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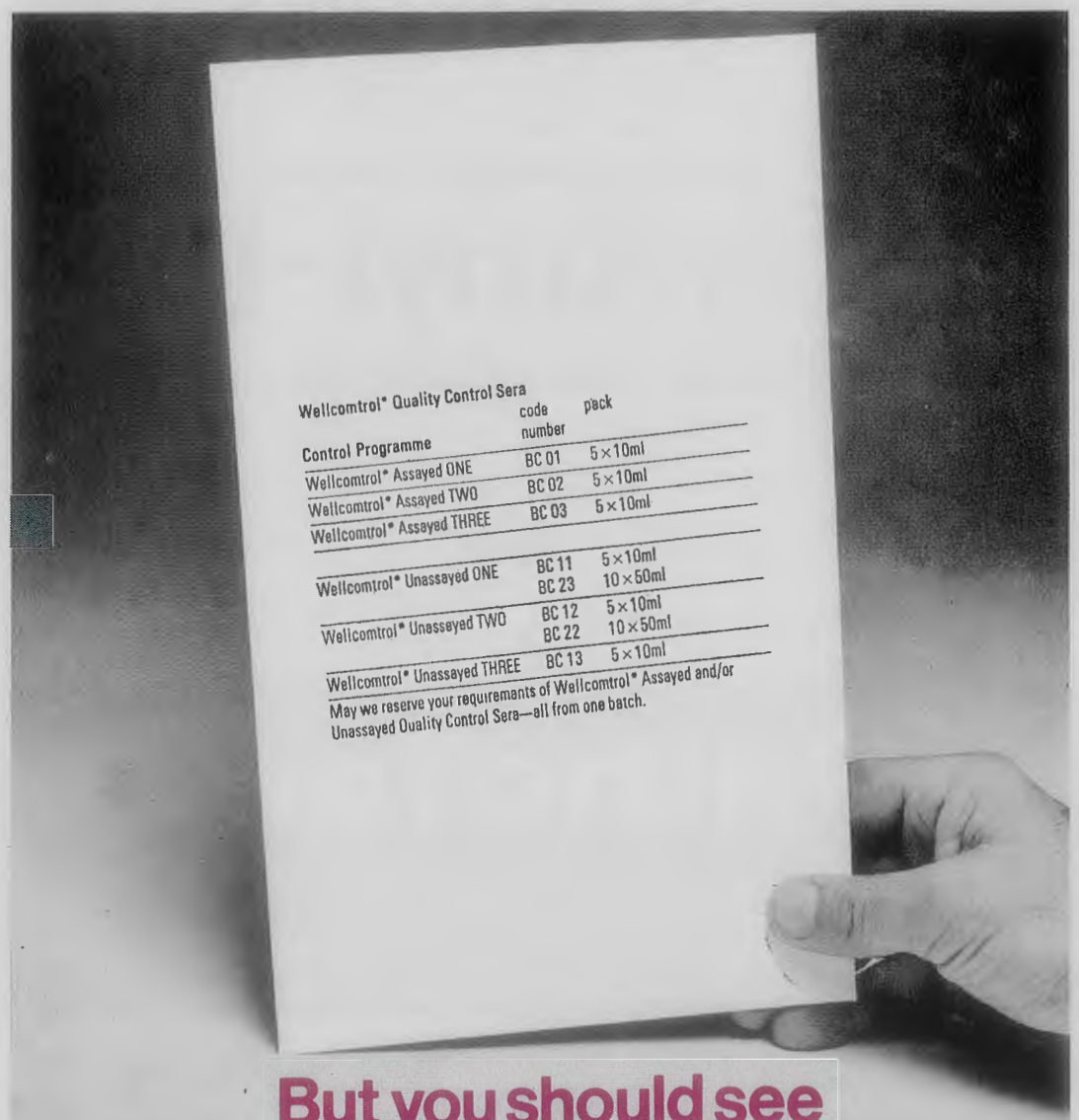

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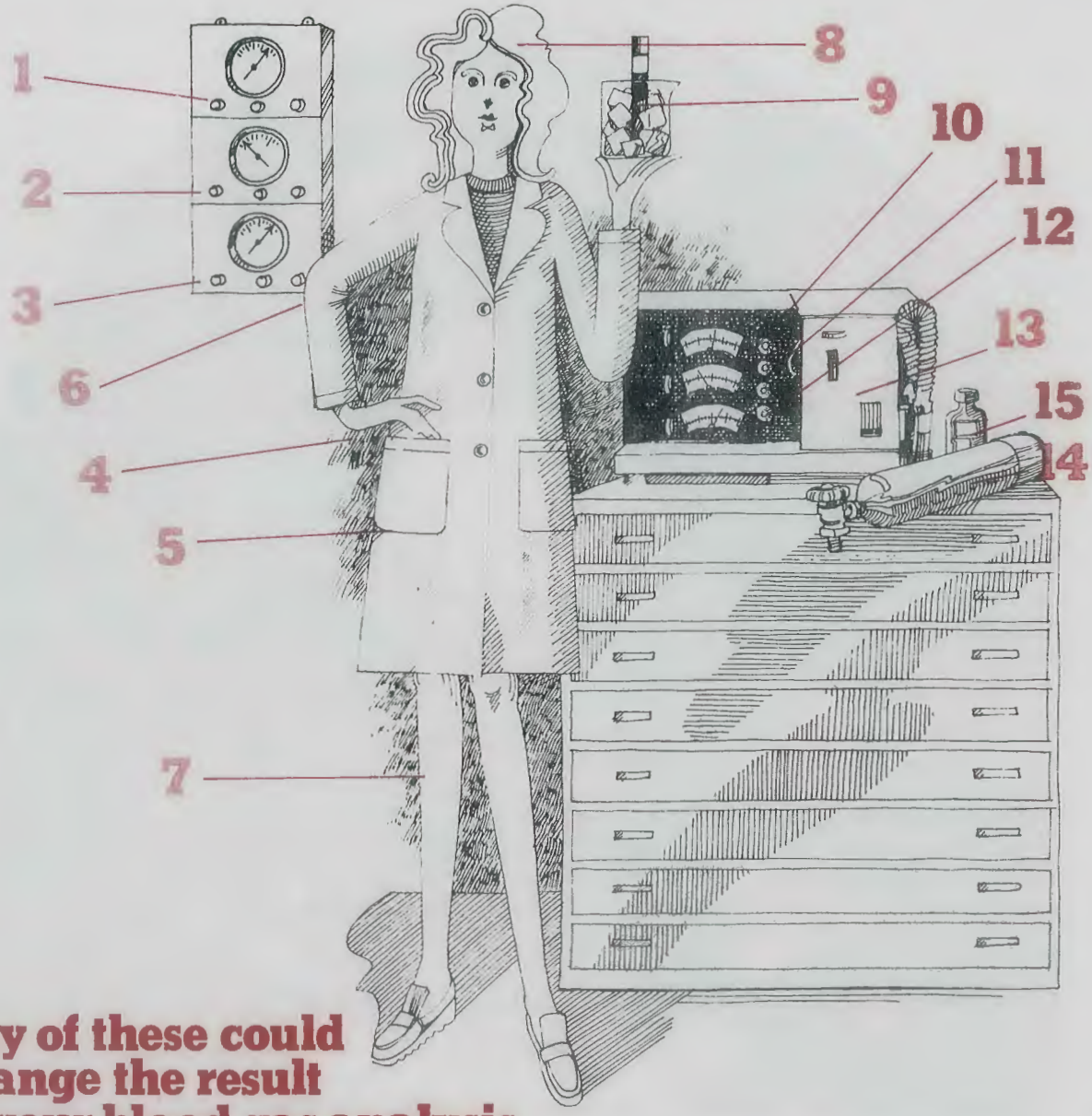

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T. H. PULLAR MEMORIAL ADDRESS

A Look Towards the Past . . . and a Glimpse in Levity Towards the Future

J. Case

Presented at the 31st Annual Conference of the New Zealand Institute of Medical Laboratory Technology, Palmerston North, August, 1975

It is now nine years since the death of Dr Thomas Henry Pullar, in whose name, honour and memory this annual address was established, and it is no doubt inevitable that successive Conferences since then have seen the attendance of a decreasing proportion of delegates who knew Dr Pullar personally. Many medical technologists have gained senior positions who began their training less than nine years ago, and the system of training itself has changed radically from that with which Dr Pullar was familiar and which he played a prominent part in developing. Indeed, there have been so many changes in recent years — and promise to be so many more in the future — that I sometimes wonder how Dr Pullar would have viewed things had he been spared to see them.

One thing for certain is that he would have contributed actively to developments that have taken place, for he was much devoted to participation in these areas, a true friend and adviser to our profession, which he viewed as complementary to his own. It is not really surprising that of eight previous T. H. Pullar Memorial Addresses the majority have taken as their theme some aspect of these matters in which Dr Pullar was so much interested, and I thought it would be fitting in the circumstances if I did not attempt to depart significantly from that trend.

My main trouble in that regard, however, is that I do not profess to be an authority on the subject of medical technology training, nor have I been connected with it in New Zealand for over four years, so it would be most inappropriate to venture opinions as to the manner in which the training programme should be developed in the future. I am fearful, into the bargain, of making portentous observations which may, read years later, seem ridiculous, although the temptation to venture prophecies which may conceivably come to pass, is almost irresistible. I thought it would be safest, in the long run, simply to review some of the various changes that have occurred since 1966, to mention options for the future and perhaps to introduce the odd controversial point for discussion, if not for serious consideration.

1966 was the year in which a new salary determination was circulated by the Department of Health. It offered $2,230 as the minimum starting salary for a staff medical laboratory technologist, rising by incremental steps that must have seemed niggardly even in those days, to a maximum of $2,520. We were still using the pound as our unit of currency at that time, and the figures mentioned have been derived by simply multiplying the stated salaries in the relevant departmental circular by two. At the same time, the annual salaries of grade laboratory officers were from $2,420 at the bottom of what was then Grade (d) to $4,010 at the top of Grade (a), with all kinds of impenetrable barriers preventing progression up the scale. I may not be completely justified to say that the purchasing power of our present salaries is vastly greater than those we were getting then, but it was certainly a significant breakthrough when we succeeded in establishing the right to negotiate with the Department of Health, to make our representations directly instead of through an intermediary body not invariably sympathetic towards our aspirations. This was the means whereby we were able to secure a reform of the Grading Committee, a restructuring of the graded scales and the removal of some of the bars to salary advancement, together with due recognition, in terms of both monetary reward and status, for the ever-increasing complexity of the jobs we are required to undertake.

1966 was also the year in which we became acquainted with the recommendations of an advisory working group appointed to study the feasibility of a new system of certification for medical laboratory technologists in the United Kingdom. The examination system administered by the Institute of Medical Laboratory Technology was plainly no longer adequate. It was recognised in the findings of the Advisory Working Group that a system involving formal education and examination in basic science subjects was required, given in the environment of an educational institution, and serving as a foundation for the teaching of technique subjects in the hospital laboratory situation. An existing system of Ordinary and Higher National Certificates was seen by the Advisory Working Group as being adaptable to the requirements of medical laboratory technologists (still called technicians in Great Britain, incidentally), and the conclusions included suggestions as to how the O.N.C. and H.N.C. courses would need to be structured for the particular requirements.

In due course the O.N.C. and H.N.C. replaced the I.M.L.T.'s Intermediate and Associateship examinations in Britain, and subsequently a similar but less complete transition occurred in New Zealand. The former Intermediate or Basic Training course began to be replaced by a New Zealand Certificate of Science course structured for the purpose, but the existing system of examination beyond the basic training level was retained for the time being, presumably while the various alternative options were explored. It would seem from recent developments that some changes in this area are imminent, but it is evident that there has been disagreement within the Council of the Institute about the details of the new plan; and I believe it would be foolish, as well as presumptuous, for me to take sides in this matter. I do, however, think it would entirely proper to make reference to developments that have been occurring in Australia, insofar as I am aware of them, as therein may lie a source of inspiration — or if not that then of solemn warning.

To begin with, the training schemes vary somewhat in the different States. The autonomy of the States of Australia in educational matters (and in sundry other matters, too) is a commodity very jealously guarded. In fact politicians devote a great deal more time and energy to protecting the rights of their respective States from the encroachments of their Federal counterparts in Canberra, than in exercising those same rights with efficiency and intelligence. Some States have progressed (if progressed is the word) to a more advanced level in the training of medical technologists than others. The ultimate aim seems to be more or less the same in all States, that being to offer a degree course in medical technology. Such a course has been available in some States for a few years, and Victoria plans to offer one shortly. Whilst this trend no doubt has much to commend it, there are certain disadvantages that are already becoming apparent. Diploma courses are still being offered in parallel, and it would seem that official thinking envisages a three-tiered structure of technical staff in diagnostic laboratories, with laboratory assistants as the bottom layer, technologists as the top layer and technicians sandwiched in between. A thing that diploma holders in New South Wales have found to their cost is that the minimum qualification for recognition as a technologist is a degree, and even holders of a Fellowship Diploma (not an easy qualification to come by) have found themselves reclassified as "Technical Officers", which is merely a polite euphemism for "technician".

Real or imagined penalties for individuals in terms of status are not the only disadvantages, however. The degree courses are very much academically oriented, with the inevitable consequence that graduates emerge without the necessary manipulative skills and practical experience to be immediately useful in the hospital laboratory. Even the full-time diploma courses have tended to underemphasise the need to develop technical ability and practical experience, and it seems to some thoughtful observers that the effect of "progress" in the field of education for medical technologists has been to generate an elite class of hospital laboratory worker who will be ideally suited to the planning and execution of research projects, but who will be worse than useless in the average routine

diagnostic laboratory, at least for the first months of his employment, possibly running into a year or two. Such a viewpoint, like most generalisations, cannot be wholly correct, of course. It no doubt does less than justice to many dedicated, capable and competent young men and women who will amply repay the funds spent on their education, but it is an opinion I have heard expressed on several occasions that New Zealand-trained medical technologists are very much in demand in Australia and elsewhere because of the in-service training still existing here.

It would seem that a proportion at least of in-service training is essential for the development of the necessary qualities to be a good medical technologist, however the rest of the course may be structured. It is obvious that technical skills cannot as effectively be gained in the artificial atmosphere of a classroom laboratory, working with contrived samples and situations, as in the everyday laboratory, working with a random scatter of the real thing.

Speaking personally, I have long been concerned at the manner in which immunohaematology tends to be neglected in the overall pattern of training, not necessarily just here in New Zealand, but worldwide. The trouble stems, perhaps, from the view of immunohaematology as but a minor offshoot of haematology proper, necessitating a mere fraction of the time apportioned in the training to other disciplines. The result has been an inadequate level of performance in the one area of the laboratory where mistakes can most readily have fatal consequences, and an inadequate proportion of trainees opting to specialise in that subject.

Things are fortunately changing in this regard, with immunohaematology coming to be recognised as a discipline in its own right, deserving of an adequate amount of training time and worthy as a subject in which to specialise. I was pleased to read recently that it is proposed to require entrants to the examinations in New Zealand to have spent a minimum of 160 hours in the crossmatching of blood for clinical use, before sitting the Basic Training Certificate examination and again before the Part II Haematology and Immunohaematology examination. This is little enough, indeed, but it is a step in the right direction. It would seem that the thinking which deprived half the trainees in New Zealand from ever having the opportunity for training in a routine blood grouping laboratory is to be a thing of the past, and I for one shall not lament its passing. The provision of a week or two-week (or whatever it was) period of practical classroom instruction was never an adequate substitute for time spent actually performing and interpreting serological tests under supervision in an actual working laboratory, and it is doubtful if anyone ever seriously believed it was. If it were so, indeed, it should be possible to train medical technologists to the level of the certificating examination without their ever entering a working laboratory at all, and the whole thing accomplished within a period of ten or a dozen weeks. The notion is palpably ridiculous, and the quality of the training should never be sacrificed as a concession to expediency.

Another significant development in recent years has been the introduction of statutory registration. This was not won without a struggle, and it may have been because the Department of Health actually wanted medical technology to become a State registered profession that this was gained on terms that were not impossibly disadvantageous. The battle is still being fought in Australia where, again, there is the additional problem posed by the autonomy of the separate States. There are powerful pressure groups opposed to the granting of statutory registration in any case, and it is entirely possible that this will never come to pass. What may achieve a similar effect in some ways, however, is the accreditation of laboratories, which is a matter currently being studied. The terms under which this measure will eventually be introduced are still open to conjecture, but it is likely that there will be regulations governing the classes and qualifications of persons who may be employed in diagnostic laboratories, as well as some kind of periodical assessment of performance, requiring certain minimum standards of accuracy to be met as a prerequisite for accreditation and for its continued retention.

