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A Review of the Association Between Australia Antigen and Hepatitis

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Introduction

Viral hepatitis, an acute inflammation of the liver, may be classified into two clinically similar infections described as infectious hepatitis (IH) or epidemic jaundice, and serum hepatitis (SH) or homologous serum jaundice.

Epidemic jaundice has been recognised for many years; references to it have been found in Hebraic writings and in the works of Hippocrates. Although this form of hepatitis is most commonly spread by the faecal-oral route it can also be transmitted by blood and blood products since viraemia occurs during the period of incubation and may persist continuously or intermittently for many months after apparent recovery.

Serum hepatitis has a comparatively short history being first described in 1885[38] following the occurrence of jaundice among a group of shipyard workers who had been inoculated against smallpox with an inoculum of human origin. Subsequently outbreaks of jaundice increased alarmingly with the greater use of inadequately sterilised syringes and needles which had become contaminated with blood from a carrier of the hepatitis virus. The transmission of hepatitis by transfusion of blood and blood products has been recognised for some time and it has now become an important complication of the transfusion of these products. The description by Blumberg *et al.*[9] in 1965 of the Australia antigen and its subsequent association with serum hepatitis has led to much investigation into this disease process and its aetiology by a multitude of research workers throughout the world. In this review the knowledge accumulated to date on this subject is discussed.

Nomenclature

Various authors have suggested names for the antigen associated with SH. Briefly these are as follows:

Name	Abbreviation	Author
Australia antigen	Au/Ag	Blumberg
Serum Hepatitis antigen	SH/Ag	Prince
Hepatitis antigen	HA/Ag	Gocke
Hepatitis associated antigen	HAA	McCallum

The term 'SH antigen' misleadingly implies restriction of the antigen to cases of classical serum hepatitis, and 'hepatitis associated antigen' is inadequate in its specificity as it may be implied that it includes the postulated antigen associated with IH. However, in this text HAA will be used specifically for the antigen associated with SH as it has now become a recognised part of the vocabulary of the majority of researchers in this subject.

Biochemistry and Morphology of HAA

HAA has been derived from sera of persons carrying this antigen and studies have shown that as a particulate object it contains protein, small amounts of lipid, has a specific gravity of less than 1.21, but as yet no nucleic acid has been demonstrated, nor carbohydrate, in the antigen particles[10]. However, a DNA polymerase has been found to be present in HAA preparations indicating weak DNA synthesising activity[27]. Positive sera for HAA have been examined under the electron microscope (EM). and various particle types have been described. The most numerous type of particles are small round forms 16 to 18 nm in diameter with an approximate 3 nm periodicity on their surface which suggests that they may be made up of subunits, but no such construction is as yet definable[1]. Long forms 20 nm in diameter and of variable length of up to 230 nm are frequently seen in the sera; these may be bent and have a varying diameter at their terminal portions. A third rare form has been described which is a double shelled or 'dough nut' form 40 to 45 nm in diameter, and it has been suggested that these represent tightly coiled, elongated long forms[18]. This 'dough nut' particle has been termed a 'Dane body'. These various particle types may be separated by ultracentrifugation and identified as having HAA specificity by the use of anti HAA with resultant clumping of particles demonstrable using the EM. Treatment of the antigen with various proteolytic enzymes or heating to 56°C does not destroy its reactivity in serological tests. It is, however, destroyed by 1 percent sodium dodecyl sulphate and by boiling. Carefully controlled heating of diluted serum to 98°C for one minute destroys its infectivity but not

its antigenicity *in vivo* or *in vitro*[34]. It has been shown that the SH infective agent passes through a filter of average pore size 54 nm[49] and it has been proposed that the 40 nm diameter particles are the virion of SH and the rest of the HAA is excess virus coat material not incorporated into the intact virus. These Dane particles have an inner component and when treated with 'Tween 80' this inner component is revealed and has a diameter of 20 nm which suggests that the HAA virus is an encapsulated virus-like herpes virus, the inner capsule being assembled within the nucleus and an outer envelope added in the cytoplasm to form the Dane particle[17].

HAA may be present in human sera where its concentration is often very high and has been estimated at about 10^{13} particles per millilitre. It has been found in blood products such as fibrinogen, thrombin, antihaemophilic globulin as well as other fractions, but not in gamma globulin (immunoglobulin fraction) nor in albumin[51]. HAA and anti HAA have been demonstrated in the sera of chimpanzees and other primates, also in guinea pigs, rabbits and mice[15]. The presence of HAA in synovial fluid[39], bile[6], and urine [4, 7] has been demonstrated as it has in faeces[24, 32], although one group of workers were unable to confirm this finding[25]. HAA infectivity of urine and faecal samples has been clearly demonstrated and the transmission of SH by 0.001 ml of antiserum shows that nasopharyngeal secretions may be a further method of infection[31]. HAA particles have been found in the nuclei and/or cytoplasm of hepatocytes of patients with HAA positive sera using a specific immunofluorescence technique[44]. Its apparent replication in tissue cultures of human liver cells hopefully indicates that in future culture of the HAA virus will be a reality[13]. Placental transfer is doubtful although a case has been studied where an infant $2\frac{1}{2}$ months of age had serum which was HAA positive, anti HAA negative, the child's mother having the same HAA serology. Although transplacental infection in this case is a possibility, the authors do not rule out the possibility of transmission orally or by direct maternal-foetal method through placental tears at birth[22, 41].

Studies on HAA positive and negative 'normal' subjects have shown no relationship between the presence of the antigen and ABO blood groups, levels of haemoglobin, packed cell volume, red cell count, uric acid, white blood cell galactose 1 phosphate uridyl transferase and glucose 6 phosphate dehydrogenase. However, vitamin B_{12} levels are significantly higher in HAA positive subjects [10, 26], and the levels of haemoglobin in renal dialysis patients transfused with HAA positive blood are known to rise significantly and it has been suggested that in these patients the liver damage influences erythropoietin metabolism[30].

Detection of HAA and Anti HAA

Various methods have been employed in the detection of the antigen and its antibody, the basis of each being serological or actual particle demonstration using electron microscopy. Variations in each method have been described to suit the particular situation of individual workers. In each case the basic technique is unchanged and the following is a general discussion of techniques employed and their advantages and disadvantages.

Gel Immunodiffusion

This is a modified Ouchterlony technique using slides coated with 0.3 to 0.5 ml of hot 2 percent agar, allowed to cool, and stored in aluminium foil at 4°C for several weeks if necessary. This 'precoating' of the slides is omitted by some workers. For use the precoated slides are further coated with 0.5 ml of hot 1.1 percent agar, taking care to avoid air bubbles. The slide is allowed to cool and may be stored in a moist chamber at 4°C for seven to 10 days. The technique for use is to employ a template to cut wells in the agar, removing the agar plugs so formed with a pasteur pipette fitted to a vacuum. The well pattern used may vary but generally consists of six wells 3 mm in diameter, 2 mm apart, in a circle surrounding a central well. The wells are inoculated as required and placed in a moist chamber at 25°C for up to seven days and lines of precipitation between antibody and antigen are looked for using oblique illumination in a darkened room, or by staining with a protein stain such as 0.1 percent azocarmine in 1M acetic acid[8].

A suitable agar for this technique is 'Ionagar' (Oxoid) dissolved in a barbitone buffer of pH 8.6. Advantages of gel immunodiffusion are its simplicity and economy of anti-serum with automatic identity of the reaction. Its disadvantages are that it is relatively insensitive and rather slow.

Complement Fixation

This technique is basically an adaption of the standard serological technique. The inactivated test sera are added to anti HAA and a standard amount of complement, which is fixed to HAA-anti HAA complexes if they are formed. Maximum specificity of complement fixation is achieved by incubating the mixture at 4°C overnight, although reasonable results are obtained after incubation at 37°C for one hour. Subsequently one attempts to demonstrate the utilisation of complement in the reaction by the addition of sensitised sheep cells and observe for the presence or absence of lysis. This technique is very sensitive and gives antigen and antibody titres higher than those obtained by gel diffusion. It presents a more technically demanding technique and has a few disadvantages. The specificity of positive reactions cannot be confirmed and strongly positive sera may demonstrate prozoning which may be combated by testing three dilutions of test serum which are suggested as being undiluted, one in 16 and 1 in 256. Anti-complementary activity is often found in jaundiced sera which may be due to unspecific factors or HAA antibody-antigen complexes[23]. By heating diluted sera to 85°C for one hour HAA appears to be released from antibody-antigen complexes resulting in higher titres of HAA and higher detection rates by complement fixation[45].

Latex Agglutination

In this technique latex particles are precoated with anti HAA. The serum under test is added to one drop of a one in 20 dilution of guinea pig serum and one drop of precoated latex on a slide and observed for agglutination which is detectable in two minutes if the serum is HAA positive. The presence of guinea pig serum prevents false positive reactions but how this is achieved is as yet inexplicable. Strongly positive sera give agglutination within 15 to 20 seconds. The sensitivity of this technique is as good as complement fixation; this plus the speed of the method would appear to justify its use in some immunohaematology laboratories[85].

Crossover Electrophoresis

Hot agarose gel is placed on microscope slides, allowed to cool and two rows of wells 2 mm diameter and 4 mm apart are punched in the gel using a template. The size of well may be varied, some workers prefer a slightly larger well for antigen and a smaller one for antibody. Antiserum is placed in the wells of one row and sera under test for antigen in the other. Once more the pattern of seeding the wells may be varied with antigen and antibody placed in alternate wells of each row. The slides are placed, with the antigen on the cathodal side, in an electrophoretic field for one and a-half hours at 22°C using 12 to 15 volts per cm of slide, and a barbitone buffer of pH 8.4 which is the isoelectric point of immune gamma globulin. The antigen, being an α_2 globulin, moves by electrophoretic migration and the antibody by endosmosis in the opposite direction. Precipitation of the two indicates a probable positive result which should be confirmed by specific identification using a known HAA positive control to give the typical precipitation arc of identification. Precipitation may be viewed by using an oblique light or a protein stain. This technique is rapid but is only slightly more sensitive than the gel diffusion technique; its rapidity and simplicity show it to be a technique of choice in the screening of large numbers of sera from blood donors[14].

A modification (Laurell's modification) of this technique uses cellulose acetate instead of agar. The acetate is saturated with antibody and test sera are 'spotted' onto the strip and electrophoresed. This is as sensitive as the immunodiffusion technique but uses a lot of anti HAA compared to the standard crossover electrophoresis which uses 10 to 15 μl per test well[47].

Simultaneous results of HAA and anti HAA may be achieved by using a system of three rows of wells in the agar gel as proposed by Pesendofer[15]. The test sera are placed in the centre row, antigen to one side and antibody to the other. Manani[15] incorporated the antiserum in the agar gel at a pH of 7.6 using a tris buffer. Once again the amount of antiserum used does not appear to be justified by any increase in test sensitivity or specificity.

Electron Microscopy

Negative staining of electron dense material, for example a virus or viral particles, may be achieved using 3 percent phosphotungstic acid. This gives a high viewing contrast but requires an optimum particle concentration of about 10^9 to 10^{12} particles per ml which may

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4	15.2	46	34	8,700	15.4	46	34	7,600
5	12.0	37	33	5,900	11.8	35	33	6,200
6	14.2	43	34	8,600	14.2	42	35	8,200
7	15.5	45	34	6,500	15.5	45	34	6,800
8	16.1	47	34	5,000	15.8	46	34	4,800
9	12.2	36	34	8,000	12.1	36	34	8,800
10	15.9	48	33	12,500	16.1	49	33	12,400
11	13.2	39	34	6,700	13.7	40	34	6,900
12	11.9	35	34	11,600	11.9	35	34	12,200
13	11.9	33	36	8,100	12.1	33	36	8,000
14	7.4	23	32	8,900	8.1	23	34	9,200
15	12.8	36	36	4,700	13.0	36	36	4,900
16	8.0	24	33	5,500	8.5	24	34	5,200
17	14.6	42	34	3,500	14.7	43	34	4,500
18	22.1	65	34	9,900	22.7	65	34	9,400
19	11.5	34	34	6,300	11.7	33	34	7,000
20	16.4	48	34	5,300	16.3	47	34	5,800
21	12.2	35	34	6,600	12.0	36	33	6,700
22	10.1	31	32	9,200	10.0	30	33	9,000
23	9.0	27	33	4,100	9.0	28	32	4,400
24	13.0	41	32	8,900	13.2	41	32	8,500
25	15.0	46	32	10,000	15.2	46	32	9,800

solution to blood possible in routine counts, all parameters measured by the Coulter S remained unaffected (Table 3). The same experiments were repeated using di-sodium EDTA powder to obviate any variations arising from dilution factors when using a solution. The results showed insignificant differences between powder and solution. It should be noted that either Lithium or di-sodium EDTA solution should be used with the Coulter S. Other anti-coagulants such as ammonium or potassium oxalate distort cell size, and sodium citrate has an effect on ionic concentration, which would alter the electric field between the electrodes of the aperture bath.

The manufacturers of the Coulter S give an allowable limit of variation from the value of ± two standard deviations, for all tests and results calculated by the machine. The results of 50 repetitive and sequential tests on ten separate patients lie well within the range given. The results endorse the impression gained with daily routine work that the Coulter S gives accurate and reproducible results.

In the Medical Laboratory, blood counts are sometimes tested the next day on samples delivered to the laboratory during the night from country areas. These samples are kept refrigerated at four degrees centigrade from time of delivery to counting. It was found that measurement of Hb, Rbc, Hct, MCV, MCH and MCHC were unaltered, but the total leucocyte count between the ranges of 4,000 cmm and 10,000 cmm showed a reduction after six hours refrigeration. This difference is not significant in practical haematology and in fact after six hours refrigeration was still within the allowable limit of variation for the Coulter S. After fifteen hours refrigeration however the fall-off in total white count varied significantly from the original (10.4 percent to 13 percent). It was concluded that after fifteen hours the total leucocyte count is of little value, but was the result of refrigeration and not the Coulter itself.

Routine maintenance of the Coulter S and cleaning procedures can be carried out quickly and efficiently. It is preferable to have a particular person responsible for this.

Numbers of Tests

According to the manufacturers, the Coulter S can test a new specimen every twenty seconds, giving a theoretical rating of 180 tests per hour. In this survey we found the maximum number of tests a single technician, not necessarily senior or especially trained, has been able to achieve, is 150 per hour, when handling specimens and print out forms singly. Beyond this level there was a rapid fall-off in accuracy.

However it became apparent shortly after the introduction of the Coulter S into the Haematology Department that the increased test rate gave a much more steady flow of work in the routine counts and examination of blood films to follow. The bank-up of specimens when a larger number were received at one time from outlying depots, was also obviated. Conversely as tests could be done so rapidly, samples could be accumulated in slack periods, leaving time for other tests. The Coulter S counter, together with automated blood film staining, enabled results to be returned to the medical practitioner much sooner. A routine day's work of some 400 blood counts was easily carried out by one technician when using the machine; previously three to four technicians were required.

