate weighing 2,090g was delivered at this hospital after induction. Clinical impressions were that she was a compromised small for gestational age infant. No meconium was passed up to 48 hrs post delivery, and a barium enema was given which revealed a meconium block in the low large bowel.

A provisional diagnosis of Hirschprung's Disease or meconium ileus was made. At laparotomy an ileal atresia was found and was corrected.

The infant progressed well then suddenly deteriorated 48 hour post-operatively, with abdominal distension and bile stained vomiting. At 72h post operation no visible trace of the barium enema were seen in small bowel motions. Over a period of 48 hours the infant made variable progress and the possibility of cystic fibrosis was considered. A faeces specimen was obtained, at 106 hour post-operation, and tryptic activity was determined using the

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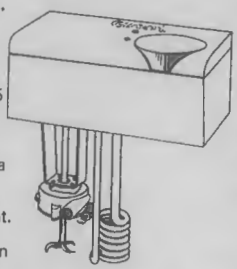

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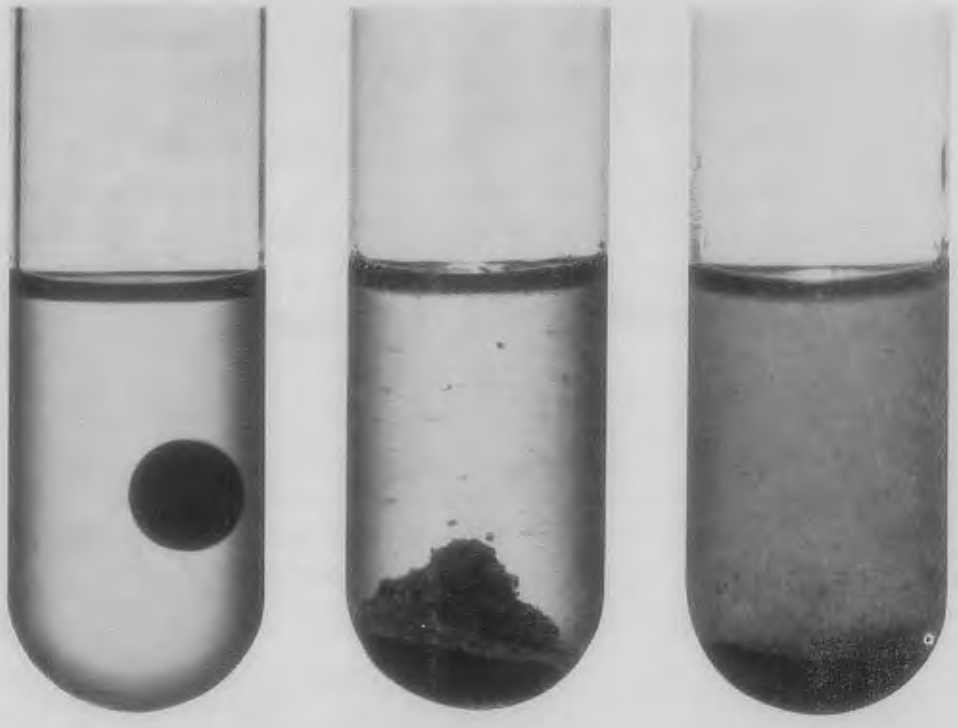

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X-ray film technique.[2] A result of less than 1 in 5 was obtained which indicated the possibility of fibrocystic disease, however, immunochemical detection of albumin, trypsin and chymotrypsin indicated normal results. Up to this time very little faecal material had been passed *per rectum*. At 130 hours post operation the infant was progressing well and was passing normal, soft faeces. At 154 hours post operation a second faecal specimen was collected for tryptic activity and was greater than 1 in 80, immunochemical analysis for albumin trypsin and chymotrypsin were normal.

The two faecal specimens were tested for the presence of barium using an aqueous solution of sodium rhodizinate. The first faecal specimen gave a reddish brown precipitate indicating the presence of barium, the second specimen was negative. Burke (1961)[1] demonstrated that the tryptic enzymes were inhibited by certain metals one of which was barium.

Whilst this infant was ill she presented with the signs and symptoms of cystic fibrosis and a faecal tryptic activity of less than 1 in 5 would appear to agree with clinical finding. As sweat tests are unreliable in the neonate the only technically simple methods available are assessment of tryptic/chymotryptic activity and quantitation of albumin, trypsin and chymotrypsin preferably by immunochemical techniques. As some 10% of infants with cystic fibrosis develop a meconium ileus it is not unreasonable to investigate any gut atresia as a potential case of cystic fibrosis. It is important therefore, if faecal enzymatic assessments of pancreatic function are used that the faecal specimens are totally free of any heavy metals before using these tests to assist in the diagnosis of cystic fibrosis. No effects have been observed to date with regard to heavy metals interfering with the immunochemical determination of trypsin and chymotrypsin concentration.

16-1-78

M. Legge,
Perinatal Biochemistry Unit,
Pathology Services,
Christchurch Women's Hospital,
Christchurch, New Zealand.

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An Investigation of the Cause of Artefactual Atypical Lymphocytes in Routine Haematological Blood Films

Summary

The use of fans to rapidly cool-air-dry blood smears prepared for routine haematological examination markedly increases the incidence of artefact atypical lymphocytes.

Introduction

The high incidence of atypical lymphocytes in the routine blood smears at our haematology laboratory has caused concern for some time. This study was undertaken to determine the cause—the following being hypothesised as possible causes: staining method ("Hema-Tek" system), anticoagulant distortion (EDTA) method of film preparation (fan drying compared with still air drying). A double blind technique was used to eliminate personal bias.