I have made no reference here to the achievement of automation and to the

increasing use of computer technology in the laboratory, much of which has taken place during the time span under review. Perhaps, indeed, we should be more profitably occupied if we were looking towards the *next* nine years, rather than indulging in nostalgia about the last nine. Maybe we shall become redundant within that time, or at least reduced to mere machine-minders. What with these test kits the commercial companies are constantly developing, the ever-expanding range of the Ames Company's stick tests and even the biochemical reactions of bacteria being determined by means of expensively packaged and gimmicky commercial reagent strips, an ever increasingly academic education would seem to be becoming unnecessary. With no reagents to make up, no complex chemical reactions to understand, an array of foolproof machinery to start up each day and the dipping in of a stick, followed by the recording of a simple colour change, the principal manual manipulation required, we could even find ourselves replaced by an army of partly trained chimpanzees. Truly the year 1984 could prove more horrifying for us than George Orwell could ever have imagined.

Glucose-6-Phosphate Dehydrogenase Zymograms on Cellogel

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Received for publication, August 1975

Summary

A technique is described for electrophoretic separation of G6-PD on Cellogel 200 thus avoiding starch gel electrophoresis. The zymogram development is through a conventional oxido-reductase coupling reagent phenazine methosulphate and colour change is produced by the reduction of a tetrazolium salt. The method is simple to operate and could be adapted to suit electrophoretic equipment in most clinical laboratories.

Introduction

Glucose 6-phosphate dehydrogenase (G6-PD) (D-glucose-6-phosphate: NADP oxidoreductase E.C. 1.1.1.49) is the first enzyme on the pentose phosphate pathway. It is through this pathway that cells generate much of their NADPH required for biosynthetic pathways. In the erythrocyte, NADPH produced by this enzyme reaction is utilised as the coenzyme for glutathione reductase (E.C. 1.6.4.2.) which maintains glutathione in its reduced form, and this enables the cell to protect itself from oxidative damage. Apart from possible involvement in amino acid transportation, this appears to be the main role of glutathione. A deficiency in the power to generate NADPH will thus leave the cells prone to oxidative destruction. Over eighty variants of G6-PD have been reported in humans (Yoshida 1966).[3]

Rattazzi *et al.* (1967)[2] described a method for electrophoretic identification of G6-PD isozymes on cellogel. The technique described below is based on the same methodology but incorporates semipurification of the enzyme and a shorter separation time.

Materials and Methods

All chemicals used were of Analar quality

Sodium phosphate buffer A: 0.005M, pH 6.4-6.5

Sodium phosphate buffer B: 0.1M, pH 5.8, containing 0.5M sodium chloride

Cellogel 200: a modified cellulose acetate (Chemetron, via 6 Modena 24, 20129, Milano. Italy)

A2 Protion: an ion exchange cellulose resin (Tasman Vaccine Laboratory Ltd., N.Z.)

Power Source: Shandon Vokam SAE -2761 (stabilised power output, 0-400V and 0-80 mA.)

Electrophoresis: Shandon model U77 (internal dimension 24 x 26 cm with variable bridge length)

Electrolyte buffer: TRIS 0.0614 M; EDTA (acid) 0.004M; Citric Acid 0.0136 M, pH 7.5.

Solutions for zymogram reaction: Meera Khan (1971)[1]

A. Tris/HCl/EDTA. Buffer
Tris 12.11 g
Disodium EDTA 0.149 g
Adjust the pH to 8.6 using 1:1 HCl, bring the final volume to 100 ml.
Store at 4°C.

B. NADP
NADP disodium salt 4 mg/ml
Store at -20°C

C. G6-PD
G6-PD disodium salt 20 mg/ml
Store at -20°C

D. MTT tetrazolium bromide
MTT 2.0 mg/ml
Store at 4°C in the dark

E. PMS
Phenazine methosulphate 0.4 mg/ml
Store at 4°C in the dark

F. Ionic solution
$MgCl_26H_20$ 2.033 g/100 ml

G. Zymogram reaction solution:
Mix 1.25 ml of A and 0.5 ml of B to F inclusive
Prepare fresh and keep in the dark until used
(This is sufficient for one 5.7 x 14 cm Cellogel strip).

Haemolysing solution:

10 ml of neutralised 10% EDTA
5 ml of 2 mM NADP
0.5 ml of β-mercaptoethanol

Make up to one litre with distilled water.

Preparation of haemolysates: (all procedures carried out at 4°C unless otherwise stated).

The plasma is removed from 5 ml of blood and the cells washed three times in physiological saline. The volume is then made to 10 ml with haemolysing solution, mixed well and allowed to stand in an ice bath for 15 min, with occasional mixing. One ml of carbon tetrachloride is added, followed by rapid mixing on a vortex test tube mixer. The haemolysate is allowed to stand for 15 min with occasional mixing during this period. The solution is then centrifuged at 15 000 g for 20 min, and the supernatant is removed and semipurified as described below.

Semipurification of G6-PD using A2-Protion

The barrels of 10 ml syringes are used as chromatography columns, being filled with washed A2 Protion. The columns are equilibrated with 50 ml of phosphate buffer A. Five ml of haemolysate is applied to the column and eluted with phosphate buffer A until all red colouration is removed. This step removes nearly all the haemoglobin and most of the 6-phospho-gluconate dehydrogenase (Yoshida, 1973)[4].

Glucose 6-phosphate dehydrogenase is then eluted from the column with phosphate buffer B. Fractions of 3 ml are collected, most of the activity being in the first five tubes. The tube with the maximal activity or maximal absorbance at 280 nm is used for the zymogram.

Electrophoretic procedure

One strip of 5.7 x 14 cm Cellogel 200 is soaked in the electrolyte buffer in the electrophoresis tank for 30 min, then removed and excess buffer blotted off before placing in position across the tank. Wicks 24 cm wide are made from Whatman 3MM chromatography paper. The current is applied for 10 min followed by application of the nine samples, the two end ones being controls. Approximately 4-5 μl of sample is applied for each test. A marked dye, bromophenol blue, is spotted onto the origin and the power turned on to 200 volts using the constant current mode. The run is stopped when the marker dye has reached the opposite wick (generally at 45 min).

Zymogram colour development

One piece of Cellogel is placed on a glass slide with its absorbent side uppermost, covered with solution G and the excess blotted off with a strip of Whatman number 1 filter paper cut to the size of the Cellogel. After electrophoresis the Cellogel strip is placed on top of the impregnated Cellogel (absorbent sides facing) and covered with the filter paper. These are compressed between two glass plates which are then wrapped in aluminium foil and placed in the 37°C incubator. After 30 m incubation the colour has developed sufficiently well to measure the bands or photographically record the results.

Results and discussion

This technique was used to study over 320 sheep in a trial on biochemical differences in Romney sheep selected for high and low

fertility since 1948. Only the one phenotype was detected in these sheep but comparisons of electrophoretic mobilities between cows, humans and two types of yeasts were made. It was found that the electrophoretic patterns obtained on the 45-minute run on Cellogel gave separation equal to several different starch gel techniques which required eight hours running.

The electrophoretic procedure described above is an improvement upon the method described by Rattazzi *et al.* (1967)[2]. The two major advantages of this modified procedure are: semipurification of the G6-PD prior to electrophoresis and the reduced running time required to separate the different G6-PD isozymes and enzymes obtained from different yeasts and animals.

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4. Yoshida, Akira (1973), *Science, N.Y.* **179**, 532.

Addendum. Clarification was sought on three points arising from this article.

1. Could heterozygotes be missed by taking only one of the 3 ml samples? The small column was very quickly equilibrated with the elution buffer. When this was tested experimentally on many of the sheep only one form was found.

2. How is protion washed? According to the manufacturer's recommendations. Wash A2 protion with 0.5M NaOH, then with water until about pH9. Wash in 0.5M HCl and then with water until about pH4. Wash again in 0.5M NaOH, and then with water until about pH9. It is then ready for equilibration.

3. Should the current rather than the voltage be given if a constant current is being used? For this system, setting the current according to the voltage reading of 200V at the start of the run gave very good standardisation between the runs.—*Editor.*

G-6-PD Deficiency: A Case Report

J. A. Pountney, ANZIMLT.

Haematology Department, Hamilton Medical Laboratory, Hamilton.

Based on a paper presented to Hamilton Branch Seminar, May, 1974.

Summary:

A 5-year-old Caucasian male developed an acute haemolytic anaemia which responded to blood transfusion therapy.

10 months after the onset of this illness, a routine blood count was carried out in this Laboratory. This showed a haemoglobin of 11.1 g/100 ml, reticulocyte count 9 percent and examination of the blood film showed slight polychromasia with an occasional spherocyte.

In view of the reticulocyte count with only slight indications of haemolysis in the film, an enzyme screen was carried out. This revealed a markedly reduced glucose-6-phosphate dehydrogenase (G-6-PD) level.

Further tests, including full haemolytic surveys and family studies, were performed.

Introduction:

Erythrocytes are virtually completely dependent on the pentose phosphate pathway for supplies of reduced nicotinamideadenine dinucleotide phosphate (NADPH). The importance of NADPH to the economy of the cell is related to the fact that it acts as a co-enzyme for the reduction of oxidised glutathione (GSSG) to reduced glutathione (GSH). GSH in turn protects protein sulphydryl groups within the erythrocyte, primarily those of haemoglobin, against oxidation.

G-6-PD deficiency is normally sex-linked, i.e., the gene for the defect is carried on the X chromosome. The fact that it is sex-linked has been confirmed by showing close linkage of G-6-PD deficiency with colour blindness — another X-linked gene. Beutler (1971)[1]. Full expression of the trait occurs in hemizygous males where the single X chromo-

some carries the mutant gene, and in homozygous females where both the X chromosomes carry a mutant gene. Intermediate expression is found in heterozygous females.

The defect has a high incidence in Negroes but also occurs in a number of non-Negro races, and is seen most commonly in Mediterranean peoples, e.g., Sardinians, Greeks and Sephardic Jews[3]. In general, the deficiency of the enzyme is more marked in non-Negroes and may not be self-limiting as with Negroes. Individuals who inherit the deficiency appear to be clinically normal unless they ingest certain drugs, e.g., antimalarial drugs. Other predisposing factors to a haemolytic attack in the absence of drugs include infections and diabetic acidosis[3].

There are two major molecular forms of human G-6-PD in erythrocytes. These are genetically controlled and are designated B+ and its major variant A+. They are distinguishable electrophoretically. Molecular difference is limited to a single amino acid substitution, i.e., asparagine in B+ for aspartic in A+. Harris *et al.* (1970)[4]. All Caucasians, with rare exceptions, have the B type enzyme: B+ being normal activity. B— being deficient activity. 18 percent of American Negroes have the A+ enzyme. All American Negro males hemizygous with respect to G-6-PD deficiency have the A type which is labelled A—. The remaining American Negro males have the B enzyme.

In addition, there is a group composed of Negroes and Caucasians in whom there are a number of variant G-6-PD molecules based on electrophoretic mobility, i.e., fast, normal or slow, pH optimum, temperature stability and Km values for nicotinamideadenine dinucleotide phosphate (NADP) and glucose-6-phosphate (G-6-PO_4). Km is a constant derived from the level of substrate at which the enzyme manifests one half of its maximum velocity during electrophoresis[4]. In the final analysis, differentiation of variants from one another will depend upon identification of the specific amino acid substitution involved.

Case Report:

Investigation of the patient's history showed that in November, 1972, he was admitted to hospital with severe anaemia and vomiting. The haemoglobin was 4.9g/100ml and leucocyte count 40,000/cmm with a few metamyelocytes and an occasional blast cell. The platelets were normal. A bone marrow examination showed normoblastic hyperplasia (myeloid/erythroid ratio 0.34:1) and increased iron stores. These findings were considered to be consistent with acute acquired haemolytic anaemia in childhood, or Lederer's anaemia.

He was given a blood transfusion, to which he responded well, and was then discharged from hospital. However, 9 days later the patient was readmitted with pallor, tonsillitis and vomiting. He was said to have been quite well 4 days prior to admission, but then became very pale, weak and anorexic, and had a palpable spleen as well as palpable nodes in the neck and groin. The haemoglobin was 4.5 g/100 ml, leucocyte count 28,000/cmm, platelets 510,000/cmm and there were 12 erythroblasts/100 leucocytes. Two units of blood were given and he again responded well. The following month, and from then on, regular haematological assessments were made: Table I. There was found to be a continuing, persistent reticulocytosis with a few spherocytes and polychromatic cells in the blood film. A 24-hour Osmotic Fragility Test was done and found to be slightly increased.

In March, 1973, the patient's tonsillar lymph nodes became palpable once more and a course of penicillin was suggested. The following month he developed tonsillitis again, and it was considered that these infections were probably contributing to his anaemia.

Early in June, 1973, the patient was admitted to hospital for a tonsillectomy. He was mildly anaemic and was given one unit of fresh blood on the day prior to his operation and another unit post-operatively. His haemoglobin on being discharged was 14.5 g/100 ml.

In July he was again anaemic and the following month was tested at this Laboratory, when his G-6-PD level was shown to be reduced. A week later blood counts and full haemolytic studies were carried out on the patient and his mother, father and 3-year-old sister. Results of the haemolytic study on the patient are shown in Table II.

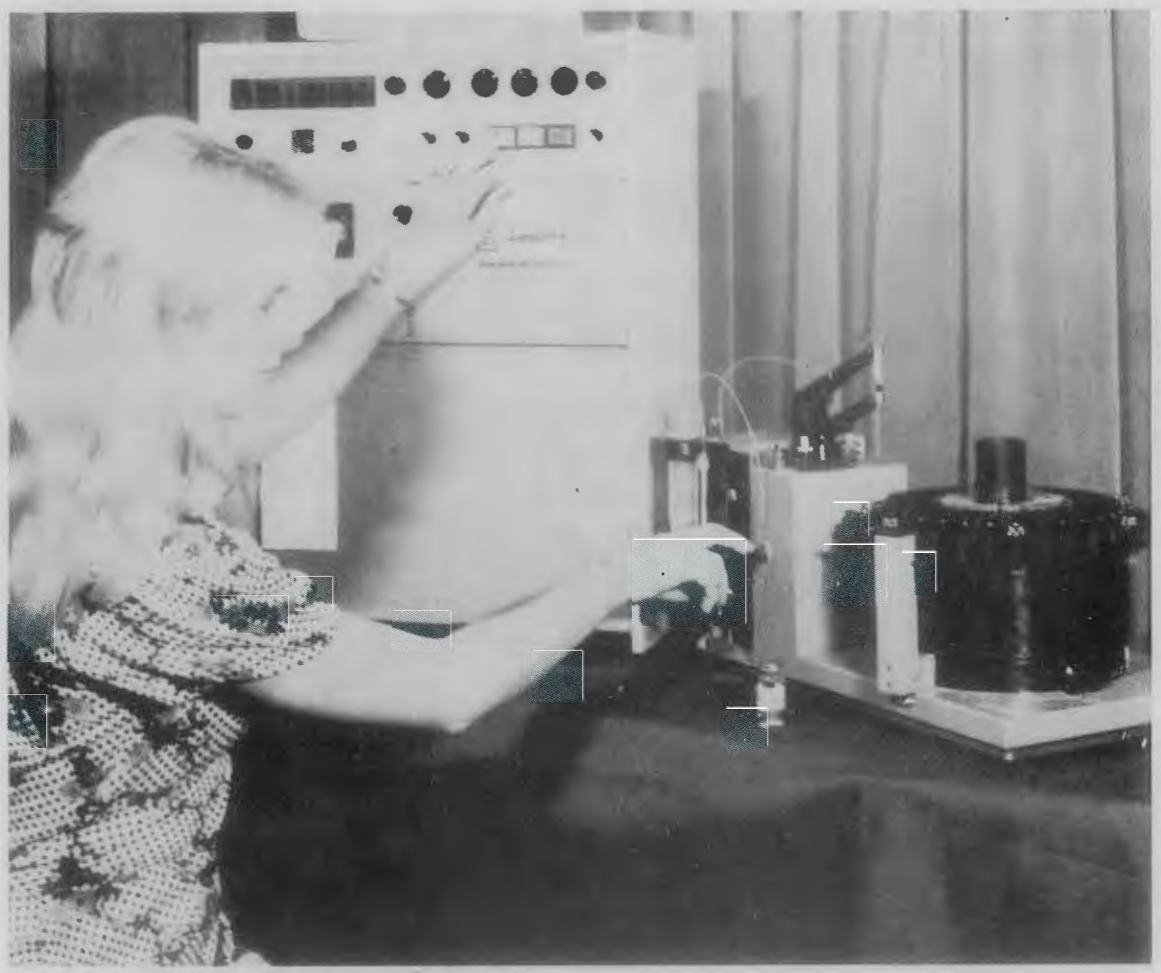

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Table 1

	Haemoglobin g/100ml	Reticulocytes %	Leucocyte Count/cmm	Platelet Count/cmm	Bilirubin mg%	Haptoglobin mg%
November, '72	4.5	—	28,000	510,000	0.6	—
Post Transfusion	13.0	—	9,000	—	—	—
December, '72	11.5	8.1	10,000	—	—	—
January, '73	10.7	9.9	7,000	—	0.9	50
March, '73	10.5	10.2	—	270,000	1.4	160
April, '73	10.1	8.2	7,000	—	0.8	95
June, '73						
Pre-operative	9.7	5.8	12.000	215,000	—	—
Post Tranfusion	14.7					
Post-operative	10.0					
Post Transfusion	14.5	2.4	7,000	160,000	—	—

The Direct Coombs was negative on three occasions during this time and in both January and March the Osmotic Fragility Test was slightly increased.