Results and Discussion

Comparisons

A comparison between manual results and Coulter S results was made on the same specimen—taken at random from the daily flow of work. Results correlated well at all levels for haemoglobin and haematocrit and for total white blood counts between 3,000 cmm and 12,500 cmm (Table 4). Low white counts carried out in duplicate similarly showed good correlation for counts between 500 cmm and 3,200 cmm. This count remained good however, only if the background count of the Coulter S was kept below 200 cmm. The same precaution applied to total red cell counts. The importance of this and the necessity of checking the background count of the Isoton diluent each morning cannot be over-emphasised. Once the Coulter is standardised the background count remained satisfactorily low, i.e., below 200 cmm for every white blood count and 20,000 cmm for red blood counts, provided that particle-free Isoton is used during that day. One batch of Isoton supplied by the manufacturers contained so much debris that a background count of 1,600 cmm leucocytes and 330,000 cmm for red cells was given.

When the leucocyte count is 30,000 cmm or more a large error is introduced with manual methods, simply because of the greater number of cells to be counted. The manual count was often higher on elevated counts than the Coulter count. Whether this was due to repetitive counting of the same cell in manual counts or whether it was the result of several leucocytes or a clump of leucocytes being enumerated on the counter as a single cell, was not apparent. However, as the Coulter inconsistently printed out a count of 999×10^3 with a proportion only of leucocyte counts above 84,000 cmm, the greater variation in high counts would seem to occur in the Coulter S rather than in manual counts. For this reason, as a routine, all counts above 30,000 cmm in the laboratory were done by manual methods, the Coulter S result being discarded. It was also found, as in earlier surveys, that the leucocyte count immediately following, above 30,000 cmm was fallaciously high due to 'carry over' of cells from the previous count. An even greater error was produced in the haemoglobin in the specimen immediately following a very high leucocyte count. This was due to turbidity from the white blood cells in the cyanmethaemoglobin solution in the lysing chamber. This source of error also observed by other workers, can be obviated by flushing the machine out with duplicate counts on the specimen following a very high white blood count.

Carry over from previous specimens was measured and found to be the same as in previous surveys, that is, 2 percent, an amount which had no effect on results in routine procedures.

Warm and Cold Agglutinins

The effect of cold and warm agglutinating antibodies on the results of comparative manual and Coulter counts on the same patient were tabulated and in the case of cold agglutinins, the effect of prewarming the sample at 37°C for fifteen minutes before counting was noted. Similarly comparative counts on patients showing excessive rouleaux in the blood film estimate were examined.

Common Errors

The technique of four junior laboratory assistants using the Coulter S throughout the day and under varying degrees of workload was observed without their knowledge. The commonest errors noted were:

1. Removing the blood from the aspirating tube of the Coulter S before a complete quatum was taken up by the machine.
2. Holding the specimen tube at such a distance from the tip of the aspirator that both blood and air from the top of the blood were taken into the sample valve of the machine.

3. Mixing the specimen for a period of less than three to five minutes on the rotating wheel, before transference to the Coulter S.
4. A number of technicians started the next count before the machine was ready to accept a new sample, i.e., before the light on the touch control bar changed back from red to green.
5. Occasionally a technician under pressure put a result card into the printer labelled with a different patient's name than that on the specimen tube being tested.

Mechanical and Technical Difficulties

Aperture blocking may occur several times a day, from dust and debris, presumably in the Isoton and from cell debris, or from air bubbles. On most occasions this is easily remedied without any holdups in the work flow, by pushing the 'clear' button on the control panel.

We have found by experience that the manufacturer's recommendation of simply immersing the apertures in Isoterge solution is not sufficient to prevent them blocking. To ensure that the apertures are kept clean we found a 1 percent solution of 'Protosol' (Smith Biolab, Auckland) very effective. The use of 'Protosol', however, will result in a longer cleaning period of the aperture baths, to give a zero background count.

The sampling valve can very easily be tightened excessively after cleaning, so that it is unable to turn freely and fully, allowing a complete flow of diluent. As the metal rod for adjusting the valve has to be not more than finger tight, this fault can occur readily and can be difficult to detect. This also is not stressed by the manufacturers.

The waste chamber had to be replaced once because it had a small hole punched in the glass wall by the valve ball. The replaced chamber was obtained from Sydney in 12 hours. Temporary repairs with araldite and tape were not satisfactory.

Once, wide fluctuations were noted in the haemoglobin estimation and red cell counts. Intensive testing and checking of the machine failed to give an explanation. Only after several days was it noticed that these fluctuations occurred in a macro sample put through the machine after a micro sample. The reason for this was that a portion of the micro dilution was retained from the specimen, in the front portion of the sampling valve. This residual blood in dilution was aspirated into the valve along with the following macro specimen and then washed into the white blood count mixing chamber, giving rise to errors in the haemoglobin and red counts and to a lesser degree in the white blood cell counts. This source of error (and the time wasted in detecting it) is not mentioned in the manufacturer's manual. Once obvious, however, the error was readily remedied by flushing the valve through with Isoton after a micro capillary sample or alternatively and more satisfactorily by simply repeating the macro test done after a micro and discarding the first macro result.

Over a period of two weeks, fluctuations in haemoglobin values on the same samples were outside the expected variations of the machine. Fairly extensive checking of voltage showed that the voltage output of the haemoglobin timer card varied considerably and this was assumed to be a fault of the card. A new Haemoglobin Timer Card unexpectedly gave no significant improvement. It was observed at this stage that the haemoglobin light assembly was corroded at the points of contact. This was replaced. Subsequently the assembly has been modified by Coulter Electronics Limited; it is now made of material resistant to corrosion. It would appear that variations can be produced in the voltages of the Haemoglobin Timer Card with defective contacts in the haemoglobin light assembly. It should be noted that any machine cause of fluctuations in the haemoglobin values must be either a fault in the haemoglobin light assembly or alternatively in an opaque aperture bath which needs a clean. The latter should be readily detected by moderately experienced staff, but the former requires specialised electrical knowledge.

We have found it necessary to clean the white blood count mixing chambers each day. Protein material collects on the chamber walls at the top of the fluid level and forms a 'scum ring'. If allowed to accumulate this debris can pass into the lysing chamber and finally into the aperture bath where it can easily block the apertures. The need for cleaning the white cell mixing chamber frequently is not mentioned in the Coulter manual. Soaking the chambers in Isoterge, as recommended, is not adequate to remove the debris. It has been found necessary to wash the chambers thoroughly in warm soapy water, clean the walls with a cotton wool swab on an applicator stick, and ensure thorough

rinsing with water. After reassembling, the chamber should be flushed through with Isoton before the machine is used again. Cleaning the white blood count mixing chambers does not interfere with the flow of work.

The vacuum trap connection tubes occasionally lose pressure through being blocked with crystalline material precipitated from Isoton. This can be easily avoided by disconnecting the tubes, once a month and cleaning them with a jet of hot water from a 20 ml syringe The disconnection and reassembly takes only a few minutes and the tubes do not necessarily need to be flushed through with Isoton before use. This point of maintenance is not mentioned in the manufacturer's manual.

Since this paper was started the New Zealand agents for Coulter Electronic have accumulated a comprehensive range of spare parts and have now trained personnel to carry out servicing.

The newer machines which have recently arrived in the country are modified and some of the problems outlined above will most probably not be experienced by the users of the machines.

The modifications have been made to, (1) Waste chamber; (2) Sampling valve; (3) Haemoglobin lamp assembly; (4) Vacuum trap.

Summary

After eleven months' use in the routine laboratory work, handling up to approximately 400 blood samples per day, we conclude that the Coulter S gives rapid, reliable, reproducible results provided that the points in regular maintenance mentioned in the manufacturer's manual and this thesis be observed. The Coulter S minimises the error, stresses and problems associated with the large influx of work received towards the end of the day. The machine can be operated by junior staff after minimal instruction. Since installing the machine the number of staff required to perform routine haematology, that is, haemoglobin, haematocrit, white blood count and ESR estimations, has been reduced. This is a major factor in partly offsetting the large capital outlay. Full benefit from this and other types of automated laboratory procedures can only be achieved by centralisation which is inevitable, though partly dependent on rapid and efficient transport of the sample from the bedside to the laboratory. The initial apprehension of the staff over accuracy of results from the Coulter has vanished, partly because of the ease with which any suspected result can be checked. Even so, a constant watch for possible errors in results, that do not correlate with the blood film done on every patient is always necessary.

Acknowledgments

The author wishes to thank sincerely Dr A. J. Erenstrom for his help and guidance in the preparation of this paper, Mr H. Stunzner, and the colleagues in the laboratory for their continual encouragement and assistance.

REFERENCES

1. Accuracy in Clinical Chemistry, 1968, Scientific Products, Miami, Florida.
2. Barnard, D. F., Carter, A. B., Crosland-Taylor, P. J. and Stewart, J. W. (1969), *J. Clin. Path.*, 22, 26.
3. Brittin, G. M., Brecher, G., Johnson, C. (1969), *Am. J. clin. Path.*, 52, 679.
4. Coulter Counter Service Manual, 4th Edition, 1969.
5. Dacie, J. V. and Lewis, S. M. (1968), *Practical Haematology*, 4th Edition.
6. Nelson, M. G. (1969), *J. Clin. Path.*, 22, 1.
7. Pinkerton, P. H., Spence, I., Ogilvie, J. C., Ronald, W. A., Marchant, P. and Ray, P. K. (1970), *J. Clin. Path.*, 23, 68.
8. Stewart, J. W. (1967), *Brit. J. Haemat.*, 13, 11.
9. Wintrobe, M. M. (1967), *Clinical Haematology*, 6th Edition.

A Cytological Analysis of Urine from Renal Transplant Patients

Diana J. Walker
National Women's Hospital, Auckland

Adapted from a thesis submitted in partial fulfilment of the examination for Qualified Technical Officer, September, 1971

Introduction

This study was undertaken to assess whether any useful information such as prediction of rejection episodes could be obtained on cytological grounds. Examination for evidence of cytomegalic inclusion disease was concurrently performed.

Taft and Flax[7] found that renal tubular cells were present in large numbers during acute rejection episodes and in chronic rejection in

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13	11.9	33	36	8,100	12.1	33	36	8,000
14	7.4	23	32	8,900	8.1	23	34	9,200
15	12.8	36	36	4,700	13.0	36	36	4,900
16	8.0	24	33	5,500	8.5	24	34	5,200
17	14.6	42	34	3,500	14.7	43	34	4,500
18	22.1	65	34	9,900	22.7	65	34	9,400
19	11.5	34	34	6,300	11.7	33	34	7,000
20	16.4	48	34	5,300	16.3	47	34	5,800
21	12.2	35	34	6,600	12.0	36	33	6,700
22	10.1	31	32	9,200	10.0	30	33	9,000
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25	15.0	46	32	10,000	15.2	46	32	9,800

solution to blood possible in routine counts, all parameters measured by the Coulter S remained unaffected (Table 3). The same experiments were repeated using di-sodium EDTA powder to obviate any variations arising from dilution factors when using a solution. The results showed insignificant differences between powder and solution. It should be noted that either Lithium or di-sodium EDTA solution should be used with the Coulter S. Other anti-coagulants such as ammonium or potassium oxalate distort cell size, and sodium citrate has an effect on ionic concentration, which would alter the electric field between the electrodes of the aperture bath.

The manufacturers of the Coulter S give an allowable limit of variation from the value of ± two standard deviations, for all tests and results calculated by the machine. The results of 50 repetitive and sequential tests on ten separate patients lie well within the range given. The results endorse the impression gained with daily routine work that the Coulter S gives accurate and reproducible results.

In the Medical Laboratory, blood counts are sometimes tested the next day on samples delivered to the laboratory during the night from country areas. These samples are kept refrigerated at four degrees centigrade from time of delivery to counting. It was found that measurement of Hb, Rbc, Hct, MCV, MCH and MCHC were unaltered, but the total leucocyte count between the ranges of 4,000 cmm and 10,000 cmm showed a reduction after six hours refrigeration. This difference is not significant in practical haematology and in fact after six hours refrigeration was still within the allowable limit of variation for the Coulter S. After fifteen hours refrigeration however the fall-off in total white count varied significantly from the original (10.4 percent to 13 percent). It was concluded that after fifteen hours the total leucocyte count is of little value, but was the result of refrigeration and not the Coulter itself.

Routine maintenance of the Coulter S and cleaning procedures can be carried out quickly and efficiently. It is preferable to have a particular person responsible for this.

Numbers of Tests

According to the manufacturers, the Coulter S can test a new specimen every twenty seconds, giving a theoretical rating of 180 tests per hour. In this survey we found the maximum number of tests a single technician, not necessarily senior or especially trained, has been able to achieve, is 150 per hour, when handling specimens and print out forms singly. Beyond this level there was a rapid fall-off in accuracy.

However it became apparent shortly after the introduction of the Coulter S into the Haematology Department that the increased test rate gave a much more steady flow of work in the routine counts and examination of blood films to follow. The bank-up of specimens when a larger number were received at one time from outlying depots, was also obviated. Conversely as tests could be done so rapidly, samples could be accumulated in slack periods, leaving time for other tests. The Coulter S counter, together with automated blood film staining, enabled results to be returned to the medical practitioner much sooner. A routine day's work of some 400 blood counts was easily carried out by one technician when using the machine; previously three to four technicians were required.

Results and Discussion

Comparisons

A comparison between manual results and Coulter S results was made on the same specimen—taken at random from the daily flow of work. Results correlated well at all levels for haemoglobin and haematocrit and for total white blood counts between 3,000 cmm and 12,500 cmm (Table 4). Low white counts carried out in duplicate similarly showed good correlation for counts between 500 cmm and 3,200 cmm. This count remained good however, only if the background count of the Coulter S was kept below 200 cmm. The same precaution applied to total red cell counts. The importance of this and the necessity of checking the background count of the Isoton diluent each morning cannot be over-emphasised. Once the Coulter is standardised the background count remained satisfactorily low, i.e., below 200 cmm for every white blood count and 20,000 cmm for red blood counts, provided that particle-free Isoton is used during that day. One batch of Isoton supplied by the manufacturers contained so much debris that a background count of 1,600 cmm leucocytes and 330,000 cmm for red cells was given.

When the leucocyte count is 30,000 cmm or more a large error is introduced with manual methods, simply because of the greater number of cells to be counted. The manual count was often higher on elevated counts than the Coulter count. Whether this was due to repetitive counting of the same cell in manual counts or whether it was the result of several leucocytes or a clump of leucocytes being enumerated on the counter as a single cell, was not apparent. However, as the Coulter inconsistently printed out a count of 999×10^3 with a proportion only of leucocyte counts above 84,000 cmm, the greater variation in high counts would seem to occur in the Coulter S rather than in manual counts. For this reason, as a routine, all counts above 30,000 cmm in the laboratory were done by manual methods, the Coulter S result being discarded. It was also found, as in earlier surveys, that the leucocyte count immediately following, above 30,000 cmm was fallaciously high due to 'carry over' of cells from the previous count. An even greater error was produced in the haemoglobin in the specimen immediately following a very high leucocyte count. This was due to turbidity from the white blood cells in the cyanmethaemoglobin solution in the lysing chamber. This source of error also observed by other workers, can be obviated by flushing the machine out with duplicate counts on the specimen following a very high white blood count.

Carry over from previous specimens was measured and found to be the same as in previous surveys, that is, 2 percent, an amount which had no effect on results in routine procedures.

Warm and Cold Agglutinins

The effect of cold and warm agglutinating antibodies on the results of comparative manual and Coulter counts on the same patient were tabulated and in the case of cold agglutinins, the effect of prewarming the sample at 37°C for fifteen minutes before counting was noted. Similarly comparative counts on patients showing excessive rouleaux in the blood film estimate were examined.