Method

A total of sixty blood films were prepared, twelve from each of five normal people. From each person six films were made from "finger prick" blood and six were made from EDTA anticoagulated blood. Three films from each of the above two classes were left to air dry on the bench, and the remaining three films from each of the two classes were dried using a commercial hair drier blowing cold (room temperature) air. Thus there were three films each for each of the following four parameters from each of five people.

EDTA SPECIMEN: STILL-AIR DRIED
EDTA SPECIMEN: FAN BLOWER DRIED
FINGER PRICK SPECIMEN: STILL-AIR DRIED
FINGER PRICK SPECIMEN: FAN BLOWER DRIED

The sixty films were stained in random order using Leishman's stain in a "Hema-Tek" staining machine. An independent person code numbered the films from 1-60 randomly and the films were examined.

One hundred consecutive lymphocytes were differentiated into normal lymphocytes and

atypical lymphocytes, for each of the sixty films — a total of 6000 lymphocytes. The criteria used to define "atypical lymphocytes" were:

(a) Larger than normal lymphocytes with pleomorphic nuclei
(b) Irregular cell margins with abundant cytoplasm which was vacuolated, foamy or basophilic
(c) A concentration of basophilia at the periphery of the cytoplasm, which shaded off towards the centre.

Results

Table 1

	Number of Atypical forms seen in 1500 lymphs	%
STILL-AIR dried films from EDTA Specimen	24	1.6
FAN dried films from EDTA Specimen	259	17
STILL-AIR dried films from FINGER PRICK Specimen	21	1.4
FAN dried films from FINGER PRICK Specimen	360	24

Discussion

In this study a total of 6,000 lymphocytes were classified. Of these, 664 or 11.1% were classed as atypical.

Of these atypical lymphocytes, 619 or 93.3% were from films dried with the fan while 45 or 6.7% were in still-air dried films. This means that when the fan is used 10.3% of lymphocytes are atypical compared with only 0.75% in films still-air dried.

It is pleasing to note that there is little difference between results obtained from EDTA versus finger prick blood, indicating that there is little or no anticoagulant related distortion of the lymphocytes. Also the results so clearly incriminate the fan method of drying films that it exonerates the staining machine of contributing to the problem.

We have now ceased using the fan to fast dry our films and the problem is solved.

January, 1978

B. D. Hokin, ANZIMLT,
Haematology Department,
Middlemore Hospital,
Auckland.

Correspondence

Sir,—I have read the paper "Evaluation of a Serum Latex Pregnancy Test" in the March issue of the Journal[9], and wish to pass comment on it.

The author states "A tube haemagglutination test is required in the hospital laboratory to quantitate HCG levels in patients with choriocarcinoma or hydatidiform mole . . the tube haemagglutination inhibition test assists in the diagnosis of these conditions because of its greater sensitivity and should not be replaced in these situations."

I do not agree with this statement and I am unaware of any evidence either in this paper, or elsewhere, which supports it. To distinguish patients with trophoblastic disease from those with normal pregnancy requires estimation of HCG. In normal pregnancy HCG levels do not generally rise above 300,000 iu/l[1] while in trophoblastic disease levels well above this value are found[4]. It would, therefore, seem to me that one specification not required of a test used for this purpose is high sensitivity.

One of the references[5] (reference 4 in her paper) supporting the statement "Latex tests are generally not suitable for quantitative assays" compares HCG levels found by using both tube and latex tests. The reference quotes McGregor and others[6], who found HCG excretion in normal pregnancy to range from 50,000-100,000 iu/l using a haemagglutination inhibition procedure, and Taymor and others[8], who found levels of 64,000-110,00 iu/l using a latex agglutination inhibition procedure. The agreement between these two findings is excellent and suggests that slide tests give results comparable to tube tests for the estimation of HCG. This finding is in agreement with published figures obtained in our laboratory[2], and elsewhere[7].

I therefore consider that Latex tests are well suited to both the detection and estimation of HCG in urine samples. Latex tests with a sensitivity equal to tube tests are readily available[3], and have a high degree of reliability.

These findings do not support the claims of this paper that haemagglutination inhibition

tests are "the method of choice for accurate quantitative results."

April, 1978

M. Killip,
National Women's Hospital,
Auckland.

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Sir,—In reply to M. Killip's letter re comments on the paper "Evaluation of a Serum Latex Pregnancy Test".

I agree with the statement that in the case of normal pregnancy latex tests are well suited to the detection of HCG.

However, we were evaluating a test for use when the HCG level is very low, e.g., in cases of threatened abortion and follow-up of choriocarcinoma. I agree that the initial level of HCG in choriocarcinoma does not require a sensitive test, but follow-up after chemotherapy does, as low levels of HCG are very significant.

The aim of the paper was to evaluate a *serum* latex test which could be easily substituted for a urine estimation, giving the same sensitivity as current methodology.

Sheryl Young,
Microbiology Department,
Christchurch Hospital.

May, 1978.

A Potential Error in Screening for Galactosaemia

Galactosaemia is an autosomal recessive disease with an approximate incidence of 1 in 40,000 in New Zealand. Recently a Caucasian female infant was referred to Christchurch Women's Hospital at 10 days of age with a heart murmur, hepatosplenomelagy and hyperbilirubinaemia (bilirubin 220 μmol/litre). Clinically the infant presented as a galactosaemia and the urine was tested for reducing substances which were positive. Thin layer chromatography revealed glucose only, because of the similar Rf's of glucose and galactose the urine was also incubated with glucose oxidase prior to the run, this sample revealed no spots at all; thereby indicating the spot located was glucose.