Table II.

		Normal Range
Haemoglobin	10.8 g/100 ml	
Reticulocytes	9.1%	0.2-2.0%
24hr Osmotic Fragility	Test MCF — 0.6% NaCl	
	Control MCF — 0.54% NaCl	0.465.-0.590% NaCl
48hr Autohaemolysis	Test Control	
With Glucose	2.5% 0.7%	0.0.9%
Without Glucose	6.0% 0.7%	0.2-2.0%
G-6-PD	Gross deficiency	
Pyruvate Kinase	Normal	
Heinz Bodies	None demonstrated	
Hb 'H' Inclusions	None demonstrated	
Haemosiderin	Negative	
Sucrose Lysis Test	Negative	
Hb Electrophoresis	Normal	
Hb F	Less than 1%	Less than 2%
Total Bilirubin	0.8 mg/100 ml	0.1-1.0 mg/100 ml
Direct Bilirubin	0.1 mg/100 ml	0.1-.0.2 mg/100 ml
Haptoglobins	108 mg/100 ml	40 - 180 mg/100 ml
Direct Coombs	Negative	
Antibody Screen	Negative	
Other Tests		
Iron	54 μg/100 ml	60 - 180 μg/100 ml
Iron Binding Capacity	290 μg/100 ml	240-420 μg/100 ml
Alkaline Phosphatase	212 units	50 - 300 units
SGPT	6 units	5 - 55 units
SGOT	9 units	5 - 48 units

Results of Haemolytic Study on Patien

Table III shows the first group of reactions of the pentose phosphate pathway where, in normal erythrocytes, about 10 percent of their glucose is metabolised. In our screen method for G-6-PD (Sigma Kitset), a quantity of haemolysate is added to G-6-PO4, NADP and cresyl blue contained in a vial. As G-6-PD catalyses the reaction, the NADPH formed reduces the blue dye to a wine colour. The rate at which reduction takes place is proportional to the G-6-PD content of the erythrocytes.

Normal: complete decolourisation in 20 - 100 minutes.

Table III

$$\text{Glucose} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G-6-PD}} \text{6-phosphogluconate} + \text{NADPH}$$

$$\text{6-phosphogluconate} + \text{NADP} \xrightarrow{\text{6-P-GD*}} \text{ribulose-5-phosphate} + \text{NADPH} + CO_2$$

* 6-phosphogluconic dehydrogenase

Partial Deficiency: partial decolourisation in 20 - 100 minutes, with complete decolourisation in 6 hours.

Gross Deficiency: dark blue colour remaining after 6 hours.

The patient's cells demonstrated a gross deficiency. All results obtained on testing his mother's, father's and sister's blood were completely normal. Heparinised blood samples were also obtained and sent to the Department of Haematology, Wallace Laboratory, Auckland Hospital, for G-6-PD and glutathione reductaste (GSSG-R) assays[6]. Table IV shows the results obtained. The Wallace Laboratory also carried out methaemoglobin reduction tests[2] to demonstrate intracellular enzyme activity with the aim of showing an abnormality in the patient's mother. However, no abnormality was found in the cells of his mother, father or sister. The majority of the patient's cells were abnormal.

Investigation of the patient's family history has shown that both parents have had no history of anaemia. Both sets of great-grandparents are deceased — one set having come from Scotland — the other from Ireland. Both sets of grandparents live in New Zealand but have not been tested and, at this stage, no other relatives have been contacted.

Since the diagnosis was established his haemoglobin has ranged from 10.8 - 11.3 g/100 ml, reticulocytes from 4.1 - 9.1 percent and his blood film has consistently shown slight polychromasia with a few spherocytes.

In September, 1973, blood samples from the patient and his mother and father were sent to the United States for testing to see whether the deficient enzyme fitted into any of the documented variant groups. The tests were carried out under the direction of Dr Mentzner at the School of Medicine, University of California, San Francisco.

The G-6-PD and 6 phosphogluconic dehydrogenase (6-P-GD) content of the erythrocytes and leucocytes was determined spectrophotometrically. There was found to be virtually no G-6-PD activity in the patient's cells, whereas the 6-P-GD activity was normal.

Table IV

G-6-PD and Glutathione Reductase Assays

	G-6-PD Screen	G-6-PD Assay	GSSG-R Assay
Patient	Abnormal	1.0EU	10.2EU
Mother	Normal	15.6EU	6.5EU
Father	Normal	19.9EU	8.0EU
Sister	Normal	18.4EU	10.7EU
Control	Normal	18.8EU	6.5EU

Normal Ranges: G-6-PD 14.2 - 22.0 EU
GSSG -R 4.6 - 9.4 EU

EU = μ moles of NAD (NADH) or NADP (NADPH) reduced (or oxidised) per minute per 3.2g of haemoglobin.

Because there was no active G-6-PD, partial purification of the enzyme for more definitive biochemical studies could not be carried out. However, an attempt was made to demonstrate the presence of mutant G-6-PD on cellulose acetate electrophoresis at alkaline pH. The patient had no stainable G-6-PD activity, whereas his mother and father exhibited normally migrating G-6-PD in the B position.

The School of Medicine feels that these results are consistent with the patient's mother being a heterozygote for a mutant and normal G-6-PD, with the mutant enzyme failing to appear on electrophoresis. From these studies, they are certain that there is either a marked suppression in the synthesis of G-6-PD or, more likely, the enzyme synthesised is remarkably unstable. In their experience, the only way to obtain information regarding the nature of the mutant G-6-PD in this circumstance is by immediate partial purification of the enzyme from freshly obtained leucocytes.

Unfortunately the long transit time between New Zealand and San Francisco limits the possibilities of further studies.

Conclusion

Early diagnosis of G-6-PD deficiency is important to prevent the institution of unnecessary treatment, and although G-6-PD deficiency is a rare disorder, enzyme screens should always be included in haemolytic studies, especially when the cause of a haemolytic process is in question.

Acknowledgments

I wish to thank the Pathologists of Hamilton Medical Laboratory for allowing this work to be published, Mr C. S. Shepherd for his encouragement and advice, and Mr R. Anderson of Auckland Hospital Laboratory for carrying out some of the assays; also Dr W. Mentzner of the School of Medicine, University of California, San Francisco, for the work carried out in his laboratory and for his advice, and Miss Mary Oliver for typing the article.

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The Haemolytic Anaemias — Part 1

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Introduction

A haemolytic anaemia may be defined as an anaemia resulting from an increase in red cell destruction. Normally the average life span of a red cell is about 120 days and the normal state of the blood is maintained by a balance of production and destruction rates. However, shortening the erythrocyte lifespan does not necessarily result in an anaemia as compensatory bone marrow hyperplasia may increase red cell production sufficiently to maintain a normal haemoglobin level. Anaemia therefore develops when destruction exceeds the rate of production. Haemolytic anaemias are characterised by:

(1) Increased rate of breakdown of haemoglobin.

(2) Increased rate of production of erythrocytes.

Clinically the above principles are represented by the triad of anaemia, splenomegaly and acholuric jaundice and as such may present in four principal ways:

(A) As the classical triad

(B) With one or more of the triad missing
  (1) Obscure anaemia
  (2) Splenomegaly without anaemia
  (3) Jaundice

(C) As a secondary complication of some primary disease process

(D) As an aplastic crisis.

Classification of Haemolytic Anaemias

Various classifications have been attempted for the numerous categories of haemolytic anaemias. Dameshek[10] was the first to define them on the basis of a congenital or acquired aetiology and this approach has been favoured by Dacie[6]. However De Gruchy[11] considers it better to define haemolytic anaemias on the basis of whether or not the anaemia is due to an intracorpuscular, intrinsic abnormality or an extracorpuscular, extrinsic abnormality. However, agreement only appears between haematologists on the failings of all classification methods.

Normal Erythrocyte Destruction and Breakdown

As stated previously the normal erythrocyte life expectancy is about 120 days. The mechanism of erythrocyte destruction is poorly understood but it is thought that changes occur in the erythrocyte which render it susceptible to phagocytosis by the cells of the reticuloendothelial system, particularly those of the spleen. It has been clearly shown that enzymatic systems upon which the erythrocyte depends for energy production fail upon cell aging.

Upon phagocytosis the haemoglobin of the effete erythrocyte is released and split into its constituent parts within the phagocytic cell. Protein globin is split from haem and returned to the body protein pool where it is utilised. Haem is broken down to porphyrin and iron, the iron fraction passing into the blood where bound to transferrin it is taken to the bone marrow or centre iron stores for utilisation. However porphyrin is broken down within the phagocytes to bilirubin which passes into plasma, loosely complexes with albumin to be taken to the liver where it is conjugated with glucuronic acid to form bilirubin glucuronide. This is excreted via the hepatic ducts to the intestine where it is reduced to stercobilinogen by bacterial flora of the colon and either excreted as such or reabsorbed, passing to the liver and thus completing the enterohepatic circulation of bile pigments. Hence it is re-excreted, although a small quantity of absorbed stercobilinogen passes to the kidney and there is passively excreted in urine as urobilinogen.

Diagnosis of Haemolytic Anaemia

Basically one initially must decide whether the anaemia present is haemolytic in nature, and if so what the aetiology is of that process. The haemolytic nature of the process may be seen in evidence of increased haemoglobin breakdown and bone marrow regeneration. Also present may be evidence of direct or indirect damage to erythrocytes. Present at all times will be a diminished erythrocyte lifespan.

Increased haemoglobin breakdown may be demonstrated by hyperbilirubinaemia, increased faecal and urinary bile pigments, reduced plasma haptoglobins and complement. Various attempts have been made to correlate the degree of haemolysis with these parameters[14]. Evidence of intravascular haemolysis may be demonstrated by the presence of haemoglobinaemia, haemoglobinuria, haemosiderinuria and methaemoglobinaemia. Compensatory erythropoietic hyperplasia may be noted by a reticulocytosis, erythro-blastaemia and erythroid hyperplasia of the bone marrow. In cases of congenital haemolytic anaemia erythropoietic hyperplasia may result in radiological changes such as skull vault thickening. Damage to erythrocytes may be noted in a peripherial blood smear by the presence of fragmented cells or by specific laboratory techniques such as the osmotic fragility test. Decreased survival of erythrocytes may be demonstrated using radio-active chromium, ^{51}Cr. [17, 35].

The various types of haemolytic anaemias are numerous and discussion of the clinical and laboratory findings of the most commonly found haemolytic anaemias follows.

Hereditary Spherocytosis

This form of haemolytic anaemia occurs due to an intrinsic defect in the erythrocyte which results in the cell being spherical in shape. It is most probable that this defect lies in the erythrocyte membrane and relates to an increased permeability to sodium with loss of surface lipid leading to spherocytosis. Selective trapping of the spherocyte occurs in the spleen and if compensatory mechanisms fail an anaemia results. Recently an animal model very similar to the disease in man has been demonstrated[15].

Clinical Findings

This disorder is a Mendelian dominant, being commonest in those of European stock and rare in Negroes. Patients may present with the symptoms of anaemia and/or jaundice, splenomegaly or complications of cholelithiasis. Splenic infarction, leg ulceration, haemolytic crises or repeated epistaxis are rarer presentations. The more severe the degree of spherocytosis the earlier in life the presentation generally, and most cases present before the age of ten years.

Jaundice which occurs does not necessarily parallel the degree of anaemia and seldom is greater than 5 mg dl^{-1} except in a haemolytic crisis. There is no bilirubinuria unless there is associated biliary obstruction. Urobilinogen levels may be increased. The spleen is usually palpable and invariably is firm and nontender although tenderness may occur during a crisis.

The degree of haemolytic crisis may vary in intensity and duration and if severe may be predominantly due to a failure of erythropoiesis. Gallstones if present are of a pigmented nature and occur in about 50 percent of cases. Leg ulceration, invariably

above the malleoli, may occur and this readily heals after definitive treatment. Rarely skeletal abnormalities occur, the most common being a tower skull; epistaxis of a recurrent nature is predominantly found in childhood.

Blood Picture

This is typically an anaemia with spherocytosis, increased osmotic fragility, reticulocytosis, hyperbilirubinaemia and a negative antihuman globulin (Coombs) test.

The level of haemoglobin varies depending on the degree of haemolysis and compensation, and usually lies above 7 g dl^{-1}. Spherocytes are typically seen in the blood picture, with anisocytosis and polychromasia. The mean cell volume (MCV) and mean cell haemoglobin (MCH) are usually normal but the mean cell haemoglobin concentration (MCHC) is invariably increased. The reticulocyte count is raised with values of between 5 to 70 percent being found. Normoblasts may be seen in the peripheral blood. Osmotic fragility is increased with various curve shapes dependent on the degree of spherocytosis. Tests for erythrocyte mechanical fragility and autohaemolysis are also increased. Haemoglobin electrophoresis is normal.

Diagnosis

This is based on the clinical and haematological findings plus family history. Diagnostic difficulty may occur with milder cases but testing for autohaemolysis in most of these cases is conclusive. Specific diagnosis from other haemolytic anaemias is important because of the specific treatment available.

Treatment

Splenectomy is the treatment of choice and results in a sustained clinical remission. However splenectomy in children is contraindicated because of resultant susceptibility to severe infection. Cholecystectomy may be necessary if cholelithiasis has occurred.

Splenectomy results in cessation of haemolysis, loss of anaemia, normal growth where this has been retarded, and healing of leg ulceration. Spherocytosis however remains but the cells have a virtually normal lifespan. Relapse after splenectomy is very rare and its occurrence immediately casts doubts on the initial diagnosis.

Blood transfusion may be required if anaemia is severe and as the transfused cells have a normal lifespan the temporary response to transfusion is good. In haemolytic crises hyperbaric oxygen therapy has been found to be of use[25]. Barbiturates have been used to induce glucuronyl transferase and have been noted to also reduce the degree of haemolysis[27].

Hereditary Elliptocytosis

This uncommon disorder is characterised by the finding of elliptical erythrocytes in peripherial blood. Inheritance is as a Mendelian dominant and is found to be transmitted with the gene(s) of the Rhesus blood group system. The degree of elliptocytosis is variable but is usually over 50 percent, and does not occur until after the first three months of life. Small numbers of irregular microcytes and microspherocytes may be present. The MCV is normal or slightly reduced and the MCHC is normal. Haemoglobinopathy cannot be demonstrated.

Clinical Findings

Clinically three states may be recognised:—

(1) Asymptomatic elliptocytosis with no anaemia or haemolysis.

(2) Elliptocytosis with adequate compensatory bone marrow hyperplasia.

(3) Elliptocytosis with anaemia which may be moderate. Splenomegaly may be found and splenectomy usually results in clinical cure. Laboratory findings include a moderate reticulocytosis and slightly increased osmotic fragility.

Generally there is no relationship between the degree of elliptocytosis and that of haemolysis.

Diagnosis

This depends on family history, clinical findings and more definitively on peripheral blood examination. Differential diagnosis includes symptomatic ovalocytosis which may be seen in a variety of haematological disorders such as iron deficiency anaemia, thalassaemia and leucoerythroblastic anaemia.

Treatment

In the more severe cases splenectomy may be of help but this is not a general rule. Symptomatic treatment by supplement therapy such as iron or rarely blood transfusion may be necessary. In the majority of cases which are asymptomatic only assurance is required.

Non Spherocytic Congenital Haemolytic Anaemias

This broad group of haemolytic anaemias

has several features common throughout its subgroupings.

(1) The osmotic fragility of fresh blood is usually normal.