Common Errors

The technique of four junior laboratory assistants using the Coulter S throughout the day and under varying degrees of workload was observed without their knowledge. The commonest errors noted were:

1. Removing the blood from the aspirating tube of the Coulter S before a complete quatum was taken up by the machine.
2. Holding the specimen tube at such a distance from the tip of the aspirator that both blood and air from the top of the blood were taken into the sample valve of the machine.

3. Mixing the specimen for a period of less than three to five minutes on the rotating wheel, before transference to the Coulter S.
4. A number of technicians started the next count before the machine was ready to accept a new sample, i.e., before the light on the touch control bar changed back from red to green.
5. Occasionally a technician under pressure put a result card into the printer labelled with a different patient's name than that on the specimen tube being tested.

Mechanical and Technical Difficulties

Aperture blocking may occur several times a day, from dust and debris, presumably in the Isoton and from cell debris, or from air bubbles. On most occasions this is easily remedied without any holdups in the work flow, by pushing the 'clear' button on the control panel.

We have found by experience that the manufacturer's recommendation of simply immersing the apertures in Isoterge solution is not sufficient to prevent them blocking. To ensure that the apertures are kept clean we found a 1 percent solution of 'Protosol' (Smith Biolab, Auckland) very effective. The use of 'Protosol', however, will result in a longer cleaning period of the aperture baths, to give a zero background count.

The sampling valve can very easily be tightened excessively after cleaning, so that it is unable to turn freely and fully, allowing a complete flow of diluent. As the metal rod for adjusting the valve has to be not more than finger tight, this fault can occur readily and can be difficult to detect. This also is not stressed by the manufacturers.

The waste chamber had to be replaced once because it had a small hole punched in the glass wall by the valve ball. The replaced chamber was obtained from Sydney in 12 hours. Temporary repairs with araldite and tape were not satisfactory.

Once, wide fluctuations were noted in the haemoglobin estimation and red cell counts. Intensive testing and checking of the machine failed to give an explanation. Only after several days was it noticed that these fluctuations occurred in a macro sample put through the machine after a micro sample. The reason for this was that a portion of the micro dilution was retained from the specimen, in the front portion of the sampling valve. This residual blood in dilution was aspirated into the valve along with the following macro specimen and then washed into the white blood count mixing chamber, giving rise to errors in the haemoglobin and red counts and to a lesser degree in the white blood cell counts. This source of error (and the time wasted in detecting it) is not mentioned in the manufacturer's manual. Once obvious, however, the error was readily remedied by flushing the valve through with Isoton after a micro capillary sample or alternatively and more satisfactorily by simply repeating the macro test done after a micro and discarding the first macro result.

Over a period of two weeks, fluctuations in haemoglobin values on the same samples were outside the expected variations of the machine. Fairly extensive checking of voltage showed that the voltage output of the haemoglobin timer card varied considerably and this was assumed to be a fault of the card. A new Haemoglobin Timer Card unexpectedly gave no significant improvement. It was observed at this stage that the haemoglobin light assembly was corroded at the points of contact. This was replaced. Subsequently the assembly has been modified by Coulter Electronics Limited; it is now made of material resistant to corrosion. It would appear that variations can be produced in the voltages of the Haemoglobin Timer Card with defective contacts in the haemoglobin light assembly. It should be noted that any machine cause of fluctuations in the haemoglobin values must be either a fault in the haemoglobin light assembly or alternatively in an opaque aperture bath which needs a clean. The latter should be readily detected by moderately experienced staff, but the former requires specialised electrical knowledge.

We have found it necessary to clean the white blood count mixing chambers each day. Protein material collects on the chamber walls at the top of the fluid level and forms a 'scum ring'. If allowed to accumulate this debris can pass into the lysing chamber and finally into the aperture bath where it can easily block the apertures. The need for cleaning the white cell mixing chamber frequently is not mentioned in the Coulter manual. Soaking the chambers in Isoterge, as recommended, is not adequate to remove the debris. It has been found necessary to wash the chambers thoroughly in warm soapy water, clean the walls with a cotton wool swab on an applicator stick, and ensure thorough

rinsing with water. After reassembling, the chamber should be flushed through with Isoton before the machine is used again. Cleaning the white blood count mixing chambers does not interfere with the flow of work.

The vacuum trap connection tubes occasionally lose pressure through being blocked with crystalline material precipitated from Isoton. This can be easily avoided by disconnecting the tubes, once a month and cleaning them with a jet of hot water from a 20 ml syringe The disconnection and reassembly takes only a few minutes and the tubes do not necessarily need to be flushed through with Isoton before use. This point of maintenance is not mentioned in the manufacturer's manual.

Since this paper was started the New Zealand agents for Coulter Electronic have accumulated a comprehensive range of spare parts and have now trained personnel to carry out servicing.

The newer machines which have recently arrived in the country are modified and some of the problems outlined above will most probably not be experienced by the users of the machines.

The modifications have been made to, (1) Waste chamber; (2) Sampling valve; (3) Haemoglobin lamp assembly; (4) Vacuum trap.

Summary

After eleven months' use in the routine laboratory work, handling up to approximately 400 blood samples per day, we conclude that the Coulter S gives rapid, reliable, reproducible results provided that the points in regular maintenance mentioned in the manufacturer's manual and this thesis be observed. The Coulter S minimises the error, stresses and problems associated with the large influx of work received towards the end of the day. The machine can be operated by junior staff after minimal instruction. Since installing the machine the number of staff required to perform routine haematology, that is, haemoglobin, haematocrit, white blood count and ESR estimations, has been reduced. This is a major factor in partly offsetting the large capital outlay. Full benefit from this and other types of automated laboratory procedures can only be achieved by centralisation which is inevitable, though partly dependent on rapid and efficient transport of the sample from the bedside to the laboratory. The initial apprehension of the staff over accuracy of results from the Coulter has vanished, partly because of the ease with which any suspected result can be checked. Even so, a constant watch for possible errors in results, that do not correlate with the blood film done on every patient is always necessary.

Acknowledgments

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REFERENCES

1. Accuracy in Clinical Chemistry, 1968, Scientific Products, Miami, Florida.
2. Barnard, D. F., Carter, A. B., Crosland-Taylor, P. J. and Stewart, J. W. (1969), *J. Clin. Path.*, 22, 26.
3. Brittin, G. M., Brecher, G., Johnson, C. (1969), *Am. J. clin. Path.*, 52, 679.
4. Coulter Counter Service Manual, 4th Edition, 1969.
5. Dacie, J. V. and Lewis, S. M. (1968), *Practical Haematology*, 4th Edition.
6. Nelson, M. G. (1969), *J. Clin. Path.*, 22, 1.
7. Pinkerton, P. H., Spence, I., Ogilvie, J. C., Ronald, W. A., Marchant, P. and Ray, P. K. (1970), *J. Clin. Path.*, 23, 68.
8. Stewart, J. W. (1967), *Brit. J. Haemat.*, 13, 11.
9. Wintrobe, M. M. (1967), *Clinical Haematology*, 6th Edition.

A Cytological Analysis of Urine from Renal Transplant Patients

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Introduction

This study was undertaken to assess whether any useful information such as prediction of rejection episodes could be obtained on cytological grounds. Examination for evidence of cytomegalic inclusion disease was concurrently performed.

Taft and Flax[7] found that renal tubular cells were present in large numbers during acute rejection episodes and in chronic rejection in

moderate numbers. Bossen *et al.*[1], described a cytologic profile during rejection of features such as nuclear changes, casts, RBC's, lymphocytes and tubular cells. They found more striking increases in tubular cells in patients with chronic rejection or cytomegalic inclusion disease than in acute rejection. Doak *et al.*[2] mentions in criteria for rejection the appearance of abnormal mononuclear cells in the urine. Ooi and Kincaid-Smith[6] found the most constant index in rejection episodes in the first month of transplantation was a doubling of the renal tubular cell count.

An attempt was made to compile some Auckland results.

Procedures

From January 1971 to August 1971, 202 urine specimens from 17 patients who received cadaver kidneys were examined cytologically. These patients fell into two groups:

1. Those transplanted in the period of the study, and therefore presenting a wide spectrum of change in the post-operative stages, whose function was sufficiently good to be producing adequate volume of urine for analysis.
2. Patients transplanted prior to the study period who were selected as cases of possible interest cytologically, i.e., not necessarily liable to reject but liable to involvement with cytomegalic inclusion disease.

Samples were collected daily, where possible, whilst the patients were hospitalised and thereafter at every out-patient visit. Inter-hospital collection services and helpful clinic and laboratory staff ensured prompt delivery. If there was to be any undue delay in preparation, an equal volume of 50 percent ethyl alcohol was added and the specimens were stored in the refrigerator until they could be dealt with.

The urine samples were all voided specimens and after the pH was measured 50 mls were prepared by the Millipore filter technique and stained by the Papanicolaou method. A 5μ filter was used to retain RBC's and polymorphonuclear leucocytes.

A new stock of Gelman filters became available and these were also tried. Millipore filters were inconvenient to fix and stain as they dissolved in ethanol stronger than 95 percent, so isopropyl was used as the dehydrating alcohol. The Gelman filters can be put through the normal staining used routinely in the laboratory and when mounted in Eukitt, are more transparent and show less background than do the Millipore.

Diatex, Permount, DePeX, Lab.Serv. and Eukitt were all tried with the two different types of filters and Permount was found to be most satisfactory with the Millipore and Eukitt with the Gelman. Eukitt had the added advantage of being quick drying. Also, the specimen passed through the Gelman filter somewhat faster than the Millipore, which saved considerable time in preparation.

Initially, wet films and counts in a Neubauer counting chamber were also performed on each specimen, but it was very time consuming and inaccurate in differentiating cell types, therefore it was decided that they contributed no useful additional information.

The filter preparations were examined and the renal tubular cells were counted. Other features such as background (bacteria, crystals, debris and proteinaceous material) casts, polymorphs, lymphocytes, RBC's and nuclear changes were noted.

The patients were all given Prednisone and Imuran[3] routinely after transplant and antibiotics and other drugs whenever necessary. Koss[5] states that treatment with Prednisone does not affect urinary tract cytology.

During the study period there were three acute rejection episodes just prior to the receipt of the first specimens and two early in the series of specimens. Raised serum creatinine[3] level is used by the Auckland Public Hospital team as the most reliable guide for the diagnosis of rejection if unexplained by any other means. The doses of immunosuppressive drugs are increased sharply thus reversing the process.

Specimens from two of the current patients (cases 4 and 6), were part of a series examined in this laboratory in 1968 therefore some additional information was available from these slides. These specimens were originally looked at not only for the cytological picture, but to experiment with different techniques in filter preparation and mounting methods.

Results

Of the original 17 patients, seven were discarded because of inadequate numbers of specimens. The 10 patients remaining showed a multiplicity of different cytological combinations, and in some cases varied considerably from day to day.

Cytological features seen were:

1. *Renal Tubular Cells.* These ranged from small round cells with a moderate amount of cytoplasm, exhibiting vacuolisation (Fig. 1), or elongated, small, fibre-type cells with dark staining nuclei (Fig. 2), to large bizarre cells with little or no cytoplasm, with large hyperchromatic nuclei (Figs 3 and 4). Some of these large cells showed the glassy appearance described by Johnston *et al.*[4], as being suspicious of cytomegalic inclusion disease (Fig. 5).

2. *Casts.* Almost every type was represented in the specimens. There were hyaline, granular, leucocytes, red blood cells and casts composed of renal tubular cells (Fig. 6).

3. *Crystals.* In two cases crystals were dominant in the early post-operative phases. Uric acid in one and triple phosphate in the other. Uric acid crystals also appeared in the urine of another patient 47 weeks post-operative.

4. The background material consisted of bacteria, amorphous and granular material and debris. In some cases this was present with nothing more than a few squamous epithelial calls. These squamous cells were not counted or noted in any way as the specimens were all voided, and from both males and females.

5. RBC's appeared for no good reason at various times in the post-operative phases. Leucocytes were, of course, associated with urinary tract infections, but frequently appeared in increased numbers without explanation.

6. Ooi and Kincaid-Smith[6] used lymphocyte counts in their assessment of urines but they found difficulty in differentiating them from other small cells in air-dried, May-Grunwald stained smears. We were only able to positively identify them very occasionally.

A graph on each patient demonstrated five values:

1. Serum creatinine.
2. Serum urea.
3. The number of renal tubular cells per filter.
4. Profile 1.
5. Profile 2.

Profile 1. Bossen *et al.*[1], observed and noted nuclear changes, casts, RBC's, background, lymphocytes, mixed cell clusters and tubular cells. He gave them equal value with a possible total of seven. See Table 1.

Mixed cell clusters were replaced by leucocytes by us because of difficulties of interpretation of cell types and also because it missed a significant cell component of the specimens.

TABLE 1

Spec.	Nuclear Changes	Casts	RBC	Background	Lympho-cytes	Renal Tubular Cells	Leuco-cytes	Total
1	+	+	—	+	—	+	+	5
2	—	+	+	+	—	+	+	5
3	—	—	—	+	-	+	+	3

Profile 2. It was considered that a weakness of Profile 1 was that each factor was of equal value, e.g., lymphocytes and renal tubular cells both scored as one each. To overcome this inherent weakness, the seven factors were noted over all the specimens and scored according to their frequency of occurrence as follows:

Least Frequent → Most Frequent						
1	2	3	4	5	6	7
Lymphocyte	Nuclear Changes	RBC's	Leucocytes	Background	Casts	Tubular Cells

This gave the finding of renal tubular cells and casts in a urine a much higher scoring value than lymphocytes and nuclear changes, and it was thought this would reflect a truer profile than that of Bossen *et al*[1]. See Table 2.

TABLE 2

Score	2	6	3	5	1	7	4	
Spec.	Nuclear Changes	Casts	RBC's	Background	Lympho-cytes	Renal Tubular Cells	Leuco-cytes	Total
1	+	+	—	+	—	+	+	24
2	—	++	+	+	—	±	+	28
3	—	—	—	++	—	++	++	32

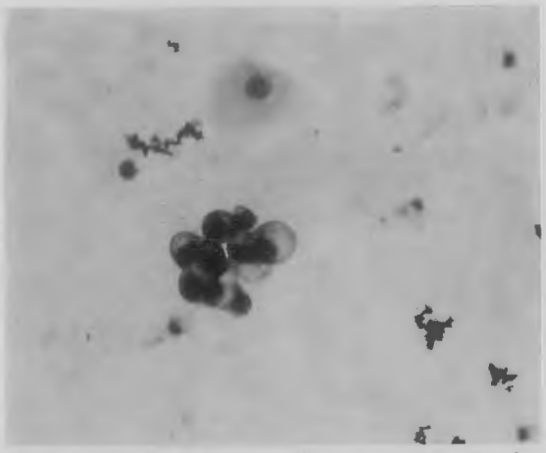


FIG. 1.—Renal tubular cells. A cluster showing vacuolisation. Pap. stain × 400.