Relevant blood biochemistry was as follows:
pH 7.11
Glucose 0.6mmol/l (glucose oxidase).
Glucose 37.9mmol/l (Auto-analyser, reducing sugars).
Galactose 22.2mmol/l (galactose oxidase).
Ketones negative.

On the basis of the elevated blood galactose the infant was diagnosed as a galactosaemia.

Two considerations should be made on the examination of the laboratory information.
1. Although the infant was hypoglycaemic (glucose 0.6mmol/litre by glucose oxidase), the auto-analyser glucose showed it to be hyperglycaemic. This would be due to the non specific reaction of galactose and perhaps other reducing sugars present in the infants blood.
2. The generally accepted technique of confirming that the positive reducing substances reaction in the urine was attributable to galactose failed. No galactose was detected by thin layer chromatography, galactose oxidase impregnated paper strips or by quantitation using a galactose oxidase method.

With regards to item 2, the infant had not received a milk feed for approximately six hours. As galactose is rapidly excreted in the urine this would probably account for the inability to detect it in the urine. In an attempt to resolve this problem a gas chromatographic technique[1] was used to identify the hexitol, galactitol which is formed by an alternative metabolic pathway from galactose. Galactitol was identified in the urine from this infant thereby confirming the diagnosis of galactosaemia. The absence of galactose-1-phosphate uridyl transferase activity also confirmed the diagnosis.

It is vital, therefore, that specific, sensitive tests should be used to identify metabolic problems such as galactosaemia and that the correct specimens are collected. This infant could have been diagnosed as a non-ketotic hyperglycaemia with no indication of the true diagnosis, galactosaemia.
30-10-77

M. Legge,
Perinatal Biochemistry Unit,
Pathology Services,
Christchurch Women's Hospital,
Christchurch, New Zealand.

REFERENCE

Wells, W. W., Pittman, T. A. and Egan, T. J. (1964). *J. biol. Chem.* **239**, 3192.

Book Reviews

Training in Medical Laboratory Technology for Islands in the South Pacific. Marilyn M. Eales, FNZIMLT. Project for the IMLS Certificate in Medical Laboratory Management, 1976. A copy of this project has been presented to the NZIMLT Library.

"The objective of this project is to provide guidelines for a training programme in Medical Laboratory Technology for the islands and territories in the Melanesian, Polynesian and Micronesian sectors of the South Pacific Ocean. The author has spent four years on medical laboratory technology assignments in the area of the Pacific. The observations and ideas presented in this project are based on personal experience and on the experience of colleagues and friends working in the area."

The introduction immediately makes the point that only relevant international experience should be transferred to developing countries and her thesis develops this theme and attempts to engender by precept and example

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what constitutes relevant experience. Too often prestige buildings and equipment are sought after in emulation of developed nations. India is quoted as an example of a country where doctors congregate in the cities, having been trained to meet the needs of a London Teaching Hospital, and are reluctant to work in primitive areas in dire need of medical assistance and of preventive medicine. This is contrasted with the effectiveness of the "barefoot doctor" in China known and trusted by his fellow workers. The application of simple remedies for the common ailments constitutes the base of the skill pyramid visualised by Maurice King, Professor of Social Medicine, University of Zambia. This is arranged in a hierarchical manner with the relatively few with most skills at the top. Skill is disseminated downwards on the basis that tasks should be performed by the least expensive member of the team capable of doing it. Surely a lesson here with more general application! The medical technology training programme outlined in the project is designed as a skill pyramid.

Conditions in the Islands are discussed and problems related to health care. Cultural background and beliefs, geography and the administration are all major factors. The various approaches to training are described. These are seldom appropriate and the tendency to send people to overseas laboratories or sophisticated courses is deprecated. Knowledge of the latest techniques or equipment may have little practical application. People giving instruction must be carefully chosen. They must have a clear idea of the objectives and their limitation. They must be able to make themselves understood, be accepted, understand their pupils who may have had a very elementary education, speak little English and react unexpectedly because of a different cultural background. The instructor must above all achieve the objectives and accomplish his assignments.

Training requirements are outlined and actual training programmes described in some detail. There is a bibliography and the appendix reproduces a working syllabus and Basic Techniques for a Medical Laboratory.

This project is required reading for anyone involved, or who anticipates being involved, in this important work.

R. D. Allan.

Nongonococcal Urethritis and Related Infections. Edited by Derek Hobson and King K. Holmes. Published by American Society for Microbiology, 1977. 391 pages. Price $US14.00.

This book is composed of the papers presented at a symposium held in New York in 1976.

Venereologists, gynaecologists, ophthalmologists, bacteriologists, virologists and immunologists from many countries met to reassess the clinical spectrum of chlamydial and ureaplasmal infections and to review recent advances in the cultivation and serological study of chlamydiae and ureaplasmas.

The book is divided into seven sessions with a chairman's summary at the end of each session.

I Nongonococcal urethritis (NGU)
II Related Genital Infections
III Oculogenital Chlamydia *trachomatis* Infections
IV Chlamydia: Biology and Experimental Pathology
V Laboratory Diagnosis of C. *trachomatis* Infections
VI Practical Problems in Isolation and Growth of C. Trachomatis in Tissue Culture
VII Ureaplasma Urealyticum

The first three sessions are clinically oriented, describing the diagnosis and pathogenesis of chlamydial and ureaplasmal infections with much discussion on whether chlamydiae and ureaplasmas play a significant role in NGU. The epidemiology of NGU in Britain and the USA is reviewed and details of antibiotic treatment given.