(2) Splenectomy is only at best of moderate benefit.

(3) Haemoglobinopathy cannot be demonstrated.

Classification

This has initially been achieved on the results of autohaemolysis with or without added glucose:—

(A) Type I cases have normal autohaemolysis and added glucose decreases autohaemolysis but not to the degree found with normal blood.

(B) Type II cases have abnormal autohaemolysis which is greatly increased and is not reduced by added glucose.

However in most cases of this group of haemolytic anaemias a specific enzyme deficiency has now been demonstrated[32], and these cases are best classified separately under the general heading of enzymopathies: two of the more common forms are discussed below.

Pyruvate Kinase (PK) Deficiency

This disease is inherited as an autosomal recessive trait, and is found predominantly in Northern European stock. Heterozygotes have no clinical or haematological manifestations of the disease.

Clinical Features

Variation in severity of the disease is typically found with greater haemolysis occurring early in life and becoming less severe upon reaching adulthood. Anaemia, jaundice, hepatosplenomegaly and cholelithiasis are common features on presentation.

Blood Picture

Anaemia may be severe with a compensatory reticulocytosis, macrocytosis, anisocytosis and slight poikilocytosis. Slight hypochromasia may be present also. Post splenectomy a sharp increase in reticulocytes occurs along with the characteristic findings of Pappenheimer bodies and siderocytes. The osmotic fragility is normal or slightly decreased: autohaemolysis may be increased and if present is not corrected by added glucose but is corrected by added ATP. The Coomb's test is negative, as are tests for Heinz bodies and acid haemolysis. Haemoglobinopathy cannot be demonstrated.

Diagnosis

This should be considered in any case of non spherocytic congenital haemolytic anaemia. PK activity of erythrocytes establishes the diagnosis.

Treatment

This consists of symptomatic treatment with blood transfusion while some benefit may be achieved by splenectomy[20].

Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD).

G6PD may be associated with haemolytic anaemia, particularly after exposure to certain drugs or chemicals. This condition is discussed later under Haemolytic Anaemias due to Drugs and Chemicals.

Hereditary Haemoglobinopathies

This grouping of haemolytic anaemias is characterised by impairment of synthesis of adult haemoglobin, and may be divided into two groups.

(1) Qualitative haemoglobinopathies which have a structurally abnormal haemoglobin molecule because of amino acid substitution and may be expressed by one of the following:

(a) A classical qualitative haemoglobinopathy, for example Hb-S disease.

(b) A haemolytic disorder associated with an unstable haemoglobin, for example congenital Heinz body anaemia.

(c) The formation of an unusually stable methaemoglobin derivative, for example Hb-M disease.

(2) Quantitative haemoglobinopathies which are due to defective production of α and β chains and hence defective production of adult haemoglobin and result in the thalassaemia syndromes.

Both quantitative and qualitative states may co-exist, for example sickle cell thalassaemia syndrome.

Haemoglobin Structure

Haemoglobin is a conjugated protein of four haem groups attached to globin. Haem is identical in all haemoglobin types but variation occurs within the protein moiety. The haemoglobin molecule consists of two halves, each consisting of two different polypeptide chains; in adult haemoglobin these are designated α and β chains. Normally occurring haemoglobin types are as follows:—

(1) Adult haemoglobin (Hb-A), composed of $\alpha 2\ \beta 2$ chains.

(2) Foetal haemoglobin (Hb-F), composed of $\alpha 2\ \gamma 2$ chains. This is predominant in the foetus and comprises up to 90 percent of haemoglobin at birth and gradually disappears by six months of age, although small amounts (less than 1 percent) may remain in adults.

(3) A minor adult haemoglobin (Hb-A_2), composed of $\alpha 2$ and $\delta 2$ chains which is normally present in amounts up to 3 percent.

The structure of a haemoglobin type may be obtained by fingerprinting and analysis of amino acid sequences of component peptides[18]. The substitution of one amino acid for another at a specific site on the haemoglobin molecule is sufficient to produce a haemglobinopathy. Haemoglobin types may be obtained by electrophoresis[8], alkali denaturation[31] and acid-elution cytochemical staining[21].

Sickle Cell Disease

This is a term applied to those hereditary disorders where the erythrocytes contain Hb-S which is less soluble than normal haemoglobin, particularly in the reduced state where classical sickle deformity of the erythrocyte results. Sickling is due to reorientation of the haemoglobin molecules which form tactoids (liquid crystalline masses). It may be demonstrated *in vitro* by the addition of reducing agents such as sodium meta-bisulphate.

The production of Hb-S is inherited as a Mendelian dominant, occurs mainly in Negroes but may be found in localised areas throughout the world. Various sickle-cell diseases have been recognised: the more common types are discussed.

Sickle Cell Trait

This asymptomatic carrier state for Hb-S may occur in up to 20 percent of certain populations, and represents the heterozygous state for Hb-S. Erythrocyte life span is normal, sickle cells are not seen in the normal blood smear, but may be readily demonstrated by reducing substances *in vitro*. Splenic infarction and haematuria may result in an hypoxic environment. Hb-S confers relative resistance to *P. falciparum* malaria.

Sickle Cell Anaemia

This represents the homozygous state for the Hb-S gene, and sickling readily occurs *in vivo* with resultant chronic haemolytic anaemia and vascular obstruction. Erythrocyte mechanical fragility is markedly increased and high viscosity results in thrombosis.

Clinical Features

Diagnosis usually occurs in childhood when symptoms of anaemia manifest clinically. Crises may occur and consist of bone or joint pain, abdominal pain, fever or nausea and vomiting, plus the features of haemolysis such as anaemia. Cholelithiasis is common and cerebral vessel obstruction may result in various neurological symptoms.

Patients are usually of slender build, of less than average height and may have a thoracic kyphosis and lumbar lordosis. Hypogonadism may be present. The conjunctiva are characteristically yellow-green, and hepatosplenomegaly is common in childhood. Compensatory cardiac enlargement may occur and recurrent pulmonary disease is common. Chronic leg ulceration is also common, and osteomyelitis susceptibility occurs in children. Skeletal x-rays usually demonstrate findings seen in haemolytic anaemias of hereditary type.

Blood Picture

Anaemia is moderate to severe, being of a normochromic, normocytic type. Anisocytosis and poikilocytosis occurs along with classical sickle cells. These is a variable reticulocytosis and occasional erythroblastaemia. Osmotic fragility is decreased and hyperbilirubinaemia occurs. Commonly found is a neutrophil leucocytosis with a shift to immaturity, and a thrombocytosis may be present. Characteristic Hb-S may be demonstrated by electrophoresis; there may also be an increase in Hb-F. Bone marrow displays erythroid hyperplasia with normal normoblasts.

Diagnosis

This is based on family, racial, clinical and haematological findings. Difficulty may be encountered when patients present with an acute abdominal emergency or with joint problems.

Prognosis

The future outlook for the patient with sickle cell disease is poor, but prognosis improves upon reaching adulthood.

Treatment

Basically this is supportive by means of transfusion and avoidance of precipitating causes such as fatigue.

To be continued in next issue

Examination of Renal Biopsies by Immunofluorescence

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Introduction

A national study of primary glomerular disease initiated, in July, 1973, has two objectives — to determine the incidence of different forms of primary glomerular disease and to study the aetiology and pathogenesis of this disorder in New Zealand. The achievement of these aims is largely dependent on the study of renal biopsy material. Where possible tissue from each biopsy is processed for light, fluorescence and electron microscopy. This paper describes the procedure developed in this laboratory for the examination of renal biopsies by immunofluorescence.

Division of Biopsy Material

Immediately a biopsy is obtained it is placed on dental wax and apportioned into four fragments as shown in Figure 1. This is done using a new single-edged razor blade (Gem), from which residual grease has been removed with alcohol or acetone. A dissecting microscope may be of assistance. The portion for fluorescence microscopy is placed in normal saline for transport to the laboratory.

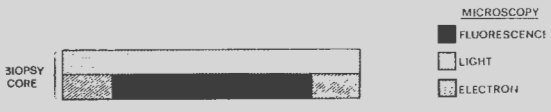

FIG 1 Division of renal biopsies for study

Embedding Technique

Embedding and sectioning of tissue for immunofluorescence is performed in conjunction with a Damon IEC Microtome-Cryostat, Model CTF. A brass specimen holder is placed in the refrigerated microtome cabinet, layered with a resinous embedding medium (Tissue Tek O.C.T. Compound, Ames) and allowed to freeze. A further smaller portion of resin is added and in this the biopsy is gently embedded. Freezing may be accelerated by the use of dichlorodifluoromethane (Freon), although care is needed to ensure that the force of the aerosol does not disrupt the position of the tissue in the embedding material. It is important that the biopsy is located on one plane to ensure that each section is representative of the fragment as a whole. Sectioning of small portions of tissue embedded in this manner is most effective as minimal trimming is required and the area of resin included with each biopsy section is small.

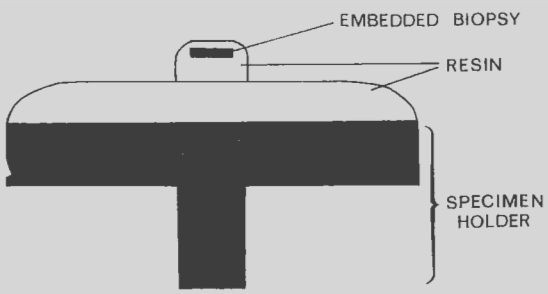

Fig 2: Preparation of biopsy for sectioning

Sectioning

The block is cut to expose the total area of embedded tissue and 24 sections are cut. These are placed on new numbered glass slides previously stored in surgical spirits. Each slide should also be etched with the histology and tissue number of the specimen. Slides 1, 12 and 24 are stained, using a modified haematoxylin and eosin (Paragon, Gurr) and examined for glomeruli. If no glomeruli are present a further numbered series of sections must be cut until glomeruli are detected or the biopsy is exhausted. Those sections containing the greatest number of glomeruli are selected for fluorescence microscopy and are stored under fanned air at 6°C overnight. Any renal tissue remaining after sectioning is complete is stored in appropriately labelled ¼oz McCartney (bijou) bottles at —75°C.

Staining Procedure

Test sections are removed from the refrigerator, placed in a perspex staining box, immersed in phosphate buffered saline (PBS, pH 7.3) and agitated periodically for 15 minutes. During this time unwanted embedding material is removed. The slides are drained and the sections thoroughly air-dried under fanned air at room temperature. The area of nine slides occupied by the sectioned biopsy is demarked on the underneath side with a black "Vivid" marker pen and the top end labelled similarly with the identity of the conjugates used. Fluorescein conjugated antisera are applied and slides are incubated in moist conditions at room temperature for 30 minutes. Excess conjugate is removed under a stream of PBS applied, using a polypropylene "squeeze" bottle. The slides are drained briefly, unwanted PBS removed with an absorbent cloth and the sections mounted under glycerol-PBS (pH 7.3) using 22 × 22mm or 18 × 18mm glass coverslips. The preparations should be examined microscopically as soon as possible after staining is complete. For this purpose a Reichart Zetopan Research microscope fitted with a 200 watt high pressure mercury vapour lamp and employing transmitted light fluorescence in conjunction with a dark-ground substage condenser is used.

The washing of sections prior to staining is important. It has been shown that O.C.T. compound contributes measurably to the development of nonspecific fluorescence. If problems with sections detaching from the slides during washing are encountered, a non-fluorescent slide adhesive can be utilised. 0.5 percent gelatin fixed with 10 percent formalin after application to the slide is most effective.[4]

Preparation and Use of Fluorescein Conjugated Antisera

Wellcome and Behringwerke AG reagents are purchased in 1.0ml lyophilised quantities, rehydrated with distilled water and working dilutions, if required, prepared with PBS. 0.25ml aliquots of each antiserum are placed in 75 × 11mm corked disposable tubes and conjugate panels, thus obtained, are stored at —30°C.

After thawing and prior to use, each conjugate is centrifuged at 2500rpm for five minutes to remove any fluorescent particulate debris which may accumulate during reconstitution and storage. The clear supernatants are transferred to clean labelled tubes. A new Pasteur pipette is used in conjunction with each reagent. The panel of fluorescein conjugated antisera currently used to stain renal biopsies is shown in Table I. Conjugated anti-hepatitis B antigen (HBAb) is a recent addition following reports[1, 2, 3] which have suggested a high incidence of HBsAg in chronic glomerulonephritis.

TABLE I.—Conjugated Antisera Panel

Manufacturer	Fluorescein Conjugated Antiserum to:	Working Dilution
Wellcome	IgG + IgA + IgM	10
Behringwerke	IgG + IgA + IgM	0
"	IgG	"
"	IgA	"
"	IgM	"
"	Beta I C globulin (C3)	"
"	Fibrin	"
"	Albumin	"
"	HBAg	"

Recording of Results

A cyclostyled worksheet (actual size, 21 × 15cm) shown in Figure 3 is used to record any immunofluorescence observed in the glomeruli, tubules and vessels of the stained biopsies. From this the final report is prepared and typed.

RENAL BIOPSY

PATIENT: AGE: SEX: WARD: H. / T. /
NUMBER: RIGHT: LEFT: PIECES

Conjugates	GLOMERULI	Tubules	Vessels
Polyvalent			
"			
IgG			
IgA			
IgM			
βIC			
Fibrin			
Albumin			
HBAg			
HISTORY (given)			
COMMENT			

Figure 3: Renal Biopsy Worksheet

Figures 4 - 6 are examples of specific glomerular staining using the technique described above.

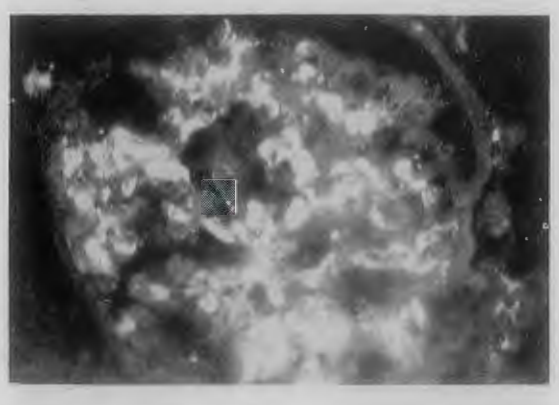

Figure 4.—IgA × 400. Widespread scattered collections of IgA are preesnt in the mesangium of a patient with IgA nephropathy.

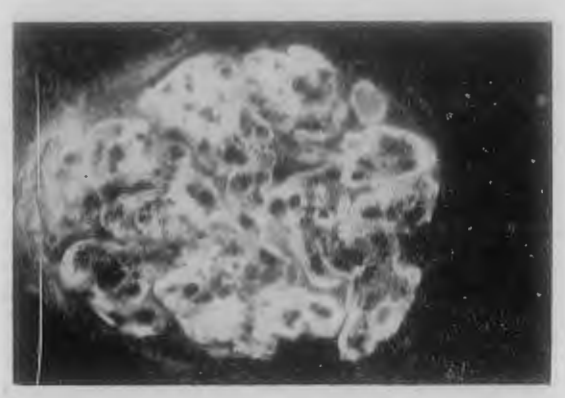

Figure 5.—IgG × 400. Extensive discrete and confluent granular deposits of IgG are present within the capillary loops of a patient with systemic lupus erythematosis.

Figure 6.—BIC × 400. Fine granular and coarse focal deposits are seen in the periphery of capillary loops of a patient with glomerulonephritis.

Acknowledgments

I am indebted to Professor A. R. McGiven for his assistance and constructive criticism.

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Addendum: Since this paper was forwarded for publication further fluorescein isothiocyanate conjugated astisera have been added to the panel. These include anti-IgA secretory piece (Dakopatts AS) used at zero and 10 dilutions and anti-C3 Activator (Behringwerke AG) which is conjugated domestically and used at zero and five dilutions. Conjugated anti-$C1_q$ serum will also be utilised routinely when it becomes available.

A Survey of Techniques in Anaerobic Bacteriology

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and D. Macdonald, ANZIMLT

Microbiology Department, Green Lane Hospital, Auckland.

Received for publication, April, 1975

Introduction

In November, 1974, a questionnaire on anaerobic techniques was circulated to 79 public hospitals and private medical laboratories in New Zealand. The purpose of this questionnaire was to obtain information from throughout the country on the type and popularity of anaerobic techniques at present being used in bacteriology departments. 56 (71 percent) laboratories replied and a summary is presented.