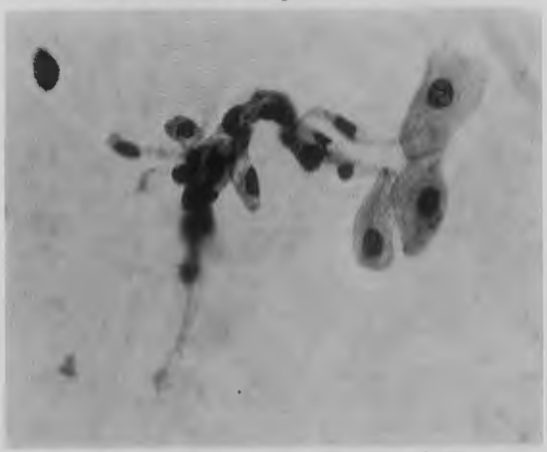


FIG. 2.—Renal tubular cells. Fronds of fibre type cells. Pap. stain × 400.

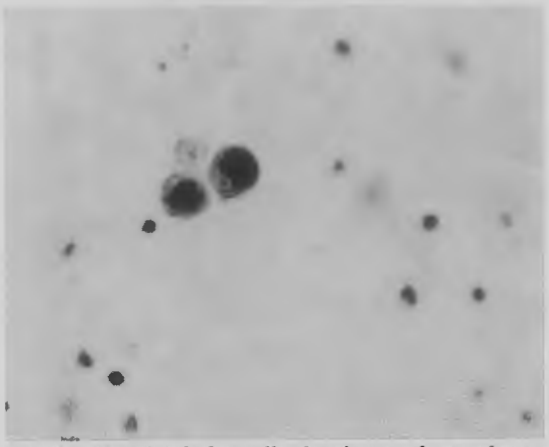


FIG. 3.—Renal tubular cells showing nuclear enlargement and hyperchromasia. Pap. stain × 400.

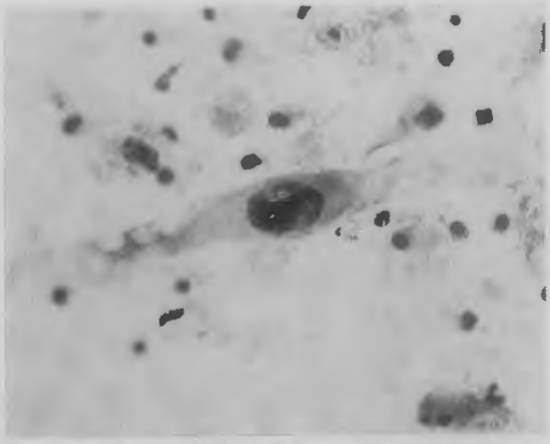


FIG. 4.—Renal tubular cell. A degenerate bizzare cell with ragged vacuolated cytoplasm. Pap. stain × 400.

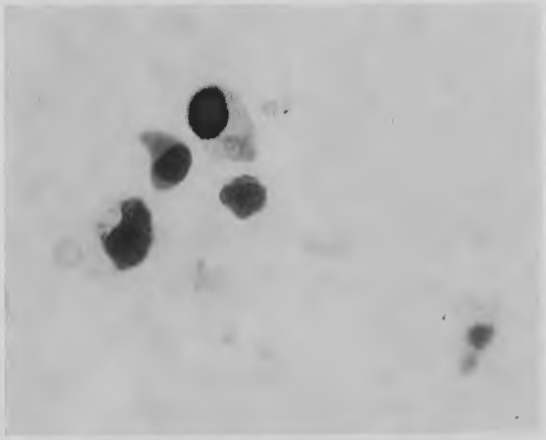


FIG. 5.—Large cells showing glassy nuclei. Pap. stain × 400.

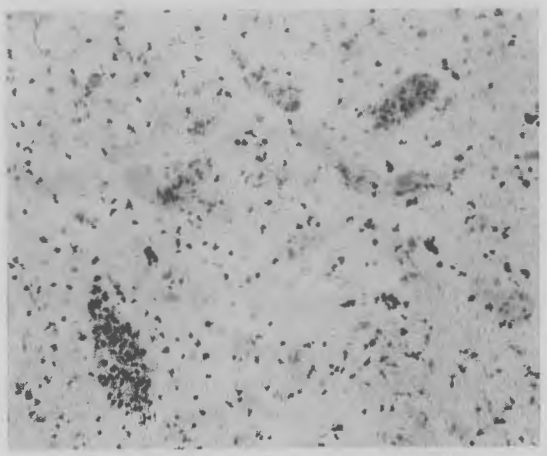
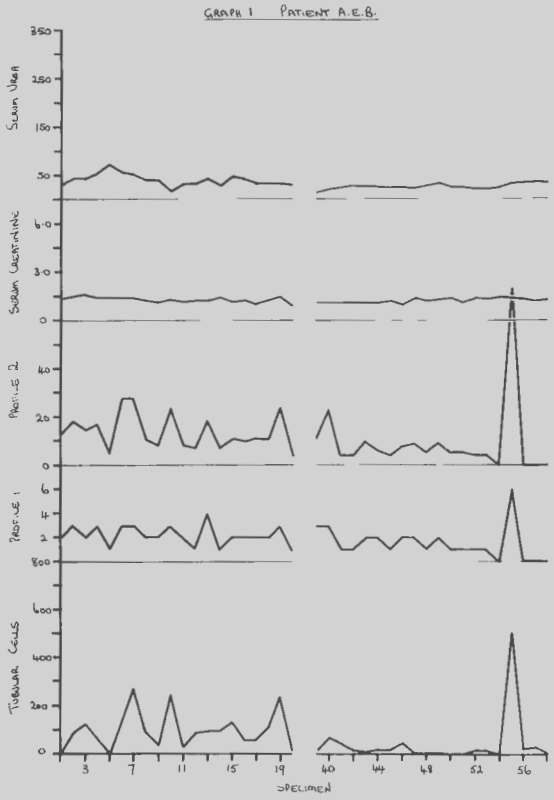


FIG. 6.—Casts. Pap. stain × 100.



Renal tubular cells were noted on patient score sheets according to Taft and Flax[7], i.e., ± less than 50; + more than 50 but less than 200; ++ more than 200 but less than 400; +++ more than 400 but less than 800; ++++ more than 800.

Case 1.—Patient A.E.B., aged 39, received a kidney flown from Australia, and was the first transplant done in Auckland for some months. Urines were received from the sixth day post-operative and 61 specimens in all were examined. A rejection episode on the tenth day was treated with increased doses of Prednisone and Imuran and this was the only such episode during the study period.

Cytologically, early specimens showed moderate numbers of leucocytes, relatively small numbers of renal tubular cells, with a background of bacteria and triple phosphate crystals. Peaks of renal tubular cells occurred in the seventh, tenth and nineteenth specimens but these were not considered unduly high as there was no increase in the other factors. A relatively large peak occurred later in the fifty-fifth specimen (twenty-eighth week) and there were also increases in background, casts, RBC's and leucocytes. There were no corresponding rises in the serum creatinine or serum urea levels.

Apart from these minor 'crises', in general the specimens showed a clear background and contained only a few leucocytes and renal tubular cells showing no nuclear abnormalities. Casts were seen very occasionally.

There were no particular changes noted during the rejection episode which occurred between specimens 4 and 5.

The graphs showed that Profiles 1 and 2 were very similar to the trends in tubular cells and there was no correlation with the serum ureas and serum creatinine. (See Graph 1.)

Patient A.E.B. is well and has been working part-time for several months doing light work.

The results of nine further patients set out originally as in Case 1 are summarised below in table form by means of symbols.

TABLE 3

		Renal Tubular Cells	Profile 1	Profile 2	Serum Creatinine	Serum Urea
Case 1		+	+	+	×	×
Case 2		×	+	+	—	—
Case 3		×	+	+	—	—
Case 4	1968	—	+	+	×	×
Case 4	1971	—	+	+	—	—
Case 5		—	+	+	×	×
Case 6	1968		+	+	—	—
Case 6	1971	—	+	+	—	—
Case 7		+	×	+	×	—
Case 8		+	×	+	—	×
Case 9		+	+	+	×	×
Case 10		—	—	×	—	×

\+ Similar peaks.
× Similar peaks but no correlation to those shown as +.
— No correlation to any other result.

The clinical particulars of the patients are summarised as follows:

Case 2 Male, aged 34, received his transplant 25 weeks prior to the study period. He had one rejection episode four days before specimens were being examined, so a 'Before' and 'After' picture cannot be demonstrated. Fifteen specimens were received for examination.

Case 3 Male, aged 26, received a transplant twelve months prior to the receipt of the first specimen. His only rejection

episode occurred six days before this in the fifty-first week post-operative. Fifteen specimens were received for examination.

Case 4 Male, aged 33, received a transplant three years prior to the study period. Five specimens from the 1968 series and 11 specimens from the current series were examined.

Case 5 Female, aged 31, received a kidney transplant in Wellington 23 months prior to the study period. There were no acute rejection episodes in the study period, but the patient had several urinary tract infections which were treated with Bactrim. Eleven specimens were examined.

Case 6 Male, aged 52, received a kidney transplant in 1968 and eight specimens were examined from the twentieth-thirtieth week post-operative in the previous study. There were three acute rejection episodes in the twelfth, fiftieth and sixty-ninth days post-operative, but the patient has been well since and has good function. Eleven specimens were received in the current study period. Because of variables such as types of filter used, unknown volumes of urine and numbers of slides being unavailable, accurate counts of renal tubular cells were not made on the 1968 slides for this patient.

Case 7 Male, aged 26, received a kidney transplant 23 months prior to the study period. There were four acute rejection episodes in that time but since then the patient's renal function has remained poor, but just adequate, so that transplant nephrectomy is thought to be inevitable. Fifteen urine specimens were examined over the six-month period.

Case 8 Male, aged 33, received a second renal transplant early in the study period and 11 urine specimens were examined from the twenty-third to fifty-eighth day post-operative. The only rejection episode occurred on day 24, so a 'Before' and 'After' picture cannot be demonstrated.

Case 9 Male, aged 56, received a renal transplant 48 weeks prior to examination. He had one rejection episode in the fifteenth week but had been well since. Nine urine specimens were examined over a period of 14 weeks. The patient died suddenly in the sixty-third week post-operative of other causes.

Case 10 Male, aged 31, received a renal transplant within the study period. There was some difficulty stabilising this patient on his drugs and diet in the early stages, so no specimens were received until 26 days post-operative. One acute rejection episode was experienced 12 days prior to the receipt of the first specimen. Twenty-four specimens were examined over a period of 79 days, some of which contained cells described by Johnston *et al.*[4], as being suspicious of cytomegalic inclusion disease. Virus culture was performed but was reported as negative.

Discussion

The criteria for the diagnosis of rejection is generally accepted as including features such as malaise, fever, tachycardia, hypertension, fall in urine volume, rise in serum urea and serum creatinine, fall in creatinine clearance, proteinuria and a fall in PAH clearance. What is not made clear in the literature, is, at what stage treatment to reverse the rejection process should be initiated. Doak *et al.*[2], state that 'Because it is certain that the earlier rejection can be detected and treated the more easily it can be reversed, we have tended to treat these episodes before unequivocal evidence for the process is obvious, and it is almost certain that on some occasions we have treated non-existent attacks'. Also when their patients are well enough, they are sent home to distant cities where they attend for checks with a local doctor. The appropriate tests are performed and the results relayed to the centre in Auckland where they are assessed and, if necessary, treatment ordered. So the diagnosis can be made on an outpatient basis.

Taft and Flax[7] showed an increased number of renal tubular cells which correlated with the serum urea and serum creatinine in four patients who had rejection episodes. In our

series there were two patients with extraordinarily high renal tubular cell counts but with no evidence of rejection. We could not show any correlation between the renal tubular cell counts and the serum urea and serum creatinine in the 10 patients.

Ooi and Kincaid-Smith[6] estimated hourly excretion rates of lymphocytes, leucocytes and tubular epithelial cells and found that the renal tubular cell count doubled in 14 out of 21 rejection episodes, particularly in the first month after transplantation. Lymphocytes increased in four of six patients who had a rejection episode during the first month and leucocytes doubled in seven out of 21 episodes. Only two rejection episodes occurred within the present study period and in neither case did they show the changes reported by Ooi and Kincaid-Smith[6], however, there were massive increases in several cases that were not associated with rejection episodes.

Bossen *et al.*[1], found that reliance on single features such as lymphocytes or renal tubular cells was more likely to lead to a false positive diagnosis than a profile of several features. Although similar trends in some, or all of the five values were sometimes noted, no clearly defined pattern emerged.

It is possible that this failure to duplicate the findings of other workers could be due in some small measure to the likelihood that rejection episodes in their patients were permitted to progress longer without treatment than were those in our own series. Obviously the rejection process is accompanied by a more or less profound local cellular reaction, and the early application of immunosuppressive treatment could well alter the relationship of the cellular response to the patient's clinical progress. Intensive cytological study of patients undergoing transplantations can provide a useful indication of local tissue responses, but clearly more work will be required to evaluate its precise contribution.

Conclusion

Over a period of seven months, 202 urine specimens from 17 patients who received cadaver kidney transplants were examined Within this period two rejection episodes occurred and were treated. The specimens were prepared by the Millipore filter technique, stained Papanicolaou and examined cytologically.

Urinary cytological profiles and renal tubular cell counts were compared with the serum creatinine and serum urea values. It was clearly shown that serum creatinine and serum urea values were not reflected by any consistent cytological picture, therefore, cytological assessment of urine specimens of this nature had little to offer in the clinical diagnosis of rejection.

Acknowledgments

I wish to thank Dr S. E. Williams and Mr M. J. Churchouse for their help and encouragement in the preparation of the text, Miss R. Romano and Mrs P. Smith of the Medical Unit, Auckland Public Hospital, for cooperation and collection of specimens and other outside departments who have assisted me. I also wish to thank Mrs R. Spottiswood for typing this manuscript and Mr M. J. Churchouse for the photomicrographs.

REFERENCES

1. Bossen, E. H., Johnston, W. W., Amatulli, J. and Rowlands, D. T. Jr. (1970), *Acta Cytol.*, 14, 176-181.
2. Doak, P. B., Maclaurin, C. H., Duke, A. J., Montgomerie, T. Z. and North, J. D. K. (1968), *NZ med. J.*, **68**, 221-227.
3. Doak, P. B., Bowie, E. A., Dalton, N. T., Douglas, R., Duke, A. J., Maclaurin, C. H., Montgomerie, J. Z., North, J. D. K., Smith, H. L. and Sorrell, V. F. (1971), *NZ med. J.*, 73, 117-123.
4. Johnston, W. W., Bossen, E. H., Amatulli, J. and Rowlands, D. T. Jr. (1969), *Acta Cytol.*, 13, 605-610.
5. Koss, L. G., MD, *Diagnostic Cytology and its Histopathologic Bases.* Second edition 1968. J. B. Lippincott Co., Philadelphia.
6. Ooi, B. S. and Kincaid-Smith, Priscilla (1970), *Med. J. Aust.* 2, 667-670.
7. Taft, P. D. and Flax, M. H. (1966), *Transplantation*, 4, 194-204.