The papers presented in sessions IV and VII describe the biology, biochemistry and antigenic structure of *C. trachomatis* and *U. urealyticum* including the use of animal models for studying the infectious nature of these agents. Some interesting methods for determining the antibiotic sensitivity of chlamydiae using cell culture sensitivity tests and relating these to treatment of the clinical disease are given. Sessions V and VI concern the laboratory diagnosis of chlamydial infection by direct demonstration with stains, fluorescent antibody techniques and electron microscopy, isolation in tissue culture and demonstration of antibody responses in serum and tears. Up to date information is given on the simplification of

tests, increasing their sensitivity and overcoming some of the problems in tissue culture. Because most of our laboratories do not have the facilities for the culture of these organisms, this book is of very limited value. However, it can be recommended to venereologists and ophthalmologists because of the detailed information on clinical diagnosis and treatment and to those laboratories with the ability to diagnose these infections because of the up to date technical data.

S. Gainsford.

Blood Group Serology — Theory, Techniques, Practical Applications. Kathleen E. Boorman, Barbara E. Dodd, P. J. Lincoln. Fifth Edition. Published by Churchill Livingstone. Price $32.30. 495 pages, illustrated. Obtained from N. M. Peryer Ltd., Box 833, Christchurch.

All technologists employed in Immunohaematology departments will be familiar with "Boorman and Dodd," a standard text-book that has been in circulation since 1957. This, the fifth edition, has undergone some changes which I am sure will be most welcome. The most notable change is the splitting of the book into three parts and also the words "An introduction to" have now been dropped from the title as in the author's preface the introduction only applies to part 1. Presumably this move is to encourage a wider readership.

The next change is that a third author has joined the team, namely Dr P. J. Lincoln, the Senior Lecturer in Blood Group Serology at the London Hospital Medical College. Unfortunately for Dr Lincoln this reviewer feels that the publication will still be familiarly known as "Boorman and Dodd" and it will be a time before the name of Lincoln will be associated with this work.

Part I—An Introduction to Blood Group Serology, Part II, A more Advanced Account of some aspects of Blood Groups, Part III, Practical Application of Blood Group Theory. This new layout is intended to cater for all levels of experience. Part I being easily readable by all students whatever their individual specialty and then encouraging more advanced reading in the latter two parts. Parts II and III are intended for the student specialising in the field of Immunohaematology to be used as a supplement to the other readings.

Part I, the introduction includes sections on the AB0 blood group system, the Rhesus system and a brief account of the other systems.

Part II, as the heading suggests, develops those subjects introduced to the reader in Part I.

Part III covers the practical application of the theory aimed at the technologist employed in a blood transfusion laboratory whether it is a Regional Centre or a hospital blood bank.

A number of new chapters have been added, one on Australia Antigen which outlines in broad terms a description of the subject then gives an account of methods routinely used for its detection. Another new chapter is devoted to the production and use of blood products. Two expanded sections each devoted a chapter relating to automation in the blood transfusion laboratory which includes the use of autoanalysers for antibody screening techniques and blood grouping. The other expanded section is that relating to the HLA system.

The final sections of the book include the familiar appendices devoted to a practical guide, reagents and the glossary.

The overall layout has been retained with which most people will be familiar in that each chapter commences with the theoretical aspects of the topic which is then followed by pertinent techniques. Neither has the layout of the book lost any of its earlier appeal, most chapters being headed with the familiar quotations and extracts from various nursery rhymes.

Generally this reviewer finds this latest edition of a standard test-book to be most acceptable and I am sure that even without this review the book will quickly appear on blood bank shelves. It is further recommended that students of Immunohaematology familiarise themselves with this latest edition rather than refer to earlier ones.

A. E. Knight.

Fundamentals of Clinical Immunology. J. Wesley Alexander, M.D., Sc.D., and Robert A. Good, Ph.D. 1st Ed, 1977. Published by W. B. Saunders and obtained from N. M. Peryer, C.P.O. Box 813, Christchurch. 338 pages, illustrated. $NZ17.70. Paper covers.

The purpose of this book is to provide a short and comprehensive review of the principles of immunology as they apply to clinical

practice. It is intended as an aid to both students and practitioners.

The initial chapter gives a very concise historical introduction. The basics of Immunology cover, immune response, origin and function of lymphoctyes, monocytes, granulocytes, the immunoglobulins, complement, mechanisms of immunological injury, immunosuppression and immunopotentiation.

There is a brief section on laboratory and clinical tests and the latter half of the book covers clinical applications.

The basics are thoroughly discussed and illustrated with diagrams and photographic plates. The immunoglobulins mention the J chains which are small pieces with a molecular weight of 15,000 which bind the units of the polymeric IgA and IgM together by the heavy chains. It has long been recognised that inappropriate immune reactions can cause tissue damage. Anaphylactic shock through the release of histamine from the basophils is one, angioneurotic oedema is another and indeed all the autoimmune processes could be so regarded. The mechanisms of immunological injury are discussed and the mechanisms are illustrated with details of experimental animal models.

Immunopotentiators which enhance immunological actions are of particular interest in the control of cancer. Not only BCG vaccine but vaccines from other organisms, particularly *C. parvum* and *B. pertussis,* have been used. Chemical substances are also listed. These substances have specific effects on T and B cells and macrophage activity.

The section on laboratory and clinical tests offers a brief survey of general concepts. Technical details are not provided since these can be found in the standard texts.

The final section starts with a lengthy chapter on infection. The limitations in the use of antibiotics which have become apparent through the emergence of resistant strains notably gonococcus, are remarked on and the need to rely on immunological methods of treatment. There has been a great increase in deaths in the United States from gram negative septicaemia, the number exceeding deaths due to cancer of the alimentary tract or motor accidents.