Results

The questionnaire consisted of three sections:

A *Blood Cultures*

All laboratories carried out routine anaerobic culture of blood. See Table I. The majority of laboratories, 43 (77 percent), incorporated liquoid in their blood culture media; 36 (64 percent) of the total also had other additives such as Para-amino benzoic acid (P.A.B.A.), penicillinase and β lactamase. 30 laboratories (54 percent) stated they had changed their methods over the last 3 years, 22 of this group now use B.D. vacutainer tubes, 5 thioglycollate, 1 Schaedlers Broth (BBL) and 2 gave no answer.

Table I

Types and brands of media used.

No.	%	Medium
26	(46%)	B.D. Supplemented peptone broth vacutainer tube (Becton Dickenson).
23	(41%)	Thioglycollate Medium
		BBL - 9 (Baltimore Biological Laboratory, Division of Becton Dickenson)
		Oxoid - 6
		No brand stated - 8
7	(13%)	Others:
		Schaedlers - 1
		BBL, BHI (Brain Heart Infusion) casteneda bottle ? 1
		Robertsons cooked meat + T.S.B. - 1
		Home-made type - 1
		No answer - 3

The period of incubation and the frequency of routine Gram staining of cultures is variable as shown in Table II. There is some overlap of total percentage as many laboratories stain on more than one of the given days. 14 (25 percent) of laboratories do not Gram stain routinely, or only if visible growth. 3 laboratories gave no answer.

23 (41 percent) laboratories isolated anaerobes, of these 17 reported *Bacteroides species* as their common isolate. 21 (38 percent) laboratories grew anaerobes, while 12 (22 percent) gave no answer. The remaining anaerobes reported were anaerobic streptococci (3), *Clostridium perfringens* (2), Bacteroides/Clostridium (1).

The type and number of anaerobes isolated compared with the common types of media used are presented in Table III.

B *Anaerobic Cultures -- other than blood*

The types of specimen cultured anaerobically are reported in Table IV.

Transport media was used by 48 (86 percent) of the laboratories surveyed; only 7 laboratories did not use transport media and one failed to answer. The most commonly used transport medium was Stuart's (39 or 70 percent). The type of primary anaerobic media used most commonly (22 laboratories, 39 percent) was a combination of blood agar anaerobically (AnO_2) and Robertsons cooked meat (RCM), followed by a number of other varieties:

12 (21%) Blood agar anaerobically
9 (16%) Robertsons cooked meat
3 (5%) Thioglycollate broth
10 (18%) Other combinations:
e.g. Cooked meat/thioglycollate
Blood agar/thioglycollate
Cooked meat/phenylethyl alcohol.

Table II

Days	1	2	5	7	10	14	>14
Length of incubation	—	—	1	6 (11%)	16 (29%)	23 (41%)	8 (14%)
Gram stain		24 44%	18 33%	11 18%	12 20%		

Table III

Thioglycollate Broth.		B.D. Supplemented P. Broth Vacutainer.	
Bacteroides sp.	11		4
Anaerobic strep.	3		0
Clostridia	2		0
No organisms	4		17
No answer	3		4
Miscellaneous	-	Clost./Bact.	1
Total:	23		26

Table IV

Specimen	Number tested	%	No. not tested	No answer
Deep wounds or abscess	56	100	—	—
Burns and ulcers	41	73	12	3
All pus	42	75	14	
Abdominal surgery specs.	54	97	—	2
Appendices	37	66	5	14
Sputa	5	9	48	3
Tracheal Aspirate	15	27	29	12
Urine	1*	1.8	53	2
CSF & related specs.	32	57	16	8
Cervical & urethral spec.	37	66	16	3
Pleural & other body fluids	50	89	5	1
Throat swabs	11	20	44	1

* Obstetrical and gynaecological specimens only.

The addition of antibiotics as a selective agent, e.g., neomycin sulphate, to the blood agar was carried out by only 7 of the 56 laboratories.

The types of agar base used were not well documented, however, those who did record the main types were: D.S.T., Oxoid No. 2, Mueller Hinton, Columbia, Tryptone soya and one laboratory used Brain Heart Infusion.

For the selective culture of anaerobes a variety of methods are reported: 22 laboratories (39 percent) use a blood agar (AnO_2), and an additive, e.g., neomycin sulphate, vancomycin, vancomycin/Kanamycin, menadione, dithiothreitol.

14 (25%) laboratories use a blood agar (AnO_2) only.
5 (9%) laboratories use Robertsons cooked meat only.
9 (16%) laboratories gave no answer.
1 laboratory used no selective medium.
1 laboratory used phenyl ethyl alcohol agar (P.E.A.).
1 laboratory used colistin, naladixic acid (CNA).
1 laboratory used Shaedlers and menadione.
1 laboratory used R.C.M./Thioglycollate.
1 laboratory used R.C.M./Clostrisel agar.

The times which laboratories maintain their primary culture are:

24 hrs. 13 (23%) laboratories
48 hrs. 36 (64%) "
72 hrs. 7 (13%) "
14 days Nil

56

The commonest anaerobes isolated from cultures other than blood are:

24 *Clostridium sp.*
11 *Bacteroides sp.*
10 *Bacteroides sp./Clostridium sp.*
2 Bacteroides / Clostridium / Anaerobic Streptococcus.
6 Clostridium/Anaerobic Streptococcus.
3 No answer.

56

Of those laboratories who maintain stock cultures of their anaerobic isolates, Robertsons cooked meat media was the most favoured, i.e., 32 (57 percent).

C *Technical Data*

The various systems and gases used to obtain an anaerobic atmosphere are documented in Table V.

12 laboratories used both Gas Pak system and McIntosh Fildes Jar. 26 laboratories, or 93 percent of those using McIntosh Fildes Jar, evacuate their jar prior to filling.

Time allowed to let catalyst of McIntosh Fildes Jar function before incubation varied considerably (see Table VI).

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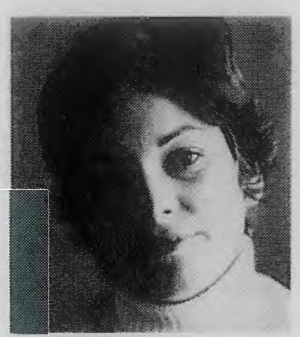
DCR/SD 5NZ/2

How would you have classified these two patients by your usual thyroid function profile.



Case 1: Female, age 32.

Presented with weight loss, palpitation, nervousness and tremulousness.
Examination revealed a restless and anxious woman with lid retraction, regular pulse and a grade 2 diffuse goitre.

Biochemical results:
January: T-3 Trilute 52%
T-4 Tetralute 6.8 mcg T-4/100 ml
FTI 7.2
Clinically hyperthyroid not confirmed by thyroid function tests.

November: ETR 0.98
T-3 RU 55%
T-4 7.0 mcg T-4/100 ml
Clinically hyperthyroid. Thyroid function tests normal.

May: T-4 5.2 mcg T-4/100 ml
T-3 RU 50%
AMES SERALUTE T-3 (RIA)* 760 ng 100 ml (N90-235). Clinically hyperthyroid. T-3 Toxic.
Treatment: Carbimazole.

Patient is an obvious T-3 thyrotoxic who could have been diagnosed 16 months previously if a total T-3 determination had been carried out using the Ames Seralute Total T-3 (RIA)* test.

Case 2: Female, age 42.

Presented February with weight loss, mild tachycardia with anxiety. Medication not known.
T-3 RU Trilute 43%
T-4 Tetralute 12.0 mcg T-4/100 ml
FTI 11.0
Hyperthyroid? Euthyroid on pill.

May: Weight loss continues, mild tachycardia with continuing anxiety.
T-3 RU Trilute 43% (40-60)
T-4 Thyrolute 12.0 (3.8-12.2)
T-4 N Thyrolute 1.25 (0.49-1.10)
Thyrolute confirms clinical finding of mild hyperthyroidism.

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THYROLUTE* – a test for total T-4 and normalised T-4 on the same column.

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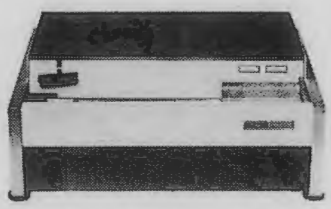
If you are shopping for a specimen delivery system that really delivers stop by the Ames window?

1: Ames Cyto-Tek* Slide Stainer

This is a fully automated system of unitized slide processing without the disadvantages of immersing racks of slides in a series of baths. A metered amount of fresh, unused stain (and

other solutions in sequence) is automatically delivered to each slide individually for a predetermined time, thus ensuring reproducibility of staining day after day, week after week.



3: Ames Hema-Tek* Slide Stainer

Automatically delivers haematological slides, stained to uniform depth, dimension and definition. It eliminates the need to prepare aged stains and gives reproducible slides of superior quality and dependability.

4: Ames Microtome Cryostat II*

Provides an optimal cutting environment to minus 30°C with improved frost-free interior, quick freeze bar and automatic defrost cycle.

2: Ames Tissue-Tek II* Embedding System

It melts, delivers and solidifies the paraffin and allows accurate orientation of the specimen in the paraffin matrix.

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Table V

	Total	Gas Employed
McIntosh Fildes Jar	28	22 pure hydrogen
		1 pure nitrogen
		1 80% N_2, 10% H_2, 10% CO_2
		1 97% CO_2, 3% H_2
		3 90% H_2, 10% CO_2
Gas Pak system	40	38 CO_2 + H_2 generator envelope.
		4 add own gas supply
		3 use both methods
		1 no answer.

Table VI

Minutes	Laboratories
5	5
10	4
15	6
30	5
Nil	7
No answer	1
Total:	28

16 laboratories (29 percent) use pre-reduced agar plates to assist in isolation of anaerobes.

13 laboratories (23 percent) indicated use of additives to assist in the reduction and enrichment of media.

8 L-cysteine
6 menadione
1 dithiothreitol
3 palladium chloride
1 sodium thioglycollate

3 laboratories used more than one of the above agents. One laboratory used, for pre-reduced plates only, oxygen impermeable bags with O_2 absorbing catalyst. All laboratories use some form of indicator for control of anaerobic environment. The majority by far use a chemical indicator, as only 7 laboratories indicated using a culture of *Cl. perfringens*, *Cl. tetani*, Bacteroides; usually in conjunction with commercial indicators. All others used methylene blue and/or commercial indicator.

Discussion

Over the past 12 months there has been a greater emphasis and awareness of techniques in anaerobic bacteriology, and it is with this in mind that the following comments are made.

It is not the intention to criticise any individual technique or method, but to gather information on procedures used throughout the country in anaerobic bacteriology.

(a) *Blood Cultures*

In this survey the most frequently used method is the B.D. Supplemented peptone broth vacutainer, of which 22 (40 percent) laboratories have changed to within the last three years. Not many laboratories gave full information on the brand and type of Thioglycollate they used, therefore limiting any comments on this. Of those laboratories having additives there is no significant breakdown of the type of isolates, e.g., liquoid inhibition of anaerobic streptococci (Shanson, 1974)[3].

The failure of the laboratories to routinely Gram stain their anaerobic blood cultures must directly affect the number and how soon they isolate their anaerobes. By the time visible growth is evident several days may have elapsed and the viability of the organism is diminished, as although glucose enhances growth it can shorten the survival of the organisms. (Forgan-Smith *et al.*)[2]. Of these 14 laboratories, 6 do grow anaerobes. Although our results indicate *Bacteroides* sp. as the commonest isolate, there is no information presented in the survey to show the percentage of these in comparison with the total number of organisms (aerobic and anaerobic) isolated in individual laboratories. The incidence of anaerobes in blood cultures has increased over the past few years, and now occurs in 8-15 percent of all positive cultures (Alex. C. Sonnenworth, p. 160, Anaerobic Bacteria)[4].

It is interesting to note that although a larger percentage of those laboratories replying used the B.D. vacutainer, this group had by far the poorest recovery of anaerobes (Table III). Reasons postulated for this are that private laboratories, which have a smaller percentage from which anaerobes can be isolated than the hospital laboratories, may use a greater number of B.D. vacutainers in proportion to the public hospitals which more readily have facilities to prepare their own blood culture media. Also, if only one aerobically vented vacutainer tube is used, possibly the recovery rate of anaerobes may be diminished. It is recommended that the two-tube technique be used for aerobic and anaerobic culture.

(b) *Anaerobic Cultures other than Blood*

From the results in Table IV several comments can be made. One of these is the obvious lack of answers in the case of such things as appendix cultures and tracheal aspirates. Possibly related to the fact that

private laboratories rarely handle these. It is evident that a majority of laboratories carry out a wide range of *routine* anaerobic work, perhaps the large range could be reduced with a more intensive work-up on those specimens specifically needing anaerobic culture. In other words, let us be more selective with our anaerobic bacteriology. 27 percent of laboratories culture tracheal aspirates anaerobically, which is not essential routinely. The same applies to routine anaerobic culture of burns and ulcers. It is interesting to note that 86 percent of laboratories use some form of Transport media, although this may not be the best of methods advocated by some authorities (Virginia Polytechnic Institute Anaerobic Laboratory)[7] it is a step in the right direction. Stuart's media appears to be the most popular. However, Amies medium supports the growth of strict anaerobes, *C. novyi B.* survives for up to 72 hrs (Ellner, Granato *et al.* 1973)[1] very well. In our laboratory *Bacteroides* sp. have been recovered after 2 - 4 hrs in Amies.

Although a majority of laboratories use a combination of Robertsons cooked meat and blood agar for primary anaerobic isolation, a number of others use unusual combinations which may not exactly increase their isolation of anaerobes. Neomycin sulphate appears to be used to a great extent for selective isolation of anaerobes, with vancomycin and kanamycin being used to a lesser degree. The authors feel that many mixed infections could be missed when using only blood agar for selective culture (25 percent). It is somewhat surprising that as many as 23 percent of laboratories maintain their primary cultures for 24 hrs. Many of the slow-growing anaerobes may not be recovered in this time. (Anaerobic Bacteria — Role in Disease — Balows, Deltow *et al.*). (American Lecture Series)[5]. The high isolation rate of *Clostridia* sp. (24) makes one wonder if it is related to the fact that it is one of the more easily isolated anaerobes.

(c) *Technical Data*

The popularity of the commercially available Gas Pak system is evident in that 40 laboratories use it in one way or another. It is interesting to note the high percentage of those using McIntosh Fildes Jars who use pure hydrogen as their gas. A mixture containing at least 5 - 10 percent carbon dioxide is justifiable for enhanced isolation of such anaerobes as *B. melaninogenicus*. Unfortunately, it appears that 7 percent of those using these jars do not evacuate prior to the addition of their gas, which is not acceptable. Further to that, 7 of the 28 laboratories do not allow their catalyst to react and take up excess gas, consequently reducing the efficiency of their anaerobic system, and possibly their isolates. However, the figures show that these people still isolate *Clostridia* and *Bacteroides* sp. It is gratifying to see 23 percent of the laboratories using a reducing agent and/or additives in their media to enhance their isolation rate.

It would appear that in general a special attempt is made to isolate anaerobes. Although their isolation does not suggest their clinical significance, it indicates that the bacteriologists are aware of this aspect.

One of the most important points is that the specimens must be correctly collected and transported to the laboratory before one can be expected to isolate significant organisms. No high-powered anaerobic chamber or system can isolate or rejuvenate non-viable organisms. Given the right specimen and a good primary method of anaerobic culture using an enriched medium, anaerobes can be isolated. A good liaison with the medical and surgical staff is also important to ensure that samples are handled correctly.

Obviously, from the survey most laboratories who replied carry out a reasonable level of anaerobic bacteriology as it applies to their situation. A number of laboratories reported their intention of reviewing their methods.

If the questionnaire has given people the incentive to look at and assess their methodology, it has been of some value.

The authors wish to thank formally all those laboratories who contributed. They realised that the survey could have been more extensive and searching, but felt that this would have perhaps limited the number of replies.

The National Health Institute has recently established an anaerobic section and carries out a useful role in reference work (including gas liquid chromatographs). Cultures should be sent on cooked meat media, but only those considered to be of clinical importance, with relevant information. A wide range of reference cultures are also available on request.[6]

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Editorial comment: Many people consider it essential to culture tracheal aspirates and burn swabs anaerobically. The reasons are that in chronic lung infections such as bronchiectasis, the basic "long-term" pathogen is invariably an anaerobic organism and in cases of burns who die from infection the main organism is often an anaerobic streptococcus. (Pseudomonas, once thought to be the causative organism, is now regarded as a secondary invader).—A. E. White.

Technical Communications

A Micro-method for Reversed Passive Haemagglutination to detect HB_sAg.

The reverse passive haemagglutination technique described, using a micro-method employing turkey red cells, has been extensively tested in the Auckland, Christchurch and Dunedin Blood Transfusion Laboratories, and the results have been entirely satisfactory. Additional information can be gleaned from the instruction booklet issued with the Hepatest screening kit. The method of reading the tests, as set out below, was suggested by Hopkins and Das[1] (1973).