Variations in Creatinine Concentration in 24h Urine Collections Made in Acid

J. D. Newton

Christchurch Hospital Laboratory

From a paper presented at the Twenty-seventh Annual Conference of the NZIMLT in Wellington, August 1971

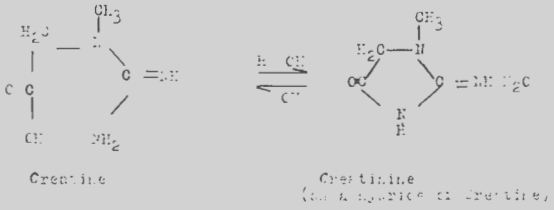
Introduction

In certain estimations and screening tests performed on 24h urine collections it is necessary to add either acetic or hydrochloric acid as a preservative. Some such tests are catecholamines, 5-hydroxyindoleacetic acid and vanilmandelic acid. In some cases it is necessary to know the creatinine concentration either because the results of the test are expressed in terms of creatinine excreted or because a creatinine clearance is to be done on the same specimen. In this laboratory it has always been accepted that a creatinine could not be done on such a specimen as the result would be affected by the acid. It was decided, therefore, that a brief investigation into the effect of acid, if any, on creatinine concentration and an evaluation of any observed affect would be carried out.

In aqueous solutions of creatine and creatinine an equilibrium will exist between the two compounds. The position of and rate of attainment of equilibrium is dependent on several factors:

1, pH; 2, Time; 3, Temperature; 4, Initial concentrations of the two compounds.

The reaction which occurs is as follows:



Creatine Creatinine

At refrigeration temperatures and neutral pH, the ratio of the two compounds approaches unity after about one year, the time becoming shorter as the temperature is raised[1]. Creatinine is formed from creatine rather quickly at acid pH and complete conversion is accomplished if sufficient time is allowed, the conversion being faster at higher temperatures[2]. Alkaline conditions favour a rapid attainment of equilibrium, the rate once again being temperature dependent[3]. Under the conditions of the investigation the pH is quite acid and so the effect of alkali will be disregarded.

The normal range of 24h excretion of creatinine is 1.0-1.8 g/24h, and that of creatine is up to 150 mg/24h[4]. The *maximum* expected error in creatinine level as measured would therefore be in the region of 15 percent assuming complete conversion of creatine to creatinine. The error would in fact be considerably less as complete conversion could not possibly take place under the normal conditions of urine collection and testing. The conditions of collection in the wards are generally (unfortunately) beyond the control of the laboratory and so only the changes which can occur in the urine as it is received can be investigated. Normally a collection once it is received is measured and aliquoted quite quickly and is then stored at 4°C, or else is refrigerated until it is aliquoted, so throughout this investigation the urine was refrigerated.

An attempt was made to determine the effect of hydrochloric and acetic acid in varying concentrations on the creatinine concentration of a normal 24h collection of urine, over a period of three days.

Procedure

A 24h collection of urine was made on a normal subject, the collection being refrigerated during collection to minimise changes during collection. Serial creatinine estimations were made each day on aliquots which had varying amounts of hydrochloric acid and acetic acid added to correspond to 24h volumes of from 200 ml to 2,000 ml. A control urine without acid was run each time. Results obtained were corrected for dilution by acid so that they could be directly compared to one another and to the control value. An attempt was then made to correlate creatinine concentration as measured, amount and type of acid added, the period of storage, pH, and the established precision of this laboratory using an Autoanalyser method which utilises the Jaffe reaction.

Volumes of acid added routinely to a winchester bottle (2.5L) are 10 ml of concentrated

hydrochloric acid or 25 ml glacial acetic acid. Aliquots of urine had amounts of acid added to them to correspond to the standard volumes of acid added to a bottle and 24h volumes of 200, 300, 500, 1,000, and 2,000 ml.

The results are tabulated.

Sample No.	Vol. acid added	Vol. urine added	Corresponding 24h Vol.	DAY 1			DAY 2			DAY 3		
				Creatinine read	corrected	pH	Creatinine read	corrected	pH	Creatinine read	corrected	pH
1	0.5 ml Acetic	4 ml	200 ml	185 mg%	208 mg%	3.3	193 mg%	217 mg%	3.2	198 mg%	223 mg%	3.2
2	"	6 ml	300 ml	195	211	3.4	198	215	3.4	213	231	3.4
3	"	10 ml	500 ml	203	213	3.7	207	212	3.9	223	229	3.9
4	"	20 ml	1,000 ml	210	215	3.9	205	215	3.6	218	229	3.6
5	"	40 ml	2,000 ml	215	218	4.2	213	216	4.2	223	226	4.2
6	0.2 ml HCl	4 ml	200 ml	205	215	1.2	210	221	1.2	218	229	1.1
7	"	6 ml	300 ml	205	212	1.3	206	215	1.2	228	236	1.2
8	"	10 ml	500 ml	210	214	1.6	215	217	2.4	225	230	1.5
9	"	20 ml	1,000 ml	210	212	2.3	210	214	1.5	230	232	2.3
10	"	40 ml	2,000 ml	210	211	4.0	213	214	4.0	230	231	4.0
11	No Acid, Control			215	215	6.6	213	213	6.6	230	230	6.6

Discussion

Looking at the table the first thing to be noticed is the considerable dilution by the acid when 24h urine volumes are low. On reflection, however, it will be appreciated that this effect is unimportant if the result is to be used with the 24h volume to calculate 24h excretion as the decrease in concentration is balanced by the increase in volume. There seems to be little effect caused by the pH or the acid used. The only obvious variation is the rather large increase of results on the third day over the previous two days, this increase is, however, followed by the control without acid and cannot therefore be attributed to the acid.

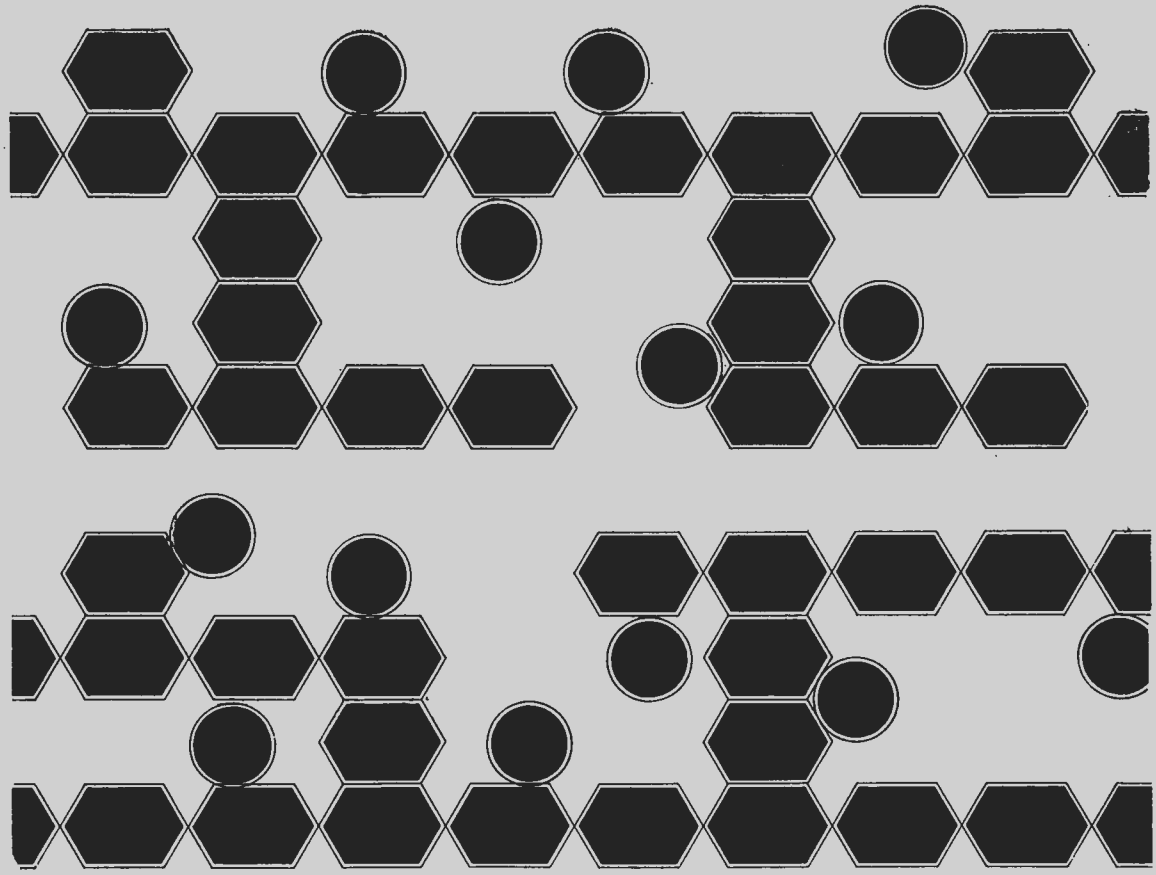
The day-to-day precision of the method was determined over a period of 42 days using the same frozen urine pool. The coefficient of variation was found to be 10 percent which gives a range (95 percent limits) of from 172-258 mg/100 ml at a true concentration of 215 mg/100 ml in which any particular control could fall and still be acceptable.

Conclusion

When the results were viewed in the light of the precision of the method it was obvious that any effect caused by the use of acid during collection and short-term storage was completely insignificant. In fact the most useful information obtained was that the method of creatinine estimation was in need of investigation. Subsequently, a manual dilution step has been omitted and analyses performed on undiluted urine. As a result, the precision on a further 42 controls showed a coefficient of variation of 3.9 percent.

REFERENCES

1. Harper, H. (1969), *Review of Physiological Chemistry*, 12th Edition.
2. Henry, R. J. (1964), *Clinical Chemistry, Principles and Techniques.*
3. *The Merck Index*, 8th Edition (1968).
4. Tietz, N. W. (1970), *Fundamentals of Clinical Chemistry.*



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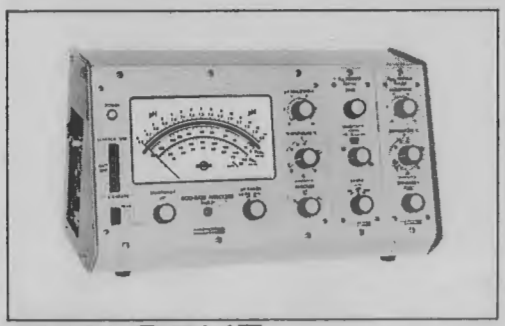

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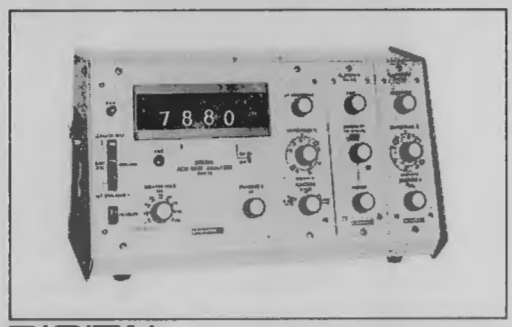

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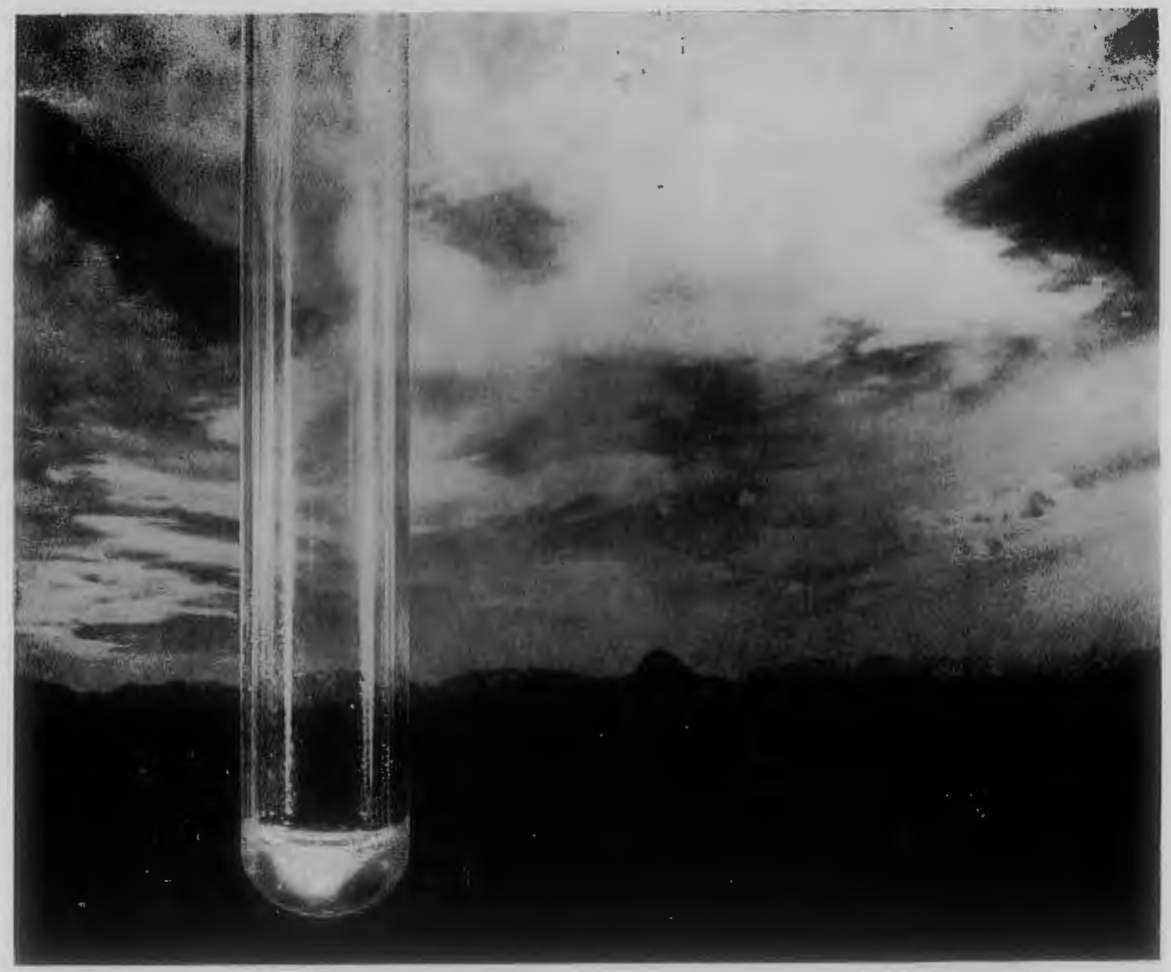

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TECHNICAL COMMUNICATIONS

A Vertical Shaker for Brown Cuvettes

Sir,

The Brown fluorometer cuvette[1] has recently become more widely used in clinical laboratories[2]. With several techniques poor reproducibility has been noted. For example, in the assay of oestrogens[1] the fluorogens are more stable in the Ittrich reagent than in the diluted acid reagent, so that prolonged shaking leads to variable losses and brief shaking must be vigorous for complete extraction. In the cortisol assay[3] prolonged shaking extracts more non-corticosteroid fluorogens and best results are obtained by short, hard shaking. However, extractions by hand shaking may also cause variable results in batches of tests. The constriction in the Brown cuvette means that the angle at which it is held is critical and variation of the vigour with which left and right hands can shake the tubes determine the reproducibility of extraction. Experiments with horizontal shaking apparatus did not solve the problem.

Below are specifications for a vertical shaker (Fig. 1) which will shake Brown cuvettes vigorously and improves the reliability of results obtained in fluorometric techniques.

Specifications

A one-quarter horsepower single phase 1,425 rpm electric motor with two-inch pulley drives a six and a-half inch pulley through a V belt. This pulley is connected through a bearing to the crank which gives a throw of one and a-half inches to the table. The terry clips for the cuvettes are size 80/0.