Antibiotics can cause depression of the normal immunological mechanisms and advice is given on a rational approach to the use of antibiotics, particularly in prophylaxis.

A chapter on transplantation of organs gives details of histocompatibility testing, preoperative evaluation of immune responsiveness and related matters. Other chapters deal with autoimmune disease, transfusion, nutrition and immunity, HLA and disease, allergic disease and immunological deficiency diseases. The latter two chapters are very detailed and well illustrated.

Immunology is a difficult subject to encompass as it involves a great deal of qualitative description. To those of us used to the strict discipline of classical quantitative chemistry some of the dissertation seems rather conjectural. Some of the passages in this textbook are really quite vague, however this possibly relates to the subject and the present state of knowledge.

In general, the information is mainly factual and well presented and the book should fulfil its stated aim. It is good value for its size and content.

R. D. Allan.

Immunology of the Rheumatic Diseases. Aspects of Immunity. R. N. Maini, 1977. This volume is No. 7 in "Current Topics in Immunology," published by Edward Arnold, 25 Hill Street, London W1. 146 pages, a few illustrations, soft covers. £5.95 U.K.

The general preface to the series remarks on the massive amount of new material accumulating in the field of immunology and the need for an up to date treatise on the aspects of the subject relevant to a particular field of interest. The intention is to produce relatively short reviews for clinicians and those working in clinical laboratories.

The first section of the book provides an account of the biology of the immune response, the relation to tissue injury, immune reactions to autoantigens and Immunological methods.

The second sections describe various "connective tissue" diseases and therapy and the third smaller section considers genetic susceptibility.

Familiar ground is covered in "Biological Aspects of the Immune Response" describing

the interaction of the T and B lymphocytes and macrophages, Coombs and Gell classification of the hypersensitivity reactions and theories of the development of T cell tolerance (to autoantigens). Evidence does not entirely support Burnet's original concept of the elimination of immunocompetent cells during foetal life. T cells also appear to have a suppressor function preventing B cell synthesis of autoantibody. (The New Zealand black mouse suffering from autoimmune haemolytic anaemia appears to lack suppressor T cells.) The various ways in which the tolerance could be bypassed by extrinsic cross-reacting antigen (viruses), loss of suppressing T cells and the powerful adjuvant action of mycobacterial infection are discussed. It has been suggested that the structurally altered IgG prevailing in rheumatoid arthritis patients bind directly to B lymphocytes activating them to produce rheumatoid factor antibodies without T cell participation. As well as the common IgM rheumatoid factor, IgG, IgD and IgA factors have been demonstrated.

Tissue injury due to inappropriate immunological reactions may be cell mediated or due to the formation of immune complexes. The concept that union of freely dispersed antigen and antibody can mediate inflammatory reactions by initiating the complement cascade leading to tissue damage is based on clinical and experimental observations. They are a very common phenomenon in infections and are normally sequestered into the reticuloendothelial system.

No less than eighteen methods for detecting immune complexes are listed in the methods section. Reference is made to the presence of immune complexes in rheumatoid arthritis in the serum and synovial fluid. Immune complexes are, of course, associated with SLE. The agent that initiates these immune responses is still unknown, although evidence for microbiological antigens has been suggested. Recent evidence shows that Rubella specific antigens are coded on the surface of rheumatoid synovial fibroblasts. It has been reported that *Mycoplasma fermentans* could be isolated in up to 40% of synovial fluid in rheumatoid arthritis. The isolation of diphtheroid-like organisms from synovial fluid has been claimed.

The arguments for a virus aetiology for SLE are described. The immunological aspects of these diseases and also Sjogren's Syndrome, Polymyositis, Myasthenia Gravis and some others described. Mention should be made of the chapter on Vasculitis and Polyarteritis Nodosa. Vasculitis is a general term for all types of conditions which show evidence of vacular damage initiated by an inflammatory process. They show evidence of circulating immune complexes and complement activation and this could be regarded as the unifying pathogenic factor.

The accumulation of knowledge about immune mechanisms discussed in the first part of this book led one to anticipate new advances in therapy. Disappointment followed. It is evident that no breakthrough has yet occurred in the application of immunological knowledge. Immunosuppressive treatment with steroids cytostatic drugs, gold and penicillinase is described. Mention is made of attempts to correct cellular immune defects with leucocyte dialysates and the use of levamisole to stimulate cellular immunity. Reports of results are variable.

The final chapter discusses the evidence for genetic predisposition to rheumatic disease.

This is a book for the specialist and contains the latest information approaching the forefront of knowledge in the field.

R. D. Allan.

Clinical Chemical Pathology, eighth edition, 1977. C. H. Gray and P. J. N. Howorth. Published by Edward Arnold, London. 245 pages, illustrated. Price, £3.50 U.K. (paperback).

First published in 1953, this book now appears in its eighth edition. This is the second time an edition has appeared with S.I. units and in paperback. The original author Gray is joined by a colleague, P. J. N. Howorth.

The chapter on Renal Function has been rewritten and is extended to include more clinical information on renal disease and two new diagrams replace those of the last edition. The acid base section has been revised and the two chapters on fluid balance which appeared in the previous edition have now been combined. Proteins and immunoglobins contains a section on "acute phase reactant proteins" and "immunocytomas and paraproteins".