The Transfusion Advisory Committee, at a recent meeting, recommended that routine screening of blood donors for HB_sAg should be carried out by a reverse passive haemagglutination technique.

Equipment: Microtest tissue culture plates. No. 3034 Falcon Plastics. Available from Smith-Biolab.

Hamilton microlitre syringe 710-N point style 3 and Hamilton repeating dispenser PB 600-1. Available from Watvic, or Carter Chemicals.

Micropipette capable of dispensing 2 μl. The "Oxford Sampler Ultra-micro" is an example with disposable plastic tips. Available from Medic DDS.

Micropipette capable of dispensing 200 μl. As an example the SMI micro/pettor with re/pettor adapter is suitable. Available from McGaw Ethicals Ltd.

Reagents: All reagents used are from the Wellcome Hepatest kit. Resuspend test cells in distilled water at least 15 minutes before use. Freeze in small (0.1ml - 0.5ml) aliquots labelled with date of reconstitution.

When required for testing, thaw the cells and centrifuge at 3,000 rpm 5 minutes. Carefully remove all the supernatant and replace with an equal volume of Hepatest buffer. Label with the date thawed and store at 4°C.

Method: Add approximately one drop of paraffin oil to reach Terasaki tray well. Add 200 μl saline to 12 $\times$ 75 mm plastic tubes.

Add one drop (approx. 25 μl) serum to the 200 μl of saline and mix. This gives a 1:8 dilution as recommended in Hepatest instructions.

Pipette 2 μl of each serum dilution (under oil) into the wells of the Terasaki tray.

2 μl of positive control serum, similarly diluted, should be included on each tray.

Add 2 μl of Hepatest Test cells to each well containing serum using repeating dispenser and leave to settle for 45 minutes in a horizontal position.

Tilt the tray to 45° and leave for 6-8 minutes (no less than 6 and, preferably, no longer than 10).

Read the results over a light background.

Results: Hb_sAg negatives will show a tight formation of unagglutinated turkey cells on the lower side of the well.

HB_sAg positives will appear as a ring form of agglutinates distributed over the base of each well. Weak positives may show a crescent formation.

False positives. All positive results should be confirmed by using the control cells provided and submitting the sample to a reference laboratory for RIA.

The incidence of false negatives is not yet known.

Advantages: Test cost close to 5 cents.

Sensitivity approaches that of RIA.

Disadvantages: Special care is needed in sample dilution and pipetting.

REFERENCE

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A. Anderson,
Auckland B.T.C.
P. Booth,
Christchurch B.T.C.
K. McLoughlin,
Christchurch B.T.C.
P. Skidmore,
Christchurch B.T.C.
D. Ford,
Dunedin B.T.C.

September, 1975.

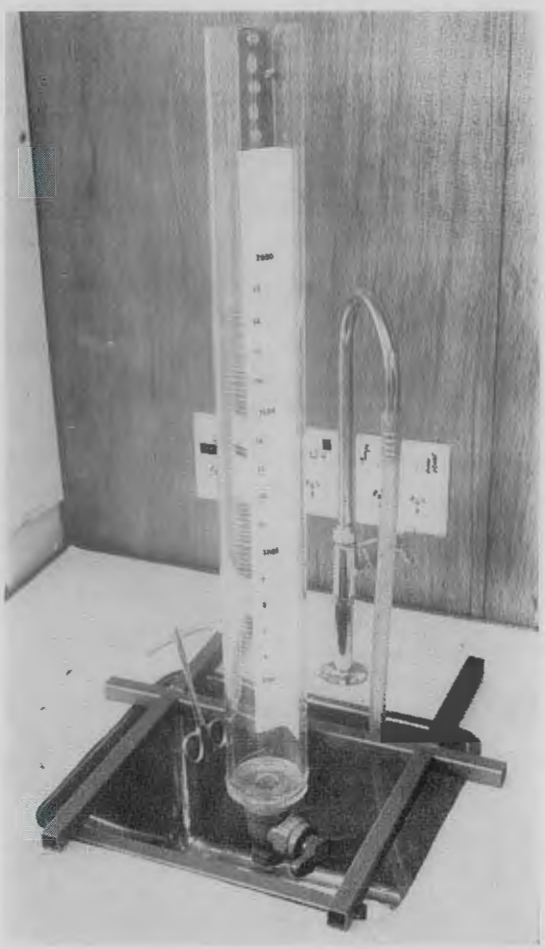
A Newly-devised Cylinder for Rapid Measurement and Sampling of 24-Hour Urine Collections

M. H. Koreman

Medical Research Council of New Zealand Mechanical Workshop, Wellcome Medical Research Institute, Department of Medicine, University of Otago Medical School, Dunedin.

Received for publication, July 1975

Twenty-four-hour urine collections were made by 1,220 adult residents of Milton, a small town 60km south of Dunedin, as part of a recent health survey of the borough. All the urines were obtained during the same 24-hour period and were delivered to the Wellcome Medical Research Institute in a single shipment. The volume of each urine collection was measured and a 60ml sample taken for electrolyte analysis. For this operation a new measuring and sampling device was constructed; overall the handling of each sample took less than one minute.



Construction details are shown in the figure. 1. A measuring cylinder was made from perspex tubing (i.d. 70mm). The base of the cylinder was turned from a piece of perspex sheet (thickness 40mm). The inner face slopes gently (10°) to a central drain hole (i.d. 13mm). 2. At the 250ml level is a side arm (i.d. 4mm) to which a short length of vinyl tubing is attached. The cylinder is graduated every 20ml from 300ml to 2,000ml; volumes can be estimated by eye to within 10ml. 3. Attached externally is a threaded collar to which a ball stopcock (Kitamura, 12.7mm) is screwed. 4. The junction is sealed with an O-ring.

Perspex parts were fused together with a 1:1 phenol-glacial acetic acid mixture and allowed to set overnight. (Face and hands must be protected from the cement and the procedure should be done in a fume cupboard.)

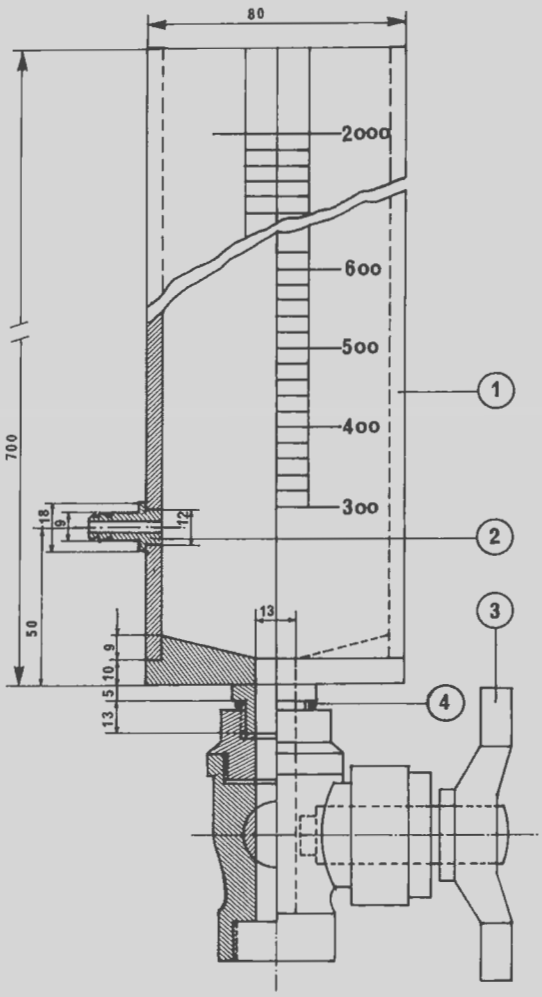
The cylinder is bolted to a piece of enamelled angle iron near the top. The angle iron is part of an enamelled iron frame which sits over a sink: the top of the cylinder is 60cm above, and the bottom of the stopcock 15cm below, bench height.

With the side arm clamped off with a Spencer Wells (haemostat) and the stopcock closed, the urine is poured in and the volume read off. The side arm is unclamped and a few ml of urine allowed to run to waste to ensure that the sample taken is adequately mixed. Opening the stopcock discharges the urine down the sink in a few seconds. Carry-over of urine from one specimen to the next is negligible. No lubrication of the stopcock is necessary.

This device is as robust as a conventional plastic measuring cylinder but because of its transparency is more easily read. It cannot be tipped over and does not need to be inverted for emptying. The side arm, with its short length of flexible tubing, makes it easy to run off well-mixed samples of the desired volumes. A model is now in use in the Diagnostic Laboratories at Dunedin Public Hospital and is proving very satisfactory.

Acknowledgments

The Milton Health Survey was initiated by the Milton Rotary Club as a community project. The author wishes to thank Mr E. L. Phelan for his advice.



A Novel Oxygen-free Solution for Standardising the pO_2 Electrode

In most types of equipment for measuring pO_2 a Clark-type oxygen electrode is used. Because a polarising voltage has to be maintained between the platinum cathode and the silver/silver chloride anode, an oxygen-free solution has to be introduced at least twice a day for zeroing the pO_2 reading on the meter. Generally, this is done with a solution of sulphite in borax which can be made up in the laboratory or obtained commercially.

During the non-usage of the electrode, we keep the electrode wet with deionised water and have noticed that after a time, the pO_2 reading returned to zero. This prompted us to investigate whether deionised water left in the contact with the electrode for some time, could be a substitute for oxygen-free solution when zeroing the electrode.

During the investigations, a Radiometer type E 5046 oxygen electrode mounted on a type AMEI Astrup micro equipment was used. On five consecutive days, the electrode was zeroed using Radiometer oxygen-free solution and then standardised with circulating water

from the thermostatted waterbath (38°C). The pO_2 of the water was calculated by the formula:

pO2 = (B-a) × O_2%/100 whereby " B " is the barometric pressure in mmHg; " a " is the partial pressure of water vapour at 38°C, and O_2 is the air oxygen of 20.92%.

The time it took for the pO_2 reading to return to zero using water was recorded. On the average this was 90 min.

After this was established, the readings of the electrode were taken after being in contact with deionised water for at least 90 min and compared with the readings of the electrode in contact with oxygen-free solution. This was done on 10 different days, both in the morning and afternoon, and the difference noted was never more than ± 3mmHg.

Utilising deionised water to zero, the pO_2 electrode can make quite a saving in use of oxygen-free solution (in expense or making up the solution) as long as it is first established with one's own equipment the time it takes for the pO_2 reading to return to zero.

R. W. L. Siebers,
Biochemistry Department,
Hastings Memorial Hospital.

May, 1975.

A Method for the Evaluation of Sputum

Problems arise when evaluating sputum, since many of the organisms causing lower respiratory tract infection are normally present in the pharynx and mouth. This makes it difficult to decide the significance of pathogens isolated in mixed culture from this area when the lower respiratory tract secretions brush past the pharynx and mouth.

An article by Bartlett (1974)[1] includes a system for the evaluation in the quality of sputum specimens submitted to the laboratory. A gram stain of the specimen is examined under low power (10 × objective) and a score is given depending on the number of epithelial cells (indicative of oral contamination), neutrophils (indicative of an infective process) and mucus present. The criteria for the evaluation of sputum is given in Table 1.

A score of 0 or less suggests excessive oral contamination and a report would be returned stating, " Specimen unsuitable for culture. Please repeat ". Specimens with a score of 1, 2 or 3 are cultured.

Fifty random samples of sputum were examined using the criteria shown in Table 1, and were routinely cultured regardless of their scores. The following results were obtained:

(a) If a score of 0 or less is taken for rejection of the specimen then 16 (32 percent) would have been rejected and therefore not cultured. Of these one (2 percent) grew a significant number of Acinetobacter (greater than 10^7 organisms per ml.). The rest grew normal mixed respiratory flora.

(b) Eleven specimens (22 percent) scored 3, of these, 3 (6 percent) grew normal respiratory flora, the rest had significant number ($7 10^7$orgs./ml) of potential pathogens present.

(c) Twelve sputums (24 percent) had insufficient specimen for a dilution culture, and it is suggested that those with a score of 2 or 3 are directly cultured for assessment, and those with a score of 1 are repeated.

We have had the method in operation for a few months and find that there is good correlation between our results and those obtained by Bartlett. He reports that he was able to eliminate speciation and susceptibility testing of 28 percent of all pathogens isolated, using this evaluation method.

The advantage of this method is in eliminating unsuitable lower respiratory tract specimens in which the significance of pathogens present is difficult to evaluate. The result is a saving of time, materials and possible misleading, irrelevant information. The system is easily put into operation and allows junior staff to discriminate easily between suitable and unsuitable specimens for culture.

N. G. Wood,
Department of Microbiology,
Green Lane Hospital.

June, 1975.

REFERENCES

1. Bartlett, R. C. (1974), *Am. J. clin. Pathol.* **61**, 867.

Table 1

Criteria for Evaluation of Sputum.

	per 10 × field	Score
Squamous epithelial cells	10 — 25	— 1
" " "	> 25	— 2
mucus		+ 1
neutrophils	10 — 25	+ 1
"	> 25	+ 2

Correspondence

Sir,—I wish to draw the attention of technologists employed in blood banks to an increasing transfusion problem caused by the spectacular increase in numbers of people of Polynesian origin who are living in the community. I refer to the relatively high instance of Jk^{a-b} phenotype among these people. Because the frequency of Jk^{a-b} is extremely low in the European community and because it would appear that Polynesians with this phenotype are active anti-Kidd antibody producers the problem of transfusion and blood donor supply is obvious. Testing of over 7,000 Polynesian people with anti-Jk^{a-b} shows that this phenotype has a frequency of about 1 percent and is more or less evenly distributed over all Polynesian groups. The antibody when it is encountered in these people has always been active at 37°C, it is usually incomplete, is enhanced slightly by enzymes and may be slightly haemolytic, it characteristically shows a considerable variation in the strength of reaction and antibody activtiy may deteriorate with the storage of serum. The antibody may contain activity against Jk^a and Jk^b plus the complex Jk^{a-b}, or it may be an entirely complex antibody. In an effort to deal with this problem, several units of Jk^{a-b} blood collected from Polynesian people have been stored in liquid nitrogen. These units are suitable for transfusion and can be obtained on request. Reference cells for the identification of anti-Jk^{a-b} are also available.

Roy Douglas,
Specialist Technologist,
New Zealand Blood Transfusion Services,
Auckland.

September, 1975.

Book Reviews

Methodological Development in Biochemistry. Volume 4. Subcellular Studies. Edited by E. Reid. Published by Longman Group Ltd., London. $NZ12.75. 438 pages. Supplied by Penguin Books (N.Z.) Ltd.

This soft-backed book is the latest in the series originating from the Wolfson Bioanalytical Centre of the University of Surrey, and its contents are derived primarily from a Subcellular Methodology Symposium held there in 1973. The 38 articles are written by various authors specialising in diverse fields of subcellular fractionation. The wide-ranging material is grouped into five categories — techniques and equipment, nucleoproteins, membrane-associated proteins, elements from liver cells, and elements from non-hepatic tissue and individual cell types.

The key to most subcellular studies is centrifugation, often with density gradients, and the first section is concerned with aspects of this, including description of apparatus for forming reproducible gradients, reorientation of gradients from vertical to horizontal layers, and theory and design of discontinuous gradients. A numerical method for a computer-derived rate-zonal centrifugation is described, plus the completely-written Fortran programme and an example of the computer printout. The often overlooked effects of high-speed centrifugation and density gradient materials on cellular components is covered, while other techniques involve immunological methods for studying cellular localisation and secretion of proteins, and methods using cell nuclei. The emphasis with all these techniques, as in other sections of the book, is very much on practical procedures with detail down to volumes and molarity of all reaction constituents. Much of the experimental work described could be reproduced directly from this book without reference to other sources.

Techniques with nucleoproteins involve the use of Metrizamide and NaI for isopyonic banding of ribonucleoproteins, the use of zonal rotors for preparative separation of RNA fragments, and studies on specific proteins associated with animal RNA. There is a short but interesting section on the purification and properties of membrane proteins and the use of membrane and subcellular fractions of lymphocytes for preparing anti-lymphocytic sera.

As might be expected, the longest section of the book deals with subcellular fractions derived from liver cells. General considerations

are given to selective criteria for markers of subcellular organelles with a comparative study of rat liver cytomembranes. Four articles deal with lysozomal membranes and constituents and two with the microsomal fraction. But the centrepiece of this section (and possibly the whole book) is a comprehensive article by D. Morré and coworkers on the isolation and quantitative characterisation of rat liver cell organelles and endomembrane components. This provides a wealth of methodological detail and results with a critical discussion of problems in this field. Many workers may find this article justifies the cost of the whole book.