Acknowledgment

This machine was constructed to the author's requirements by the engineering department, Green Lane Hospital. The author also thanks Dr M. Lever and Dr C. W. Small for discussions.

J. Powell, ANZIMLT,
Pathology Department,
Green Lane Hospital,
Auckland.

March 7, 1972

REFERENCES

1. Brown, J B., MacNaughton, Caroline, Smith, Margery A. and Smyth, Barbara (1968), *J. Endocrinol.*, 40, 175.
2. Lever, M. (1971), *NZ J. Med. Lab. Technol.*, 25, 76.
3. Lever, M. (1971), *Clin. Biochem.*, 4, 68.

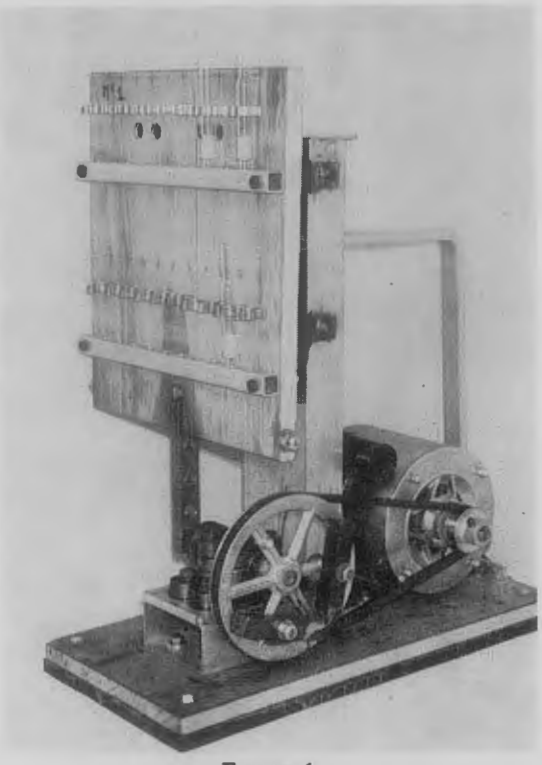

Figure 1.

A Modified Alcian Blue-PAS Technique

Sir,

When staining renal biopsies with the alcian blue/PAS technique[2, 3]; for basement membrane it was noticed that the PAS uptake was considerably reduced. This was presumably due to the basement membrane first colouring with the alcian blue and giving a weak or purplish PAS reaction. It was therefore, decided by us to modify the existing technique to allow the PAS to give a strong colour in the basement membrane whilst also allowing any other alcian blue positive material to be demonstrated. The following modification is now being used by us:

Reagents

1. 1 percent alcian blue 8GX (CI No. 74240) in 0.5 percent acetic acid.
2. 1 percent aqueous Periodic acid.
3. Barger and De Lamater's Schiff[1] (0.5 percent basic fuchsin (CI No. 42500) instead of 0.25 percent).

4. Harris' haematoxylin.

Method

Sections: Paraffin 3 u.

1. Dewax and pass slides to water.
2. Place in 1 percent alcian blue 8GX five minutes.
3. Wash in running water one minute.
4. Place in 1 percent Periodic acid five minutes.
5. Wash in running water five minutes.
6. Rinse in distilled water and place in modified Schiff reagent 20 minutes.
7. Wash in running water five minutes.
8. Stain nuclei in Harris' haematoxylin two minutes.
9. Blue and wash in running water five minutes.
10. Dehydrate in alcohol, clear in toluol and mount in DPX (or any other synthetic resin).

Results

Basement membrane—strong pink-red.
Acid mucopolysaccharides—blue-green.
Nuclei—blue.

M. J. CATTERMOLE,
Pathology Department,
Christchurch Hospital.

March 15, 1972.

REFERENCES

1. Barger, J. D. and De Lamater, E. D. (1948), *Science*, 108, 121.
2. Lison, L. (1954), *Stain Technol.*, 29, 131.
3. Steedman, H. F.. (1950), *Quart. J. Micr. Sci.*, 91, 477.

Improved Fluorometric Determination of Quinidine and Quinine

Sir,

Quinidine and quinine are commonly determined by their fluorescence in acid solution.

Cramer and Isaksson[1] used benzene to extract quinidine from plasma to which alkali has been added, followed by back-extraction into acid. Some technical improvements of this method are described here.

An Aminco-Bowman spectrophotofluorometer fitted with a xenon arc lamp and a R136 photomultiplier was used throughout this work. Mirrors and 1 mm slits were placed in the cell housing.

Add 0.3 ml 0.1 N sodium hydroxide to 0.2 ml serum and mix. Extract thoroughly with 2.5 ml of amyl acetate AR. Allow to settle or centrifuge briefly to separate the phases and then transfer 2.0 ml of the upper phase to a Brown fluorometer cuvette[2] to which 0.6 ml 0.1 N sulphuric acid has been added. Stopper the tubes and shake thoroughly. Allow to settle, or centrifuge if necessary. Read the fluorescence of the lower phase in the fluorometer with excitation wavelength 350 nm and emission wavelength 455 nm. The results are then compared with appropriate blanks and standards.

Figure 1 shows the response of the method to samples of pooled serum to which known amounts of quinidine sulphate have been added. Control sera not containing quinidine consistently gave apparent quinidine concentrations less than 0.1 μg/ml and usually less than 0.05 μg/ml. Other possible interfering components present in serum such as bilirubin are not extracted from alkaline solution.

By this procedure, clinically useful levels of sample can be determined in less than 0.05 ml serum if necessary. Brown fluorometer cuvettes minimise the number of transfer steps. Amyl acetate has low toxicity and is available free from fluorescent impurities. These features

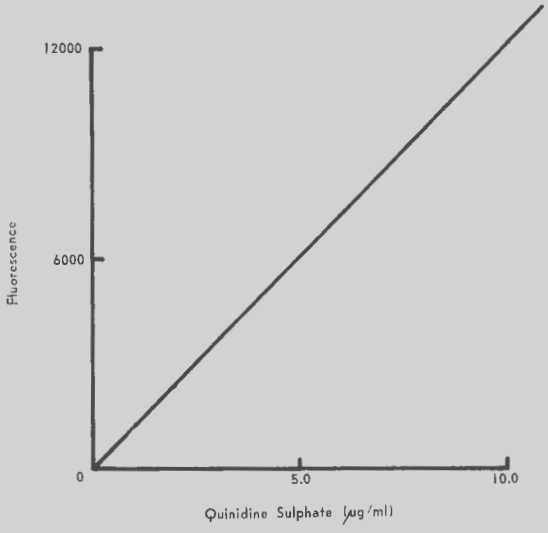

FIG. 1.—Extraction of quinidine sulphate from serum. Fluoroscence (excitation wavelength 350 nm, emission 455 nm) obtained from portions of pooled serum with added quinidine sulphate. Reagent blank subtracted.

offer significant advantages in the clinical laboratory.

D. M. Wilson and
M. Lever,
Pathology Department,
Green Lane Hospital,
Auckland.

March 17, 1972.

REFERENCES

1. Cramer, G. and Isaksson, B. (1963), *Scand. J. Clin. Lab. Invest.*, 15, 553.
2. Brown, J. B., MacNaughton, C., Smith, M. A. and Smyth, B. (1968), *J. Endocrinol.*, 40, 175.

Hepatitis Associated Antigen in Control Sera

Sir,

Recently, as an adjunct to a number of precautionary measures currently in operation, it was decided to screen commercially available control serum or plasma of human origin for Hepatitis Associated Antigen (HAA). These control preparations were tested at zero, 5, 50, and 100 dilutions using the complement fixation method of Grist *et al.*[1] adapted to a microtitre technique. Included were varying lot numbers of clinical chemistry control sera from three manufacturers, five batches of plasma coagulation control from one manufacturer and a variety of positive and negative control sera from three manufacturers. The latter were components of a number of diagnostic serology kits including those for the detection of thyroid auto antibodies, antibody to deoxyribonucleoprotein and *Echinococcus granulosus* as well as sera for the control of flocculation tests for syphilis.

Of 30 sera tested, two—a chemistry control and a plasma coagulation control from different manufacturers — were found positive for HAA. Both results were reproducible giving titres of $\frac{1}{16}$ and $\frac{1}{4}$ respectively and confirmatory values were obtained elsewhere[2].

Confirmation has been received from both manufacturers and assurances have been given that all blood components of human origin will be screened for HAA before inclusion in future control preparations.

On the basis of these findings it would appear desirable that all batches of control sera of human origin should, on receipt in the laboratory, be screened for HAA by a test of appropriate sensitivity before release for general use. It is understood that in the United States of America the Federal Government has recently recommended that all human plasma and serum used for the manufacture of reagents is screened by a licensed HAA testing system. It is not clear how stringently this recommendation will be enforced. A further advantage is seen in specific mention of such precautionary procedures being included with data accompanying control reagents.

M. J. Gratten,
Pathology Department,
Christchurch Hospital.

April 21, 1972.

REFERENCES

1. Grist, N. R., Ross, Constance A. C., Bell, Eleanor J. and Stoll, E. J. (1966), *Diagnostic Methods in Clinical Virology*, p. 54. Blackwell Scientific Publications, Oxford.
2. Manning, D., Wellington Hospital, *Personal Communication.*

Obituary

David Leigh, ANZIMLT

It is with sincere regret that the Institute records the death of David Leigh, at the age of 26 years, at Nelson. David trained in the Pathology Department of Auckland Hospital and moved to Nelson in 1969 where he qualified and commenced a most promising career in charge of the haematology department. His amiable polite personality caused him to be very well liked by his colleagues, hospital staff and patients with whom he came in contact. The rather dry sense of humour which he possessed was quite often the source of an outbreak of laughter in the laboratory, and this ability to make people laugh, both at him and themselves, helped to sustain David and his visitors during his illness. All who worked with David through the last few months will remember the greater physical effort required from him to remain a useful staff member, and his desire to organise his medical treatment to cause the minimum of inconvenience to his employers. In his chosen subject he had every opportunity to follow the progress of his own condition, well knowing the prognosis. During the last months of his life he bore his conditions with Christian fortitude. The Institute is the poorer for his premature death Our sympathy is extended to his wife, Amelia, daughter, Janet, and his family.

7.6.72 —F. C. D.

CORRESPONDENCE

Sir,—The letters of Messrs McCarthy and Bolitho in the March issue of the Journal concerning the CAP Quality Evaluation Programme were most interesting. The New Zealand Society of Pathologists has been greatly encouraged by the support given to this scheme by all participants.

I would like to join them in emphasising that a quality evaluation programme can in no way be considered a substitute for internal quality control. However, it does provide a real check on quality control as it actually functions, indicating where existing quality control programmes are deficient and even suggesting in some cases that they are non-existent.

The College of American Pathologists is aware of the less than full value caused by delays in the return of the results. Steps have been taken to speed up the turn-around this year.

There were good reasons for the timing of the introduction of the scheme. Members of our Society learned of the existence of the CAP Survey in 1969 and a few participated as individuals in 1970 in order to assess feasibility and value. In the light of this experience and following direct negotiations with both Hyland and the College of American Pathologists, group participation by New Zealand laboratories at concession rates was arranged for 1971. All known clinical laboratories in New Zealand were offered participation by the Society. Twenty-eight accepted. They reflected a cross-section of laboratories. With two exceptions the 17 hospital laboratories were in the 'small' category (fewer than 75,000 tests per annum 1970). Of the others one was a medium provincial hospital and the other a large metropolitan one. The 11 private laboratories were evenly spread from small to large but were, on the whole much larger than the hospital group. There were something approaching 2,000 laboratories participating in various aspects of the survey.

There were several factors mitigating against greater participation. Despite the concession there is no escaping the cost. To the 'Administrator' this seems to be the area which can most easily be reduced in times of economic stringency. This certainly prevented a considerable number of the larger laboratories from participating. In my personal view, this is a very short-sighted approach. Administrators seem happy to allow thousands of dollars to be spent on churning out results but are less concerned with whether the results are worth churning out. Then, some laboratories already participating in other surveys thought the basic survey was too elementary. The returns from the latter indicate that nothing is too elementary!

Mr Bolitho suggests that participation should be made compulsory. I agree personally but think that this should await clarification of some of the factors mentioned above. However, it would be extremely dangerous to accept uncritically his further suggestion that the results of surveys should be made available to outside bodies. The prime requisite of this type of survey is that the samples get no special treatment and truly measure the performance of *routine* testing. As soon as participants are aware that they are going to be judged by some outside body, no matter what its nature, then the survey sample will be given special treatment, completely vitiating the exercise. The Standards Committee up to this stage has felt that it is preferable to assist laboratories to make their own judgments on the returns and assessments, then to examine their own consciences and take the necessary steps to improve standards of performance. This approach assumes, reasonably I believe, goodwill and aims to assist, encourage and educate.

The Standards Committee of the New Zealand Society of Pathologists is currently analysing the returns for the New Zealand group and a preliminary confidential assessment has been sent on the biochemistry section to all participants. Other sections are being similarly assessed.

F. B. DESMOND,
Hon. Secretary,
The New Zealand Society of Pathologists,
16 The Terrace,
Wellington.

April 13, 1972.

Sir,—In regard to my paper, 'Variations in Creatinine Concentrations in 24h Urine Collections Made in Acid' published in the current Journal may I make a further comment?

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Further investigation into the reason for the large Coefficient of Variation found in estimating the precision of the method, seemed to indicate variation in the initial dilutions of the urine prior to creatinine estimation by the Autoanalyzer.

The difficulty was overcome by using undiluted urine and modifying the manifold by choosing pump tubes of sufficient size to give the necessary dilution automatically. The modification was to the method of Chasson, A. L., Grady, N. I. and Stanley, M. A. (1961) *Am. J. clin. Path.* 35, 83.

Yours faithfully,

J. B. NEWTON,
Pathology Department,
Christchurch Hospital.

May 9, 1972.

WHAT'S NEW?

NEW CARBON ROD ATOMIZER EXTENDS APPLICATION OF ATOMIC ABSORPTION SPECTROPHOTOMETRY TO DIFFICULT ELEMENTS

Palo Alto, California.—The Techtron Model 63 Carbon Rod Flameless Atomizer has been introduced by Varian for use with atomic absorption spectrophotometers.

With the Model 63, sample volumes of 1-30 microlitre can be precisely analysed. Sample dispensing is simple and highly reproducible with either a conventional micro-litre syringe or a cylindrical-type fixed-volume pipette.

The accessory uses either of two different types of furnace, the carbon cup and the carbon tube, both of which offer improved sample handling flexibility and lower detection limits. Both furnaces are coated with pyrolytic graphite, thus extending the Model 63's application to difficult elements such as vanadium, silicon, titanium and molybdenum. Porosity effects associated with decreased sensitivity and memory effects are also reduced.

Feasibility of additional analysis techniques, such as the analysis of solid samples and air sampling, has been demonstrated.

The Model 63 comprises the standard Varian Techtron Model 61-CRA power supply and gas control unit together with a completely new workhead.

For information in the United States, contact Varian Instrument Division, 611 Hansen Way, Palo Alto, California 94303. In Canada, Varian Techtron, 6258 Viscount Road, Malton, Ontario.

PRECISION DOSAGE, REPEATABLE ACCURACY FEATURES OF NEW MICRODOSIMETER

A new microdosimeter has been introduced to the Quickfit instrumentation range of products, marketed in Australia by James A. Jobling Pty Ltd.