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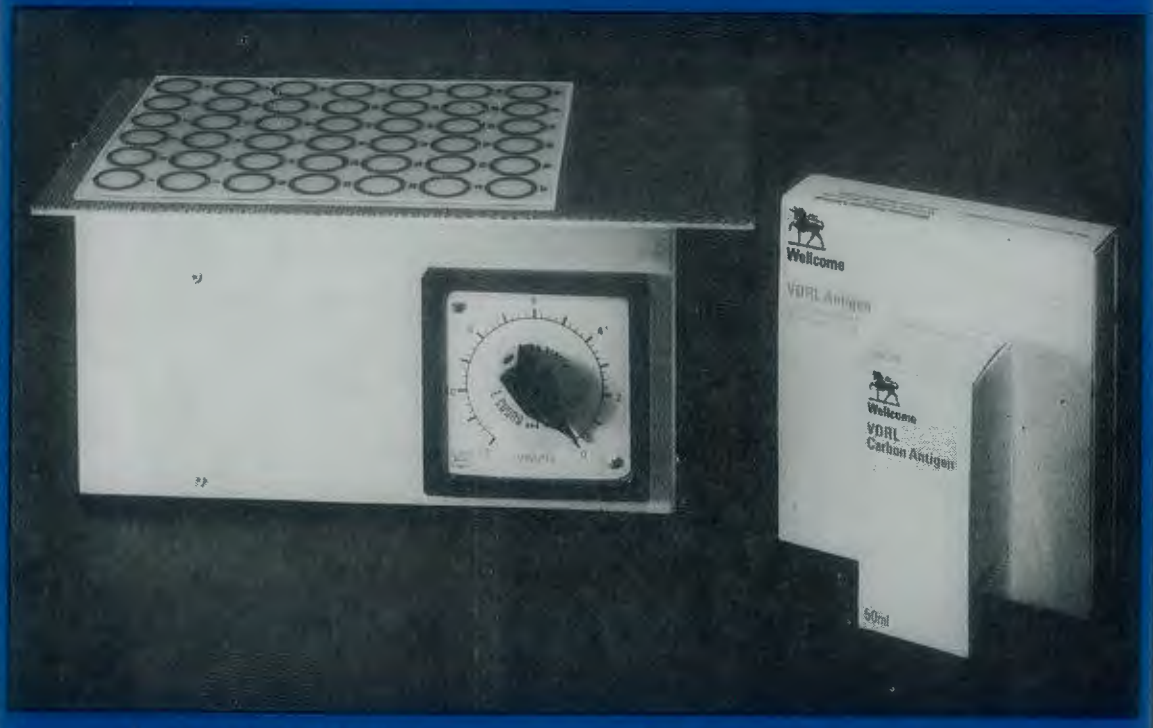

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There is a fuller section on Hormone Control in the chapter on Calcium and Phosphate and the summary on bone disease has been expanded. Causes and investigation of renal calculi is now an addition to this chapter. Endocrinology has been rewritten with revision of endocrine diseases and the addition of a section on multiple endocrine endopathy. Gastro-intestinal function has also been rewritten.

The remaining chapters are virtually the same as in the seventh edition except for the addition of a few paragraphs on recently developed topics.

Two new sections appear in the Appendix. The first is on paediatric clinical chemistry which discusses biochemical tests important in diagnosis of congenital diseases as well as problems concerned with premature infants. The second is concerned with toxicology and deals with self-poisonings, industrial and environmental poisonings and drug therapy. The Appendix also contains a comprehensive section on reference values including specimens required and conditions which give abnormal results. All values are in S.I. units except pH.

This book is not strictly a reference text, but its compact, easy to follow layout makes it a useful handbook on Clinical Chemistry.

N. J. and A. J. L. Langford.

Books Received

Recent Advances in Clinical Immunology, No. 1. Edited by R. A. Thompson. Published by Churchill Livingstone. 299 pages, illustrated.

Introduction to Research in Medical Science. Cuschieri and Baker. Obtained from Penguin Books Ltd., C.P.O. Box 4019, Auckland.

Abstracts

Contributors: E. R. Crutch, N. J. and A. J. L. Langford

CLINICAL BIOCHEMISTRY

Electronic Pipetting Device for Repetitive Deliveries. Kenny, A. P., Horne, R. G. and Fallow, T. M. (1978), *Clin. chim. Acta.* 82, 285.

An electronic dispensing pipette is described suitable for use in dispensing Quality Control materials or other liquids. Laboratory bulb pipettes are used and volumes of between 2 ml and 50 ml can be obtained with a precision of 0.2 percent. This device is an alternative to commercially available syringe type dispensers. —N. L.

A Comparative Study of Commercial Human and Bovine Albumin Preparations. Farrance, I., Dennis, P. M., Gibson, Barbara J. and Biegler, Beryl. (1978), *Annals clin. Biochem.* 15, 31.

Using seven analytical procedures, human and bovine albumin preparations are compared, and their usefulness as calibration and reference materials is assessed. Chemical purity is not taken into account. Dye binding assays in particular show that the albumin preparations should be checked for their method suitability before use. —N. L.

New Automated Dye-Binding Method for Serum Albumin Determination with Bromocresol Purple. Pinnell, A. E. and Northam, B. E. (1978), *Clin. Chem.* 24, 80.

The method described uses Auto Analyser I equipment with B.C.P. reagent at pH 5.2 in an acetate buffer. The reaction is read at 636 nm. B.C.P. is shown to be an alternative to, and have advantages over the widely used Bromocresol Green method. B.C.P. has high reactivity with human serum, negligible blank correction, good correlation with immunoelectrophoresis, was affected by bilirubin only when present in very high concentration, and salycilate did not interfere. Human serum is shown to be the most suitable for standardisation. —N. L.

Serum Alphafetoprotein in Cystic Fibrosis of the Pancreas. Brock, D. J. H., Barron, Lilias, Manson, Jean and McCrae, W. M. (1978), *Clin. chim. Acta.* 82, 101.