The variegated section on non-hepatic tissues and cells covers studies on muscle, kidney and gastric cell membranes, pig blood platelets, leukaemic cells, cultured fibroblasts, eukaryotic micro-organisms, yeasts, and plant cell organelles. As before, all these articles have a great deal of methodological detail, so much in fact that it could become a bench book for many workers. The book would also be an excellent reference beginning for a worker new to subcellular fractionation. All the articles are well referenced.

The presentation of the material is good in general, with well-drawn figures and diagrams and passable, if not outstanding, reproduction of electromicrographs. The main fault in presentation is the interspersion of legends to tables and figures (sometimes carried over from the previous page) in the general text which leads to difficulty in fluent reading. However, this arises from the amount of detail which has been crammed into a book of this size and price and is, therefore, only a minor criticism of what is a most excellent and valuable volume and one which must surely rank as one of the best "value for money" books in scientific literature.

C. Watts.

Chemical Analysis for Medical Technologists.

Clive I. Wynter, Ph.D., 1975. Published by Charles C. Thomas, Springfield, Illinois, U.S.A. 217 pages. $US14.75 (board). $9.95 (paperback).

According to the author the aims of this book are: "To familiarise the medical technologist with modern chemical instrumentation and wet analysis applicable to the field; and to provide a working knowledge of the theoretical principles involved in obtaining results from data collected by these analytical techniques." He fails to achieve either of these in this text.

The first five chapters deal with aspects of statistics and basic chemistry. These chapters emerge muddled, with example problems that are difficult to follow at times. They are too brief and the overall impression is one of a series of patchy pieces of information. The only exception is the chapter reviewing organic chemistry. This is very well presented and deals with functional group derivatives clearly and concisely. The next two chapters introduce the reader to Biochemistry and Clinical Chemistry. Both chapters are, once again, very brief and serve as poor introductions to complex and interesting subjects. The latter chapter is merely a mishmash of specimen collection protocols. The final five chapters deal with chromatography, spectrophotometry, drug identification, radiochemistry and automation. Definitions and diagrams are sparse in these chapters. The chapter on automation is particularly poor and one has the immediate impression that the author has little or no knowledge on the use of the autoanalyser. A chapter dealing with units in clinical chemistry was a noticeable omission.

The final 78 pages are devoted to laboratory experiments which appear as a pastiche of manufacturers' methods and contain no specific references for the reader.

Whilst reading this book it becomes apparent that the author has little experience in Clinical Chemistry, particularly in relation to Medical Technology. This book cannot be recommended as a text for laboratory use or for training laboratory personnel.

M. Legge.

Clinical Chemistry Conversion Scales for SI Units with Adult Normal Reference Values.

A. M. Bold and P. Wilding. 1975. Published by Blackwell Scientific Publications. 47 pages. $NZ2.30. Supplied by N. M. Peryer Ltd., Christchurch.

This booklet provides conversion scales for the common laboratory estimations using the submultiples of the SI Units currently employed in Britain. In New Zealand we

have chosen to follow the Australian conventions and it is important to note the divergent nomenclature. The intention in New Zealand is to use mmol/l for serum creatinine and urine. Clearances will be calculated as ml/second. Other differences relate to PBI (N.Z. μmol/l), Urate (N.Z. mmol/l), Cortisol (N.Z. μmol/l).

Agreement on how to express enzyme activity is still to be reached and the authors rightly state that great care should be taken to establish which method has been used and to ensure that the ranges quoted are compatible with those used in one's own environment. For example, the Bessey Lowry International Units quoted in this publication are approximately half those obtained when the commonly employed Technicon method is used.

The normal reference values quoted are subdivided into several categories, namely sex, six age groups, "healthy" ambulant and inpatients. They are derived from several thousand inpatients at the Queen Elizabeth Hospital, Birmingham, and from screening programmes. It should be noted that these values refer to adults.

The little book is probably worth acquiring for this valuable information and could easily be amended for use in New Zealand by shifting a decimal where necessary.

R. D. Allan.

Self-assessment of Current Knowledge in Haematology, Part I — Textbook Review. Samir K. Ballas, M.D. Medical Examination Publishing Company Inc. Flushing, New York. Price $NZ12.00. Supplied by N. M. Peryer Ltd., Christchurch.

This book consists of six sections covering Basic Haematology, the red cell and its disorders, benign and malignant disorders of white cells, Haemostasis, Immunohaematology lipidoses and histiocytoses, and clinical competence.

In common with other books of this type the questions are of a multiple choice nature, an answer key is supplied at the end and the questions are referenced to current textbooks so that verification and amplification of the answers can be obtained. The section on clinical competence contains a series of photomicrographs which are unfortunately in black and white, which detracts markedly from their value and in some cases the photography is rather poor. No doubt the cost of colour plates would have been prohibitive.

This type of book is very useful for examination candidates and for those wishing to keep current knowledge up-to-date. All laboratories should have a selection available to trainee technologists.

B. W. Main.

Topics in Blood Banking. Neva M. Abelson, M.D. (1974). Published by Lea and Febiger, Philadelphia. 163 pages, illustrated. Price $11.75. Supplied by ANZ Book Co. Pty. Ltd., Brookvale, NSW, 2100, Australia.

This book is designed to summarise and review various developments in blood banking, and it performs this duty in a clear and lucid manner. Little detailed methodology is included as these can easily be obtained from standard textbooks, but a few brief explanations of lesser known and recently introduced techniques are given.

The chapters on Blood Bank Organisation and Blood Donor Selection are based on programmes in the U.S.A., but provide useful information on all aspects of these topics. Some of the author's ideas could well be introduced in this country — for instance "It is doubtful if a telephone should be allowed in the laboratory . . . if one is permitted it should be of the type . . . that is answered with a pushbutton and provided with a speaker".

Both liquid storage and cryopreservation are covered in the section on Blood Storage. It discusses various anticoagulants in use and their effects on red cell enzymology, and contains clear diagrams on the latest apparatus for washing glycerolised frozen cells. Clinical indications for the use of frozen cells are also well covered.

Diagnostic reagents (typing sera, anti-human globulin, bovine albumin, etc.) are basic tools of blood banking and the reader is well informed on details of manufacture, shortcomings and quality control. The importance of anti-human globulin containing activity against complement, IgA and IgM is discussed at length, although the author's conclusion on a suitable reagent for routine compatibility testing is somewhat contentious.

Pre-transfusion tests include hepatitis screening, problems associated with autoimmune haemolytic anaemia, drug-induced abnormalities and atypical antibodies of red cells, leucocytes and platelets.

There is an excellent chapter on blood components which not only gives details of usage and preparation, but also covers dosage. Clear photographs and diagrams assist the reader in easily following modern techniques.

Exchange and foetal transfusions are less adequately discussed, and reference to more specialised publications would be advised.

At the end of each section, numerous references are given. These are well up-to-date, with a number of 1974 journals included, and gives the reader easy access to modern innovations in each field.

The main criticism is the style of writing, as throughout the book the author lapses into the first person. Repeated reference to "I consider" this and "I have done" that soon become annoying. Despite this criticism, this book would be a useful addition to the shelves of hospital blood banks, transfusion centres and medical libraries. It will never replace more authoritative works on the subject, but does add some new points of view and would encourage its readers to delve further into topics of particular interest.

D. S. Ford.

Medical Examination Review Book, Vol. 32. Haematology. Arthur A. Topilow, M.D., Merlin R. Wilson, M.D. Medical Examination Publishing Company Inc., 158 pages. Price $NZ12.00. Supplied by N. M. Peryer Ltd., Christchurch.

This book is a further one in an excellent series of Medical Examination Review Books. This volume deals with the subject of haematology in just over a thousand multiple-choice questions which are set out in sections each dealing with specific topics in haematology, e.g., haemolytic anaemia; polycythaemia. In this way the book proves to be conveniently laid out for those studying haematology wishing to review their knowledge after reading each chapter in textbooks. Each of the questions in this book is referenced to either of two standard haematology textbooks, viz., "Haematology" by Williams, Bentler, Ersler and Rundles; and "Fundamentals of Clinical Haematology" 3rd Edition, by Leavell and Thorup. This means that the reader can also readily pursue in depth information about any topic asked in the multiple-choice questions. The answers to each question are contained in a seven-page answer key at the end of the book. Many of the questions are clinically orientated and therefore, as pointed out by the authors, this book will be most valuable to those practising clinical haematology. This, however, reflects on how completely the authors have covered the field of haematology in their questions and answers and I feel that anyone who is interested in and who will be sitting examinations in this subject will find this little book of immense help.

H. Young.

Microbiology 1974. Edited by David Schlesinger for the American Society for Microbiology. This volume is a record of the proceedings of three symposia held by A.S.M. in 1974.

The first symposium recorded was held in January, 1974, and dealt with Bacterial Plasmids. Some 24 authors contributed papers on this subject and all contributions have been recorded, giving a final contribution to this volume of some 226 pages of a total of 310 pages.

A contribution of this length to this subject will be of interest to those orientated in Bacterial and Phage Genetics, but little of value can be gleaned by the "run of the mill" bench worker.

The two further symposia were held at the annual meeting of the A.S.M. and one *Vibrio para haemolyticus;* Occurrence, Identification and Clinical Significance — makes timely reading, when the N.H.I. record isolations in New Zealand associated with raw pipis.

The first paper deals with the experiences of R. R. Colwell, with strains isolated from marine sources in Chesapeake Bay and his conclusions that the organism appears to be a commensal with the crab reveals the potential public health hazard.

The second paper deals with Animal Toxicity but the third paper on "Enumeration and Identification" is a fully detailed and referenced account of the methods and minimum tests required by the F.D.A. to

The final paper in this all-too-short section classify *V. parahaemolyticus.*
deals with the epidemiology of the organism in humans.

The final symposium recorded is "Roles of Iron in Host-Parasite Interactions," and this is a collection of the data now available on bacterial infections whose outcome is determined by the availability of free iron — the host defence acting in a protective fashion by sequestering iron in proteins and the bacterial invaders producing chelators to retrieve this product.

In common with the publications of the A.S.M., the style and format are clear, concise and typographical errors appear to be kept to an absolute minimum.

This volume should be found in specialist libraries, but would have little appeal to the majority of readers in this country.

M. D. McCarthy.

Clinical Biochemistry, "Cantarow and Trumper." A. L. Latner. 7th Edition. 1975. Published by W. B. Saunders Co. and obtained from N. M. Peryer, Christchurch. 918 pages, with illustrations. $NZ30.25.

"Cantarow and Trumper" was first published in 1932 and the authors' intention was, "to provide an explanatory text so that the clinician would have an understanding of those phases of internal medicine that require the assistance of the biochemical laboratory for their complete solution." In doing this, they desired to exclude laboratory technique on the one hand and purely abstract and theoretical considerations on the other hand

There is a wide spectrum of textbooks on biochemistry with varying shades of emphasis. Quite apart from monographs on specific topics, we have basic texts with a case-orientated approach, while this book could be regarded as clinical biochemistry with a basic biochemical approach. The information explosion makes the writing of such a book an increasingly difficult task and the amount of information has the effect of making the book assume an encyclopaedic character. Explanations are generally somewhat terse. The treatment varies and some chapters are very lengthy and detailed.

Some items are dismissed briefly or are not mentioned, for example α_1 foetoprotein and α_1 antitrypsin. However, the complement cluster receives a full treatment. Much useful information does come to light. Random examples are the observation that the complete group of essential amino acids must be ingested together otherwise the nutritional effect is impaired and the suggestion for obtaining specimens for HMMA assay, namely, "to prevent false positive results the patient should avoid coffee, tea, chocolate, icecream, bananas and all drugs for 48h before urine collection." This suggestion could be applied in many instances.

Some of the technical references are rather quaint. Cephalin cholesterol, thymol and zinc turbidity tests and the icteric index have not much currency now.

The chapters on electrolytes and body fluid and endocrine functions seemed particularly useful, perhaps because the information is more directly applicable to the clinical situation.

There is a final chapter on the history and development of clinical biochemistry and a mention of SI Units.

I feel sure that this work will appeal to a greater variety of persons than physicians seeking biochemical knowledge to whom this book was originally addressed.

—R. D. Allan.

Biochemical Concepts. R. W. McGilvery. 1975.

Published by W. B. Saunders Company, Philadelphia, London, Toronto. Price $NZ14.85. 530 pages. Obtained from N. M. Peryer Ltd., Christchurch.

The contents of this book are divided into four sections entitled the proteins, the carbon cycle, the nitrogen cycle and specialised metabolism. Within this format, much of the basic material of general biochemistry is covered. The proteins relate to their structure, binding properties, enzymatic functions, and their synthesis along with nucleic acid metabolism. The rest of the book is concerned primarily with the metabolism of carbon and nitrogen compounds and covers the energy and storage of carbohydrates, fats

and amino acids, along with chapters on photosynthesis, fermentation, polyprenyl compounds and structural elements of fibres and matrices.

The conceptual approach implied in the title is not very obvious as the subjects are treated in the conventional manner. "Concepts" are stated at the beginning of each chapter but in reality are only a chapter summary. In his preface, the author states that the reader only requires some "rudimentary encounters with organic compounds" and that "no other scientific background is necessary." This would seem to imply that this is a first elementary introduction to biochemistry. But that is certainly not so, as much of the material is covered in more than rudimentary terms. For example, regulation of enzyme reactions deals with Michaelis-Menten kinetics, including data plotting, homotropic kinetics, activation and inhibition with allosteric mechanisms. While the approach is too simple for the book to be of great use to the advanced student of biochemistry, it has a lot to recommend it to those taking the subject to an intermediate level or as a subsidiary course.

The main strength of the book lies not so much with its content, but in its clarity of presentation. This is achieved by the text being liberally illustrated with diagrams and figures of a high standard. With protein and membrane structural diagrams a successful attempt has been made at three-dimensional representation. The written text, with ample headings and subheadings and keyword accentuation, is also beautifully clear. On the other hand, the book has two main weaknesses. There is a complete absence of references in the text which will influence the reader to judge what is written as dogma, whereas much of it may be open to question or have conflicting viewpoints. Also, the author has totally ignored the historical development of the given concepts. This can be crucial to a full understanding or appreciation of the subject by the student. Science presented this way loses much in impact and interest.

The author has a rather folksy style of writing, often interspersing the text with questions which he then goes on to answer. At times, his style becomes disconcerting, e.g., telling the student how to pronounce betaine ("bay-tah-een"), or describing gout as a disease in which the great toe is frequently affected and to "take special note when the mighty are felled by sore toes."

This book can be recommended particularly to the student who is content to take biochemistry at its face value as a subject which does not pose too many problems. The more earnest student will find it easily read and useful within its limitations and turn to other texts to fill in the gaps.

C. Watts.

Books Received for Review

Pathophysiology of Blood, by Erslev-Gabuzda. N. M. Peryer Ltd., Christchurch. 187 pages. 1975. $9.40.

Spores VI, by Philipp Gerhardt, Ralph N. Costilow and Harold L. Sadoff. American Society for Microbiology. 619 pages, illustrated. 1975. $US15.00.

Acid-base and Electrolyte Balance, by G. Rooth. 1st Ed., but based on two earlier books. 118 pages. 1975. $NZ7.75. N. M. Peryer Ltd.

Review of Physiological Chemistry, by H. A. Harper. 570 pages, illustrated. 1975. $NZ11.50. N. M. Peryer Ltd.

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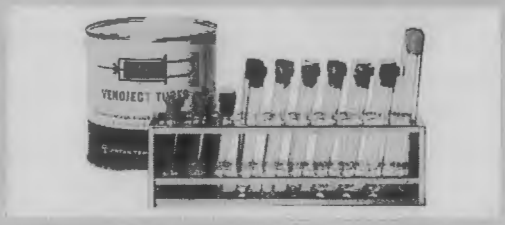

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Abstracts

Contributors: D. G. Bolitho, Lexie Friend, J. Hannan, L. M. Milligan and A. G. Wilson.

Clinical Biochemistry

Procedure for Calibrating the Technicon Colorimeter 1. Black, J. C. and Furmon, W. B. (1975). *Clinical Chemistry* **21**, 774.

The authors describe a rapid method for calibrating Autoanalyser 1 colorimeters using solutions of Bromophenol blue. The procedure is used to check linearity and stray light. —A. G. W.