It is designed for high precision dosage of liquids in the range 4 to 100 μl.

Repeatable accuracy is assured because of its simple, reliable operation.

Liquid is dispensed by syringe action, the piston travel being governed by a micrometer screw setting.

To ensure all the measured liquid is expelled, the piston movement is double acting.

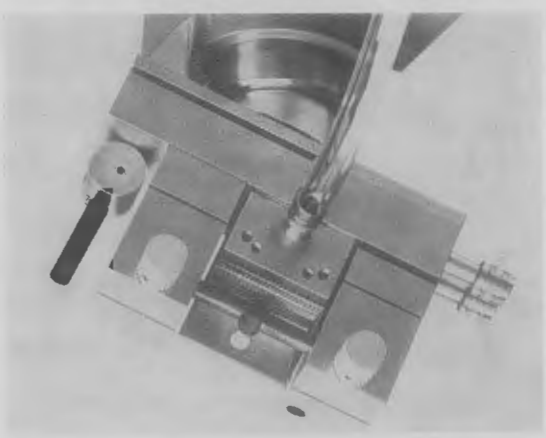
A short initial stroke fills the syringe, then a slightly longer stroke dispenses the dosage.

Four interchangeable syringes of differing capacities are available, which provide a wide range of dosage selection.

The microdosimeter is of compact, attractive design, fits easily into the hand and is extremely simple to operate.

NEW LABORATORY SCAFFOLDING

A new concept in laboratory scaffolding, introduced to the New Zealand market by James A. Jobling Pty Ltd, provides the simplest and strongest method of securing scientific apparatus to a workbench.



Called Quickrig, it is designed and manufactured for the company by John Bourke Pty Ltd, of Sydney.

The scaffolding will accommodate all special laboratory apparatus for carrying out experiments, tests or analyses, whether for scientific research, teaching or control of industrial products.

Most laboratory scaffolding has too many and too-heavy components which hinder rapid assembly and require extra bracing.

Conversely, Quickrig is economically constructed with a minimum of parts for maximum coverage.

In use, upright members are connected to cross-members by special bosshead clamps to make the system rigid and prevent lateral sag.

The scaffolding is made with stainless steel tubing of various lengths, which can be quickly fitted together at any point or position by means of the bossheads.

Assembly and dismantling is speedy and the entire scaffolding is neat and light.

Because of its few components, the new system is more easily adapted, altered, assembled or taken apart.

The unique design of the bosshead clamp permits fast changes without the need to destroy the existing scaffold.

The basic scaffolding can be built around a zinc-aluminium alloy diecast bosshead clamp, fitted with two forged steel thumbscrews, two diecast flanges for screwing to a bench, and a ½-inch diameter ball stem on a 2in x ½in alloy spindle.

These components can be rapidly and securely assembled into a wide range of scaffolding to hold a variety of scientific equipment.

Hollow stainless steel tubing for uprights and cross-members was selected to overcome corrosion problems, and will safely carry electric power wires and plastic or rubber tubes for conveying liquids inside all parts of the scaffolding.

Two multi-holed columns can be used to make a diversity of laboratory scaffolds, using bosshead clamps.

Each column has 36 holes drilled at right angles to each other.

Fitted with thumbscrews 18 holes are set at 90deg to each other, and spaced at 1in pitch, giving a completely adjustable line-up.

The extreme versatility is added to by the use of the ball stem adaptors and various lengths of tubing, to firmly mount all types of scientific equipment, meters, gauges, tables or other laboratory needs.

Components are available in individual pieces or in four kits.

They also may be custom-built to suit special requirements.

DE HAEN DIAGNOSTIC TRENDS

The scientific literature on diagnostic procedures and the use of diagnostic methods in clinical medicine is constantly growing. Papers on this subject are published in a great variety of biomedical journals. Unfortunately, the citations are not properly indexed and are difficult to retrieve. Because of the knowledge we have gained in formatting and indexing the biomedical literature on drugs and structuring such data into specific systems, we have decided to organise the literature on diagnostics.

The literature on diagnostics is complex. One cannot hope to cover all facets in great detail. It was felt that a simple annotation of a paper with a brief comment would provide the recipient with an idea of the content of the paper. Index terms in depth have been prepared according to the train of thought of a scientist concerned with diagnostic procedures in the laboratory or the clinic. These index terms appear in a conspicuous spot in each excerpt and are easily and quickly scanned. An annual index will be prepared.

The enclosed 'Sample Presentations' have been selected from the literature for their diversity of content and not in any chronological order.

The subscription is $US250 per annum.

43

TITLE EFFECT OF ORAL CONTRACEPTIVES ON SERUM LIPIDS
Schenker JG; Penson A; Foleshak #Z (Hadassah Univ. Hosp, Jerusalem, Israel)
Fertility & Sterility 22: 604-608 (Sept) 1971

ANNOTATION 375 women (ages 19-30) taking various oral contraceptives tested with control group of 66 women (ages 19-30)

COMMENT Marked rise in serum total lipids and triglycerides in those taking oral contraceptives; no significant change in cholesterol concentration.

0300

INDEX TERMS
Blood serum
Total lipids
Total Cholesterol
Triglycerides
Lipids (Kunkel)
Cholesterol (Zak)
Triglycerides (Dole)
Triglycerides Assay (Lambert)
Statistical Method: Significant difference Student "T" test
Manual test
GYNOVLAR (Schering)
METRULEN (Searle)
NOGEST-S (Ikapharm)
SEQUELAN (Leva)
Diagnosis unspecified
Chemistry

44

TITLE THE HALF HOUR SYNTHETIC 1-24 CORTICOTROPIN TEST OF ADRENOCORTICAL RESERVE IN CHILDREN
Rosenbloom AL; Savory J; Londono J (Dept. of Pediat. Pathology, Florida Coll. Med. Gainesville, Florida USA)
J. Pediat. 79: 489-493 (Sept) 1971

ANNOTATION Plasma responses 30 minutes after injection of corticotropin in 17 patients with variety of problems; 9 normal children with severe central nervous system infection also tested.

COMMENT Test useful in indicating adrenocortical reserve, could be of even greater value in study of this function under varying conditions. Normal level of 17-hydroxycorticosteroid and response to synthetic corticotropin suggest return of adrenal function.

0300

INDEX TERMS
Blood plasma
17-Hydroxycorticosteroid
Peterson method
Porter - Silber reaction
Manual test
CORTROSYN (Organon)
Meningitis
Hypoparathyroidism
CNS Stimulants, adv. eff.
Chemistry

45

TITLE CAPILLAR BLOOD SUGAR VALUES IN CHILDREN DURING ORAL-CORTISONE-PRIMED GLUCOSE TOLERANCE TEST
Cole HS; Bilder J; Epel R (NY Med Coll. Flower Fifth Hosp; Metropolitan Hosp NY)
Diabetes 20: 615-21 (Sept) 1971

ANNOTATION 3 hr. oral cortisone primed glucose tolerance test done on 113 children in 3 age groups.
Dose of glucose = 2.0 gm./kg. ages 1½ to 3 yrs.
= 1.75 gm./kg. ages 3 to 12 yrs.
Dose of cortisone acetate = 280 mg. per M^2 in two equally divided doses prior to test.

COMMENT Blood sugar values for 1½ to 4 yr. age group by COGTT be considered separately because of significant differences from other age groups. Useful for early detection of diabetes in children prior to onset of clinical symptoms.

0200

INDEX TERMS
Blood
Glucose
AUTOANALYZER (Gray, Stowe, Holden)
Statistical method - Student "T"
Manual test
KOLADEX (Custon Labs)
AUTOANALYZER (Technicon)
UNOPETTE (Becton-Dickinson)
Diabetes Mellitus
Chemistry

A WATER-REPELLENT PHASE-SEPARATION FILTER PAPER

Whatman No. 1 Phase Separating Paper is impregnated with a stable silicone, making it highly hydrophobic, while retaining the porous character of conventional cellulose filter paper. It is widely used for the analysis of steroids in blood and urine or drugs in urine, for the identification of organic additives in industrial solutions or pollutants in water, for trace metal determinations and in solvent extraction processes.

A simple conical filter funnel containing a folded circle of the paper (see Fig. 4) can be used to separate aqueous and solvent phases, in place of the conventional separating funnel. It is disposable, reducing the dangers of cross-contamination, and solids or froth present are retained by it, resulting in a clear solvent phase. The solvent phase flows quickly and cleanly through the paper, then stops automatically, leaving the aqueous phase completely retained. This is important when carrying out a

large number of routine solvent extractions at the same time. Samples can be shaken with solvent in conical flasks or test tubes and transferred directly to funnels containing No. 1 PS. No further attention is required during the separation of the phases.

The paper can be used with solvents that are lighter or heavier than water. It is not necessary to wait until the two phases have settled out into separate layers; drops of one phase suspended in the other can be separated. The paper will operate effectively at temperatures up to 90°C. Typical separation times for 25 ml solvent from 25 ml water in a 15 cm circle, quadrant folded, are 45 seconds for a heavy solvent (e.g., chloroform) or 100 seconds for a light solvent (hexane).

Whatman No. 1 PS is resistant to mineral acids up to 4N, and alkalis up to 0.4M. It is available in a wide range of sizes from Whatman distributors all over the world. Further information and samples can also be obtained from H. Reeve Angel and Co. Ltd, 14 New Bridge Street, London EC4, England.

VARIAN NMR SPECTROMETER

Varian Associates' newly consolidated Instrument Division has announced a new 60 MHz NMR spectrometer priced at only $10,700 and comparing favourably in performance with $20,000 instruments. Deliveries will commence this summer. The new EM-360 delivers such performance at 0.6 Hz resolution and a guaranteed sensitivity of 15:1 signal to noise with standard 5 mm sample tubes.

SELECTED ABSTRACTS

Contributors: D. G. Bolitho, Alison Buchanan, M. Jeanette Grey, J. Hannan, D. Tingle A. G. Wilson.

CHEMICAL PATHOLOGY

Recent Practical Methods for Typing Hyperlipoproteinemia; Advances in the Study of Blood Lipids. Herrmann, G. R. and Clarke, Sara W. (1971), *Tex. Med.*, 67 (12), 81.

A simple method has been developed by Frederickson *et al.* in which hyperlipoproteinaemia is classified by observing the physical characteristics of serum refrigerated at 4°C overnight and determining the serum levels of cholesterol and triglycerides, eliminating the expense of determining the electrophoretic pattern.

Type I is characterised by a normal or only slightly elevated cholesterol level but a high level of triglycerides of exogenous origin. The refrigerated serum present a creamy top layer and a clear infranatant.

Type II shows high levels of cholesterol (300-600 mg%) and normal or slightly elevated triglycerides. The refrigerated serum presents no top layer and the serum is clear throughout the tube.

Type III shows higher levels of cholesterol (350-800 mg%) and an elevation of endogenous abnormal triglycerides to 350-800 mg%. The refrigerated serum presents a thin, creamy supernatant layer and a cloudy, turbid infranatant.

Type IV shows a 1 mg% elevation of cholesterol for each 5mg% rise of normal or elevated endogenous triglycerides. The refrigerated serum shows no fat layer at the top of the tube, but the serum is turbid. Triglycerides may reach 1000-6000 mg%.

Type V shows moderately elevated levels of cholesterol, 300-800 mg%, and greatly elevated triglycerides and is a combination of Types I and IV. The refrigerated serum shows a heavy creamy supernatant layer over a very turbid infranatant.

—J. H.

Terminal Digit Reference in the Reading of Autoanalyser Charts. Corns, Cathryn M. and Corns, M. D. (1972), *Med. lab. Technol.* 29, 35.

Four common estimations, namely sodium, potassium chloride and urea were examined for bias in the reading of Autoanalyser charts. Sodium was later discarded from the investigation because the range normally found is narrow and the terminal digits are not encountered with the same frequency as the others. Results produced by four different analysts were analysed. Less bias was found with the more experienced operators. It was also found that the terminal digits used to set up the standard curve influenced the terminal digits of the test.

—A. G. W.

Design of an Analog Converter for use in Colorimetry. Lapidus, B. M., Tetrid, J. W. and Karmen, A. (1971), *Clin. Chem.* 17, 231.

A simple analog circuit incorporating commercially available components has been used to convert the output of a colorimeter photocell into units directly related to concentration. The converter is useful for analyses that obey Beer's Law approximately, with continuous flow or other colorimetric systems. The results obtained by analog conversion agree within 1 percent with the photocell output read in the usual manner. The cost of the components is about $200.

—A. G. W.

Analog Device for Curve Regeneration in Continuous Flow Analysis. Walker, W. H. C., Townsend, J., Keane, P. M. (1972), *Clin. Chem. Acta.* 36, 119.

An electronic analog device is described that performs on-line, the process of curve regeneration in

continuous flow systems. With high quality traces the device allows increased sampling rates with elimination of interaction. The device can be used with Technicon Mk II colorimeters or with Mk I colorimeters fitted with logarithmic converters.

—A. G. W.

Determination of Plasma α Amino-nitrogen by Atomic Absorption Spectrophotometry. Hall, F. F., Schneider, B., Culp, T. W. and Ratcliffe, C. R. (1972), *Clin. Chem.* **18,** 34.

A simple indirect method is described for this determination. After trichloracetic deproteinisation the filtrate is neutralised with alkali. The α amino groups complex with and solubilise copper from insoluble copper phosphate. The solubilised copper measured by atomic absorption spectrophotometry is proportional to the amount of α amino-nitrogen present in the plasma.

—A. G. W.

Diagnostic Values of Urinary Enzyme Determinations. A Review. Raab, W. P. (1972), *Clin. Chem.* **18,** 5.

Information of physiological and pathological significance in regard to the enzymatic activities is compiled. Attention is focused on the diagnostic applications.

—A. G. W.

Estimation of Gold in Serum by Atomic Absorption Spectroscopy. Dunckley, J. V. (1971), *Clin. Chem.* **17,** 992.

A method is described for estimating gold directly in diluted serum. Matrix effects are compensated for by adding proteins to working standards.

—A. G. W.

Currents in Renal Stone Research. A Review. King, J. S. (1971), *Clin. Chem.* **17,** 971.

Current theories of renal stone formation are reviewed together with resulting proposals for treatment. Factors influencing the initiation and development of stones are discussed.

—A. G. W.

The Determination of LSD in Human Plasma following Oral Administration. Upshall, D. G. and Wailling, D. G. (1972), *Clin Chim. Acta.* **36,** 67.

A spectrofluorimetric method is described for the assay of Lysergic acid diethylamide at nanogram levels in human plasma in the presence of a variable blank. The procedure makes use of the observation that ultra-violet light catalyses the hydration of LSD to a non-fluorescent derivative. The difference in fluorescence of plasma extracts before and after intense ultra-violet irradiation is a measure of LSD concentration.

This technique has been used for the assay of LSD in plasma following the oral administration of the drug at a dose of approximately 2μg per kilogram body weight.

—A. B.

Lipid Screening and Lipoprotein Electrophoresis by Cellogel. Messerschmidt, H. J. M. and Sedee, Philip. (1972), *Clin. Chim. Acta.* **36,** 51.

A procedure is proposed for screening lipid metabolism. A method for the separation of serum lipoproteins by electrophoresis by cellogel is described.