The authors discuss the measurement of serum alphafetoprotein by three different immunoassay techniques. Thirty patients with cystic fibrosis and 55 controls were used in the investigation. It is shown that both patients

and control values fell within the published normal limits, and large increases in serum A.F.P. in cystic fibrosis patients and in heterozygote carriers were not observed. It is pointed out that this contradicts findings reported by Chandra *et al.* (1975). *Br. Med. J.* 1, 714. —A. L.

Abnormal Electrophoretic Pattern of Albumin in Diabetic Sera. Kawaguchi, T., Tsuchida, T. and Matsushita, H. (1978). *Clin. chim. Acta.* 83, 7.

Electrophoresis was performed on sera from normal and diabetic subjects using urea containing gel. In normal patients the fast migrating bands 1 and 2 were observed before glucose was administered. After administration the slow migrating bands 4 and 5 appeared and band 1 disappeared. The slow migrating bands 4 and 5 did not appear in diabetic sera even after administration of glucose.

—A. L.

Use of Patient Data in the Control of Urea, Creatinine, and Electrolyte Estimations. White, J. D. (1978). *Clin. chim. Acta.* 84, 353.

The author discusses the use of urea, creatinine and electrolyte results from patients as a method for comparing and evaluating laboratory tests. Two district general hospitals were involved in the investigation. The data which contained normal and abnormal results was analysed in such a way so as to resolve overlapping Gaussian distributions. Thus a reference value from hospital populations may be determined and modified to be used as an indication of the precision of biochemical estimations. The information provided from the patients' results is compared with that given by the national quality control scheme.

A. L.

The Use of Glutamyl-p-Aminobenzoic Acid as the Substrate for Determination of γ Glutamyltranspeptidase Activity in Blood Serum. Szewczuk, A. and Wellman-Bednawska, M. (1978). *Clin. chim. Acta.* 84, 19.

The authors suggest the use of a synthetic amino derivative of P.A.B.A. as a substrate for γ G.T.P. estimations. This substrate is readily soluble in buffered solutions and is non-toxic which makes it valuable for use in γ G.T.P. assay. The results given by this method compared favourably with those results obtained with the method using γ-L-glutamyl-p-nitroanalide. —A. L.

A Continuous Flow Method for the Estimation of Serum Amylase Activity. Carney, J. A., Osibeluwo, S. A., Clements, J. A. and Jay, R. F. (1977). *Annals clin. Biochem.* 14, 350.

A method suitable for use on Auto Analyser II is described. Using commercially available substrate (DyAmyl-L-), the method measures enzyme activity of serum at 540 nm. After incubation of serum and substrate at 55°C, KOH is added and the coloured products are dialysed through a type "H" Technicon membrane against PO_4 buffer. Calibration is with Versatol control sera. Results obtained are compared with another commercial method. Effects of substrate dilution and substrate concentration of NaCl on sensitivity are shown.

—N. L.

Automated Procedure for Kinetic Measurement of Total Triglycerides (as Glycerol) in Serum with the Gilford System 3500. Lehnus, G. and Smith, Lynn. (1978), *Clin. Chem.* 24, 27.

A method is described for the measurement of Triglycerides using the Gilford 3500 Analyser. The decreasing concentration of NADH is measured at 340 nm after enzymatic hydrolysis at room temperature. (The glycerol kinase reaction). Standardisation is with aqueous glycerol standards and the procedure shows good correlation with the manual method.

N. L.

Rapid Loss of Factor XII and XI Activity in Ellagic Acid-Activated Normal Plasma: Role of Plasma Inhibitors and Implications for Automated Activated Partial Thromboplastin Time Recording. Joist, J. H., Cowan, J. F. and Khan, M. (1977) *J. Lab. Clin. Med.* 90, 1054.

HAEMATOLOGY

Rapid prolongation of the activated partial thromboplastin time upon incubation with ellagic acid containing activated partial thromboplastin time reagents was observed. The data presented in this paper indicate that ellagic acid containing a-PTT reagents have unfavourable properties which seriously limit their usefulness in the clinical laboratory, particularly in respect to recording of the a-PTT with certain fully automated clot timers.

—E. R. C.

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* ASO, AH, ASK, ADNase, ANADase

(1) Klein, G. C. and Jones, W. L. : Applied Microbiol. 21 : 257, 1971.
(2) Janeff, J., Janeff, D., Taranta, A., & Cohen, H. : Lab. Med., 1971 (in press).

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The Effectos of "Anti-platelet" Drugs on Bleeding Time and Platelet Aggregation in Normal Human Subjects. Buchanan, G. R., Martin, Vera, Levine, P. H., Scoon, Kristine, and Handin, R. I. (1977), *Am. J. Clin. Pathol.* **68,** 355.

The effects on Haemostasis of several commonly used drugs previously described as inhibiting platelet function were evaluated in a randomised, double-blind study of 54 normal volunteers. Results of *in vitro* platelet-drug incubations may not be directly applicable to *in vivo* haemostasis.

Evaluation of a Single-Channel Photo-optical Clot-Timing Instrument. Weisbrot, I. M. and Waldner, Deborah (1977), *Am. J. Clin. Pathol.* **68,** 360.

This paper presents an evaluation of the General Diagnositics "Coag-a-mate" single channel photo-optical clot timer.

—E. R. C.

Diagnosis of Acute Leukaemia. Variability of Morphologic Criteria. Tan, H. K., and Lamberg, J. D. (1977), *Am. J. Clin. Pathol.* **68,** 440.