Evaluation of Discrepancies in Patients' Results. An Aspect of Computer-assisted Quality Control. Whitehurst, P., Di Silvio, T. V. and Boyadjion, G. (1975). *Clinical Chemistry* **21**, 87.

A computer programme has been devised to select those clinical chemistry results that have a high probability of error. Discrepant results are printed on a report daily and evaluated by the quality control supervisor, who decides whether to accept the results or reassay. The system eliminates tedious and time-consuming manual perusal of results.

—A. G. W.

Electronic Correction of Values that are "Off Scale" in the SMA 6 System for Continuous Flow Analysis. Rosenfield, L. (1975). *Clinical Chemistry* **21**, 151.

Data are presented for an electronic device that automatically halves "off-scale" signal voltages on the "SMA Flex-6" System (Technicon). This extends the usefulness of the system by obviating the need for most repeat analyses on dilutions of specimens containing constituents in concentrations that exceed the limits of the pre-calibrated chart paper. Accurate results are obtained because the chemical reactions are shown to be linear up to nearly twice the maximum calibration on the recorder paper for the following analyses: bilirubin, total and direct (20.0 mg/dl); alkaline phosphatase (700 U/litre); lactate dehydrogenase (1200 U/litre); creatine kinase (2400 U/litre); and aspartate aminotransferase (600 U/litre). In contrast, dilution of sera 2—, 5—, and 10— fold with sodium chloride solution (8.5 g/litre) produces positive errors ranging from 6 to 38% for these enzymes, but has no significant effect on bilirubin.

—Author's Summary.

International Federation of Clinical Chemistry and Committee on Standards Provisional Recommendation on Quality Control in Clinical Chemistry. Part 1. General Principles and Terminology. *Clinical Chimica Acta* Vol. 63 (1975).

The general principles involved in quality control such as accuracy, precision and statistical methods are discussed at length. Material used for standards are discussed and graded in order of purity. A comprehensive list of terminology is provided. —A. G. W.

Aerosol Production Associated with Clinical Laboratory Procedures. Stern, E. L., Johnson, Janet W., Vesley, D., Halbert, Mary M., Williams, L. E. and Blame P. 1974). *Amer. J. clin. Pathol.* 62, 591.

The authors have investigated aerosol productions associated with a number of laboratory procedures. These included centrifugation, Vortex mixing, opening containers, Pasteur pipette, Oxford pipette, spilling on the floor and shaking, i.e., paint can and vacutainer tube.

Dropping or spilling samples was the most dangerous occurrence, as it combined extensive surface contamination with a large amount of aerosol production. Procedures involving the shaking of a specimen within a confined space resulted in abundant aerosol production. —L. R. F.

Low Serum Thyroxine in Phenothiazine-treated Psychiatric Patients. Gwinup, Grant and Rapp, Norman. (1975). *Amer. J. clin. Pathol.* 63, 94.

Five case reports of markedly depressed serum thyroxine concentration in patients being treated with phenothiazines. There were no clinical or other laboratory findings to suggest hypothyroidism.

—L. R. F.

Limitations of the Usefulness of the d-Xylose Absorption Test. Krawitt, E. L. and Beeken, Warren L. (1975). *Amer. J. clin. Pathol.* 63, 261.

The conventional five-hour d-xylose absorption test was performed on 38 patients with disease of the jejunal mucosa, giardiasis or bacterial overgrowth or no small-bowel disorder.

The test was in error in 20 - 40% of cases and the error for the entire group was 30%. The author concluded that the test yields little guidance for diagnosis or therapy of clinical problems and is superfluous when a jejunal biopsy can be obtained. —L. R. F.

Significance of Non-steady-state Serum Digoxin Concentrations. Walsh, F. M. and Sode, Jonas. (1975). *Amer. J. clin. Pathol.* 63, 446.

Serum digoxin levels are utilised as an index of digitalisation or toxicity in patients on maintenance therapy and the authors have investigated the optimal time for sample collection.

Their results indicate that blood samples should be drawn just prior to the daily dose and no sooner than six hours after administration of the drug.

—L. R. F.

Results of the 1972 CAP/NBS Study of Radionuclide Measurements. Hauser, Wolfgang. (1975). *Amer. J. clin. Pathol.* 63, 545.

Survey kits were sent from the National Bureau of Standards (NBS) to 46 laboratories. Each kit

contained a questionnaire, a multi-injection vial (with 15ml saline solution), syringe, needle, supplies and labels. The participating laboratories were asked to inject a known activity of approximately 50 microcuries of chromium-51 into the vial, seal it, and mail the bottle, syringe and needle to the National Bureau of Standards.

Results of this survey showed that the activity values of 47% of the participants were within ± 10% and 81% were within ± 20% of the activity value of the samples subsequently measured by the NBS.

The study provides information about the accuracy of radioactivity assays in nuclear medicine laboratories and indicated that there is room for improvement in the accuracy of calibration methods used for determining the activity of a radiopharmaceutical.

—L. R. F.

The Diagnostic Value of Protein Bound Serum Fucose in Cancer of the Breast. Hadjivassiliou, A., Castanaki, Anna, Hristou, G. and Lissaios, B. (1975). *Surgery Gynec. Obstet.* **140,** 239.

In contrast to some previous reports, serum fucose determination was found to be of no diagnostic value in the early stages of cancer of the breast. Only in those patients who had disseminated breast cancer was the serum fucose level elevated. —J. H.

Fluorescent Microscope Observation of Urine Exfoliative Cells in Urinary Tract Tumor. No author(s) listed. (1974). *Chin. med. J.* **8,** 137.

Observation of urine sediment smears in 118 cases with haematuria by the modified Bertalanffy technique revealed tumour cells in 48. Among these 41 had cancer, 2 were free of tumour and 5 remained uncertain. Malignant cells can be clearly differentiated by the bright fluorescent hues and morphologic characteristics when stained by fluorochrome-acridine orange. The cytoplasm of proliferating malignant cells is flame red or orange. The nuclei, bright yellow-orange, are enlarged and often have jagged outlines. The nucleus-cytoplasm ratio is shifted in favour of the nuclei.

The combination of cytologic and urologic examination is imperative in making early diagnoses and instituting timely treatment of urinary tract tumours. —J. H.

Hyperlipidaemia in Children. Lloyd, June K. (1975). *Br. Heart J.* **37,** 105.

Hyperlipidaemia in children is most commonly expressed as hypercholesterolaemia. "Normal values" for cholesterol, if defined statistically, vary between communities and levels of cholesterol in childhood above which an increased risk of coronary heart disease in adult life may be expected have not been firmly established. It is suggested that values over 250 mg/dl in a child over 1 year of age merit detailed investigation, including full lipoprotein analysis, and levels between 230 and 250 mg/dl should be repeated, with further studies if indicated. The only primary hyperlipoproteinaemia likely to be encountered in childhood is familial hyperbetalipoproteinaemia in its common heterozygous form.

No discussion of normal lipid levels in children (or adults) can be complete without mention of the methodological problems which still exist in the measurement of cholesterol and triglycerides.

—I. H.

Haematology and Immunohaematology

Immunological Aspects of Acquired B Antigen: Gerdal, A., Maslet, C. and Salmon, C. (1974). *Vox Sang.* **28,** 398.

Nine individuals of the A1 blood group were studied. The acquired B reactive structure was found to differ from that of normal B. It appears from the results obtained that the B reactive structure must have been formed at the expense of the A reactive structure. —L. M. M.

A New Case of Recombination within the HL-A System. Beng, K., Schwarjfischer, F. and Wischerath, H. (1974). *Vox Sang.* **28,** 322.

A short report on a case if recombination within the HL-A system between the first and second segregant series of antigens. —L. M. M.

Quantitative Rh Typing of r^Gr^G with Observations on the Nature of G(R12) and Anti G. Rosenfield, R. E., Levine, P. and Heuer, C. (1974). *Vox Sang.* **28,** 293.

Quantitative blood typing information was collected on a husband r^Gr^G, his wife R_1r, daughter R_1r^G and niece r^Gr — the results strongly suggested that these Rh phenotypes were directly indicative of the Rh genotypes. Tests were carried out on anticoagulated samples (CPD) and blood typing tests were performed by autoanalyser. Results and conclusions are clearly set out. —L. M. M.

Qualitative and Quantitative Study of the Antigens and Antibodies of the HL-A System — Automative Fluorochrome Method. Benajam, A., Poirier, J. C., Beraud, L., Marcelle-Race, A. and Daussett, J. (1974). *Vox Sang.* **28,** 337.

It was possible to obtain a successful evaluation of the quantity of HL-A antigens on lymphocytes using a fluorochromatic test with double marking fluorescein diacetate and ethidium bromide. It was also possible to detect anti-HL-A antibodies, to study their cross reactions and to perform tissue typing using this automatic, sensitive and repeatable method.

—Authors Abstract.

Studies on the Kell Blood Group System. Marsh, W. L. (1975). *Med. Lab. Technol.* **32,** 1.

Sixteen antigenic determinants have now been recognised that are related to the Kell blood group system. Two variant phenotypes, McLeod and Ko, are also recognised. A new antigen Kx has been identified, which appears to be required for proper expression of Kell on red cells. The relationship between the Kell blood group system and leucocytes has also been investigated. It was noted that Kx is a membrane glycoprotein and that red cells utilise Kx in the production of normal Kell antigens. Discussions are subtitled as follows: Kell Antigens of Males and Females; Kell Antigenic Activity of White Cells and Platelets; Kx Antigen Activity of Chronic Granulomatous Disease leucocytes; Characteristics of the Kx Antigen and Xg^a Blood Groups.

—L. M. M.

Morphological Changes in Maternal Lymphocytes in Pregnancy. Knobloch, V., Jouja, V. and Svobodová, Marie (1975). *Br. J. Obstet. Gynaec.* **82,** 146.

Using toluidine blue staining according to the method of Smetana (1961), changes in the nucleoli of lymphocytes of 150 women were studied. A progressive rise in the number of lymphocytes with micronucleoli was found during pregnancy and there was a drop after delivery. There was a decrease in the number of ring-shaped nucleoli associated with the increase of micronucleoli.
—J. H.

The Whole Blood Recalcification Clotting Time: A Suggested Simple and Reliable Method for Monitoring Heparin Therapy. Fanning, J. P. and Dubeau, Ann M. (1974). *J. Maine med. Ass.* **65,** 211.

The method is described in detail.

The sensitivity to heparin of the activated partial thromboplastin time (APTT) reportedly is limited to the "mid-range" of heparin effect. Activator present in the APTT reagent tends to overcome the inhibitory effect of heparin and milder degrees of hypocoagulability are essentially not detected. At high levels of heparin effect, on the other hand, the APTT frequently fails to reach an end-point.

Platelets exercise an inhibitory effect on heparin through factor 4 and the level of the platelet count affects heparin activity *in vivo*. This platelet effect is not reflected in tests performed on platelet-poor plasma (as is the APTT), while it is allowed for in methods using whole blood. —J. H.

Microbiology

Bacterial Count in Urinary Tract Infection and Its Influencing Factors. No author(s) listed. (1974). *Chin. med. J.* **11,** 197.

Contrary to the usual conception, many patients with manifest signs and symptoms of urinary tract infection or pyuria have bacterial counts $<10^5$/ml, below the traditionally required number. Among 13 species (159 strains) of bacteria isolated, the most frequently encountered were coagulase-positive staphylococci (37.7%) and coliform bacteria (27.6%). The majority of patients with counts $>10^5$ were infected with coliform bacteria (89.5%) whereas those with counts of 10^{2-4} were infected with coagulase-positive staphylococci (46.1 - 50.0%).

Growth curves of staphylococci and coliform bacilli (6 strains each) demonstrated that the former multiplied slowly in liquid media (increasing 30× in 8 h) while the latter multiplied much more rapidly (increasing 67 000× in 8 h). The minimum pH for the growth of staphylococci was 6.6 and for *Escherichia coli* 4.4. The average pH of 114 morning specimens was 5.6, too acid for the growth of staphylococci.

A count of $>10^5$ may be significant for coliform bacilli infection but staphylococci can hardly reach such a high count. Persistence of the same pathogenic organism in repeated cultures accompanied by symptoms and signs is suggestive of urinary infection, even with low bacterial counts.
—J. H.

Three Cases of Pneumococcal Bacteriuria. Rosenthal, S. L. (1974). *Amer. J. clin. Path.* **62,** 812.

Two undoubted cases and one doubtful case of bacteriuria due to pneumococci are presented. The author gives a full discussion of these most unusual findings. —D. G. B.

A New Approach to Quantitative Urine Culture. Long, G. W. (1974). *Amer. J. clin. Path.* **62,** 815.

A plate flooding technique used in conjunction with comparison with a photographic standard for the semi-quantitation of bacterial counts on urine is described. The technique is simple, but appears to have no advantage over many others which have been described. It is certainly more expensive than the blotting paper technique, for instance.
—D. G.B.

Morphologic Observations on Mycoplasmas and *Neisseria gonorrhoeae* in Association Growth Patterns. Faur, Y. C., Weisburd, M. H. and Willson, M. E. (1975). *Amer. J. clin. Path.* **63,** 106.

As is suggested by the title, this paper is mainly concerned with details of the morphology of mycoplasma species, but it does give the interesting information that the frequency of association of mycoplasma with gonococci was 84% among patients screened for gonorrhoea in several clinics in New York. The authors also use NYC medium. This medium, which is capable of supporting the simultaneous growth of *Neisseria gonorrhoea* and Mycoplasma species is a proteose peptone, cornstarch agar with a buffered base having a supplement of lysed erythrocyte suspension, horse plasma, yeast and dextrose with the usual antimicrobial agents for selecting gonococci. It appears to this abstractor that a close look at this medium and possibly its wider use is justified, as it could well reveal unsuspected epidemiological patterns of mycoplasma and neisserial association in venereal infection.
—D. G. B.

Identification of Non-fermentative Gram Negative Bacteria in the Clinical Laboratory. Kantor, L. T., Kominos, S. D. and Yee, R. B. (1975). *Amer. J. med. Technol.* **41,** 1.

An identification scheme using a dichotomous key with a selection of 18 tests to differentiate the non-fermentative organisms encountered in the routine clinical laboratory is presented. It is claimed that a wide variety of organisms, including the majority of Pseudomonas, Flavobacterium, Bordetella, Acinetobacter and Alcaligenes species can be identified rapidly by this method.

The method appears worthy of consideration as a rapid and fairly simple scheme. —D. G. B.

Role of the Nitroblue Tetrazolium Dye Test in Diagnosis of Infections. Feigin, R. D. and Pickering, L. K. (1975). *Sth. med. J., Birmingham* **68,** 237.

It is pointed out that falsely increased nitroblue tetrazolium (NBT) reduction values will be found (1) if the concentration of heparin exceeds 20 units/ml of blood in the tube used for blood collection or (2) if a concentration greater than 0.1% NBT is used during the test. —J. H.

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

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Mass: kg, g, mg, μg, ng, pg.
Mass concentrations: kg/litre, g/litre, mg/litre, μg/litre. For the present concentrations per 100 ml also accepted as are daily outputs in urine and faeces.
Molar concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre. (For the present mequiv/litre may also be used.)
Temperature: Express as °C.
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   m s = metre x second

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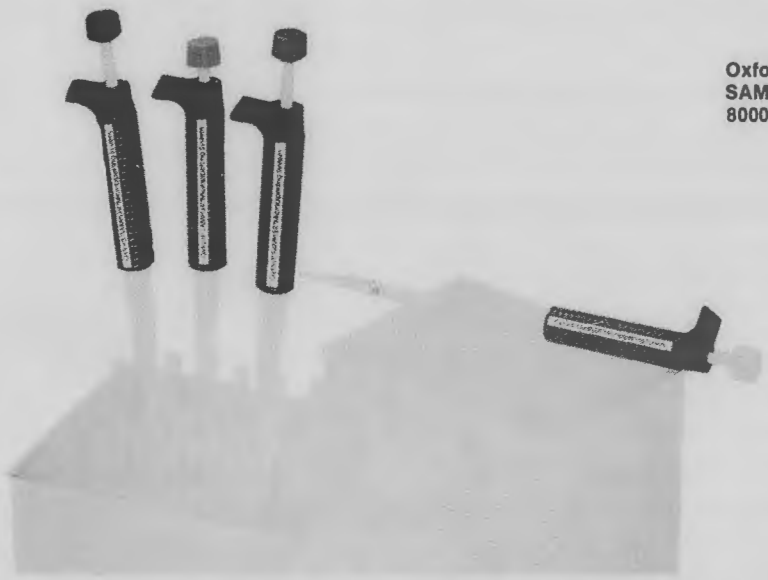

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