The authors of this paper discuss the morphologic appearances of blast cells in acute leukaemia stained by six different Romanowsky stains. They caution against generalisations regarding morphology and suggest that the exact stain employed is a significant variable in morphologic studies. —E. R. C.

Recommendation for Measurement of Erythrocyte Sedimentation Rate of Human Blood (1977), *Am. J. Clin. Pathol.* **68,** 505.

This article is published by the Expert Panel on the erythrocyte sedimentation rate under the auspices of the International Committee for Standardisation in Haematology. The Panel detail the methods and materials to use as the "standard" method.

—E. R. C.

Evaluation of the Emalog D Differential Leucocyte Counter. Cairns, J. W., Healy, M. J. R., Stafford, D. M., Vitek, P. and Waters, D. A. W. (1977), *J. Clin. Path.* **30,** 997.

The main objectives of the evaluation were to (1) to establish "normal values" for the Hemalog D, (2) compare the Hemalog D with existing manual procedures and, (3) assess the machine's reliability. The Hemalog D proved to be a practical machine for operation in a routine haematology laboratory.

—E. R. C.

A Whole Blood Control for the Coulter Model S. Morgan, L. O., Jones, W. G., Fisher, J. and Cavill, I. (1978), *J. Clin. Path.* **31,** 50.

The white cell count, red cell count, haemoglobin and mean cell volume of a new preserved whole blood preparation used as a control for the Coulter Model S were found to be stable for at least two months. This material provides a cheap and stable whole blood control which is now in routine use in 18 laboratories throughout Wales.

—E. R. C.

International Committee for Standardisation in Haematology: Protocol for Type Testing Equipment and Apparatus Used for Haematological Analysis (1978), *J. Clin. Path.* **31,** 275.

The protocol proposed in this paper could be used as a procedure for evaluation of equipment and apparatus. It is intended for use by reference laboratories appointed by appropriate authorities in the interest of the consumer. —E. R. C.

Why Has the Autohaemolysis Test Not Gone the Way of the Cephalin Flocculation Test? Beutler, E. (1978), *Blood* **51,** 109.

Beutler questions the usefulness of the autohaemolysis test in assessing various haemolytic states and suggests that this test is now outmoded and perhaps should go the way of the "cephalin flocculation, thymol turbidity, N.P.N. and the dinosaur." E. R. C.

Identification of Sources of Inter-Laboratory Variation in Factor VIII Assay. Kirkwood, T. B. L., Rizza, C. R., Snape, T. J., Phynes, I. L. and Austen, D. E. G. (1977), *Br. J. Haemat.* **37,** 559.

This paper is a report of an investigation to point out the possible sources of variation between laboratories carrying out factor VIII assays. Two important conclusions emerged from this work. The first was that differences between reagents accounted for the major part of the variation between laboratories and the second suggested that two-stage assays detected relatively more activity in the more highly purified (freeze-dried) preparations than one-stage assays. —E. R. C.

Identification of the Hairy Cells of Leukaemic Reticuloendotheliosis by an Esterase Method. Higgy, K. E., Burns, G. F., and Hayhoe, F. G. J. (1978), *Br. J. Haemat.* **38,** 99.

A distinctive pattern of alpha-naphthyl butyrate esterase positivity was observed in all of a series of 14 patients with HCL. This pattern was not observed in a range of other haematological abnormalities. The potential diagnostic value of this simple cytochemical test is discussed in relation to existing methods.

—E. R. C.

The Aspirin Tolerance Test in von Willebrand's Disease. Barbui, T., Rodeghiero, F. and Dini, E. (1977), *Thrombos. Haemostas. (Stuttg)* **38,** 510.

Opinions about the clinical value of the aspirin tolerance test proposed by Quick in von Willebrand's disease are conflicting. The results of this study seem to support the view that a platelet defect induced by aspirin causes a significant lengthening of the bleeding time only in cases with severe von Willebrand factor deficiency.

—E. R. C.

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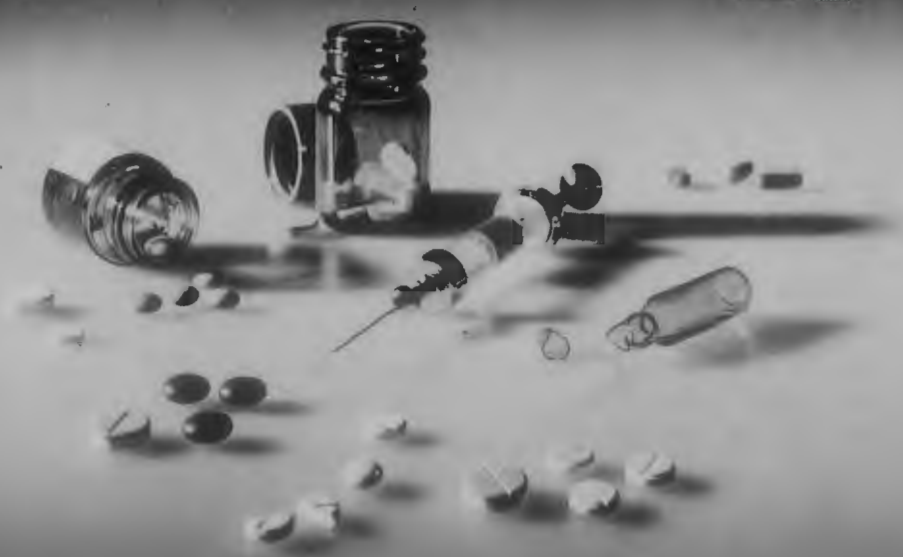

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Temperature: Express as °C.
Time: s, min, h, d, a. The latter two symbols which stand for day and year respectively are best expressed in full to avoid confusion.
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References

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