With the aid of this technique it is possible to achieve a complete resolution of chylomicrons, β-lipoproteins, pre-β-lipoproteins and two α-lipoproteins. The relative magnitude of the separated fractions can be estimated by a quantitative elution technique. It was proved by immuno-electrophoretic techniques that the two α-lipoprotein bands are probably identical. Methods and data are given of the whole procedure.

—A. B.

A simple and rapid method for quantitative determination of protein in urine. Hemingsen, L. (1972), *Clin. Chim. Acta.* **36,** 185–188.

A simple and rapid method is described; the principle is based on the 'Protein error of indicators'. The sensitivity is high and the method in routine practice has a capacity of more than 100 samples per hour. The specificity is examined and factors given for different types of proteins. Albumin (bovine) is recommended as a standard substance.

—A. B.

Lactate Dehydrogenase Isoenzymes linked to Immunoglobulin A in two cases. Mitsutaka, Nagamine (1972), *Clin. Chim. Acta.* **36,** 139–144.

Abnormal patterns of serum lactate dehydrogenase isoenzyme in two cases are reported. Investigations showed that the abnormalities were caused by an interaction between lactate dehydrogenase and immunoglobulin A. It was assumed that the lactate dehydrogenase of each case was normal and that the abnormality was due to the presence of abnormal immunoglobulin A.

—A. B.

HAEMATOLOGY

Recent Observations on Transfusion Reactions. Miller, W. V. and Schmidt, P. J. (1971), *Med. Ann. Distri. Columbia,* **40,** 419.

Urticarial reactions unaccompanied by chills, fever, etc., require only a verification of identity of recipient and donor. All other reactions are potentially haemolytic and must be investigated as such. An anticoagulated blood sample should be drawn from the patient with care to prevent haemolysis and the plasma examined carefully for free haemoglobin. Simple visual examination is all that is necessary, since amounts of plasma haemoglobin which cannot be seen are rarely significant and quantitation of higher levels does little to guide therapy. A urine sample should be examined for free haemoglobin.

The plasma of transfused blood should be examined also, since the presence of free haemoglobin indicates physical injury or bacterial contamination. If bacterial contamination is suspected, microscopic examination of a stained smear of donor blood can show organisms.

After repeating the group and type examinations on both recipient and donor blood a direct Coombs' test is performed on the patient's post-transfusion RBCs. A positive test (with a pre-transfusion sample as a control) is probably due to antigen-antibody reaction; but a negative test does not mean that such a reaction did not occur, since all of the antibody-coated cells may have already been destroyed.

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All of these examinations can be made in 15-20 minutes. Negative results make emergency treatment unnecessary, and therapy may be cautiously continued with another unit of blood. If a haemolytic process is suspected, emergency treatment should begin at once and not be withheld pending further examinations such as a repeat of group and type, repeat of crossmatches, interdonor crossmatches, serum bilirubin, etc.

Once a reaction has occurred, investigation of its nature and prompt treatment may avert tragedy. Early treatment with mannitol is strongly recommended when haemolysis is suspected.

—J. H.

Automation in Blood Banking. Myhre, B. A. (1971), *J. Ass. Adv. med. Instr.*, 5, 279.

Automation is just beginning to be introduced into immunohaematology. Only two methods of automation are now in use. These are the colorimeter recorder method (agglutinated, sedimented RBCs are separated by means of a decanting fitting, then any RBCs in the supernatant are lysed and the haemoglobin concentration recorded) and the filter paper recording method (any agglutinated, sedimented RBCs are deposited on a moving strip of filter paper). Both methods have the disadvantage of poor sample identification.

Advantages of automation include: sensitivity equal to or greater than manual methods; predictability as to number of tests performed per unit of time; ability to do multiple screenings; automatic quality control, since this must be done to assure that the machine is in adequate running condition; less expensive reagents; a certain amount of quantitation is present.

The disadvantages of automation include, in addition to poor sample identification: anticoagulated blood must be used, and anticoagulants cause complement to become unreactive, thus preventing detection of some 'complement dependent' antibodies; lack of flexibility; the machines are quite fragile and break down easily; need for a new type technologist who can work with machinery; false positive reactions due to factors such as cold agglutinins and 'Autoanalyser specific' RBC antibody, which reacts by the Autoanalyser but not manual methods; the problem of maintenance of machinery; standby equipment must be available in case of breakdown.

—J. H.

Ultrastructure of Hairy Cell Leukaemia. Ghadially, F. N. and Skinnider, L. F. (1972), *Cancer*, 29, 444.

There have been reports of several cases of an unusual type of leukaemia which is characterised by leukaemic cells having a ragged cytoplasm and numerous fine cytoplasmic projections arising from the cell surface. Schrek and Donnelly (1966) have quite aptly named this a hairy cell.

In a case studied by the authors, at one stage the neutrophil count was 92 cu mm, the platelet count 46,000 cu mm and the leukaemic cell count 9,200 cu mm.

A variety of drugs was administered to the patient, and it is speculated that one or a combination of these was responsible for the appearance of the cells.

—J. H.

Quality Control of Routine Haemoglobinometry. I. Cavill (1971), *J. clin. Path.*, 24, 701.

An analysis of haemoglobin estimations in a routine laboratory shows that quality control methods based on mean values are unsatisfactory. The use of a cusum method together with control samples is a sensitive and convenient technique for the early discovery of minor deviations in results and it is not dependent on a stable population of patients.

—M. J. G.

Haemolytic Anaemia Associated with Decreased Concentration of Reduced Glutathione in Red Cells. Lo, S. S., Marti, H. R. and Hitzig, W. H. (1971), *Acta haemat.*, 46, 14.

Three cases of haemolytic anaemia with decreased concentration of reduced glutathione in red cells are described. They all have had signs of hyperhaemolysis from early infancy and two exchange-transfusions had to be performed in one of them. In two of them stomatocytes are present in their blood smears and in one of them high sodium and low potassium in the erythrocytes is observed. Family studies reveal several apparently healthy members with the same deficiency and presenting evidence of mild to moderate hyperhaemolysis.

—M. J. G.

Variation of Specificity of Autoantibodies in Autoimmune Haemolytic Anaemia. Beck, M. L., Dixon, J. and Oberman, H. A. (1971), *Amer. J. clin. Path.*, 56, 475.

A report on a seven-year-old male patient with a severe haemolytic condition with warm autoantibodies. When first diagnosed, antibody tests showed a warm Ig G type autoantibody which reacted equally with all cells tested and was considered nonspecific. Four years later, anti-e was detected and identified and shortly after that, anti-c and anti-f specificities were demonstrated. Treatment given and possible clonal alterations are discussed.

—M. J. G.

The Effects of Immunosuppressive Drugs and Uraemia on Automated Leucocyte Counts. Luke, R. G., Koepke, J. A. and Siegel, R. R. (1971), *Amer. J. clin. Path.*, 56, 503.

Over a six-month period, 42 patients receiving azathioprine and prednisone had simultaneous leucocyte counts done by Coulter S and by the conventional visual-manual haemocytometer techniques, under carefully controlled conditions. The Coulter S leucocyte counts revealed a significantly lower count in patients on the above immunosuppressive drugs. It is suggested that the Coulter S counting system may destroy abnormally fragile leucocytes.

—M. J. G.

Comparison of Three Methods for Measuring Vitamin B_{12} in Serum: Radioisotopic, *Euglena gracilis* and *Lactobacillus leichmanii*. Raven, J. L., Robson, M. B., Morgan, J. O., and Hoffbrand, A. V. (1972), *Brit. J. Haem.*, 22, 21.

The vitamin B_{12} content of 481 sera was estimated by a radioisotopic assay and 478 of these were also assayed by the *E. gracilus* method and 396 by the *L. leichmanii* method. Normal ranges and means are given for each of the methods. In general the

radioisotope assay gave higher results than the other two methods. The *E. gracilis* assay gave the lowest results. Various aspects and discrepancies are discussed, including assays on some folate-deficient patients. The *E. gracilis* assay gave the clearest distinction between normal subjects and patients with untreated pernicious anaemia.

—M. J. G.

Mechanical Fragility of Erythrocytes in Normals and in Patients with Heart Valve Prostheses. Dale, J. and Myhre, E. (1971), *Acta. med. Scand.* **190,** 127.

This paper draws attention to patients' individual differences in haemolysis following insertion of heart ball-valves. Yet more important is the fact that it is the first paper in English to describe the haemoresistometer of Fleisch and Fleisch. A schematical diagram of this instrument is included. The haemoresistometer may have advantages over the classical glass-bead method for determining mechanical fragility. The time of trauma to red cells is shorter and more similar to the physiological trauma of red cells in the circulation. Normal values are given.

—M. J. G.

A Case of Hereditary Methaemoglobinaemia. Lay, H. and Davies, H. E. (1971), *Aust. paediat. J.* **7,** 214.

A case of methaemoglobinaemia detected in a twelve-day-old baby is described together with laboratory findings. The cause was hereditary deficiency of red cell $NADH_2$-dependent methaemoglobin reductase (diaphorase). Cyanosis first noticed on seventh day and confirmed by laboratory spectrophotometric determinations showing 20 percent methaemoglobin, with normal globin. Assay showed that the above red cell enzyme was virtually absent.

—M. J. G.

Phenacetin-induced Haemolytic Anaemia. Davidson, R. J. L. (1971), *J. Clin. Path.*, **24,** 537.

Haemolytic anaemia, rarely severe, is a common yet often unrecognised complication of the prolonged use or abuse of phenacetin-containing analgesics. Irregularly contracted (pyknocytes) or fragmented erythrocytes (schistocytes) are commonly present in the peripheral blood in this form of anaemia. It is emphasised that their recognition during screening of blood films may reveal patients previously unsuspected of analgesic abuse and at a stage before the development of the more serious complication of nephropathy. Fourteen such patients, detected during the last 18 months, are briefly described and the pathogenesis and laboratory features of the anaemia reviewed.

—M. J. G.

Disorders of Neutrophil and Monocyte Function. Douglas, S. D. (1971), *Brit. J. Haem.* **21,** 493.

An annotation that reviews part of the fast-growing field of quantitative defects of leucocytes. Various defects in phagocytosis are described and most emphasis in this paper is upon chronic granulomatous disease of childhood, but the nature of phagocytosis itself is discussed.

—M. J. G.

Cellular Changes in Chronic Myeloid Leukaemia. Pederson, B. and Mayhoe, F. G. J. (1971), *Brit. J. Haem.*, **21,** 251.

This annotation is a concise summary of current knowledge of ultrastructural, biochemical and physiological defects of polymorphs in Chronic Myeloid Leukaemia. These defects include poor phagocytic power, abnormal granular composition and low alkaline, with high acid, phosphatase activities. A proposed hypothesis suggests these defects are signs of incomplete maturation of cells when released from bone marrow into circulation.

—M. J. G.

The Biochemical, Genetic and Clinicopathological Aspects of Haptoglobin. Pintera, J. (1971), Series Haematolog. **4,** No. 2.

A whole issue of 140 pages devoted entirely to important aspects of haptoglobin, ranging through isolation and quantitation, typing, complex formation, metabolism, function and diagnostic significance. Six hundred and ninety-seven references to haptoglobins are listed.

—M. J. G.

Laboratory Control of Heparin Therapy. O'Shea, M. J., Flute, P. T. and Pannell, G. M. (1971), *J. clin. Path.*, **24,** 542.

The effect of heparin therapy was followed in 50 patients treated for thromboembolic disease. Individual response to a standard dose of 400,000 units of heparin daily showed a considerable variation and the effect was not constant on subsequent days. Five of the 50 patients developed a serious haemorrhage.

It is proposed that to ensure the adequacy of treatment detectable levels of heparin should be obtained but because of the high risk of bleeding these levels should not be excessive.

The results suggest that control of heparin therapy can be based on the thrombin clotting time. Using this test it is advised that treatment is monitored daily in order to achieve a plasma heparin level of up to 1 mg per 100 ml.

—M. J. G.

Stable Suspension of Erythrocytes suitable for Calibration of the Electronic Counters. Torlontano, G. and Tata, A. (1971), *Acta Haemat.* **45,** 325.

A simple method of preparing stable suspensions of normal erythrocytes is described. The fixative for the cells contains acetic acid, sodium sulphate and sodium chloride; stability of number, shape and volume, without aggregates is assured for at least six months and probably for two years.

—M. J. G.

Gaucher's Cells in Thalassaemia. Zaino, E. C., Rossi, M. B., Pham., T. D. and Azar, H. A. (1971), *Blood,* **38,** 457.

Gaucher or Gaucher-like cells are described in the spleen and bone-marrow of a patient with thalassaemia major. Both light and electron microscopic structure is described. It is suggested that impaired catabolism of erythrocytes may give rise to the increased glucocerebroside that accumulates within the cytoplasm to give the Gaucher cell its characteristic microscopic appearance.

—M. J. G.

Lysosomal Enzyme Cytochemistry in Acute Leukaemia. Mann, J. R., Simpson, J. S., Munkley, R. M. and Stuart, J. (1971), *J. clin. Path.* 24, 831.

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Quantitative Studies on the Salivary Flora. Ross, P. W. (1971), *J. clin. Path.*, **24**, 717–720.

A quantitative survey of the salivary flora of children is presented. The author remarks on the paucity of information available on the salivary micro-flora and stresses the importance of this information in understanding the pathogenesis of oral infections. Of interest is the high incidence of pneumococci in normal saliva (77 percent). Other pathogens found include *Staph. aureus, N. meningitidis* and *H. influenzae.*

—D. G. B.

The Effect on Sepsis Rates of Closing and Cleaning Hospital Wards. Neone, P. and Griffiths, R. J. (1971), *J. clin. Path.*, **24**, 721–725.

The closing of wards in which outbreaks of antibiotic resistant staphylococci infection occur is a common practice. This paper investigates the rationality of such a procedure. The conclusion reached is that sepsis rates are significantly reduced for a considerable period after such closure and cleaning procedures.

—D. G. B.

A Modified Nylon Bridge for a Closed Blood Culture System. Freake, R. and Patten, W. (1971), *Amer. J. med. Technol.*, **37** (6), 221–222.

A modification to the apparatus for blood culture described in 1964 by Freake and Nayman is described. The essential difference is the incorporation of two entry ports into the nylon bridge piece in place of the one port described in the original device. The authors claim that this allows the incorporation of thermolabile substances into the medium after autoclaving leaving a second entry port for injecting the blood sample.

Although this device shows promise its prohibitive price in this country makes it unlikely to gain wide acceptance.

—D. G. B.

The Decline of Antibiotic Resistance of *Staphylococcus aureus* Located in the Community. Bauer, A. W. (1971), *Amer. J. med. Technol.*, **37** (6), 247–253.

This paper discusses the drop in the number of strains of *Staphylococcus aureus* resistant to tetracycline and erythromycin over the five-year period 1964-1969. Resistance to penicillin remained unchanged during the period.

The author also discusses factors influencing the single disk, high potency technique of sensitivity testing.

—D. G. B.

Comparative Immunological Analysis of Enterobactericeae. Garcia, C. F. (1971), *Amer. J. med. Technol.*, **37** (7), 271–276.

This paper describes immunoelectrophoretic precipitation reactions carried out on *Salmonella* and *Citrobacter* species. Although the results are interesting in showing clear antigenic differences between *Citrobacter* and *Salmonella* strains the work appears to be of no value in the diagnostic laboratory.

Full technical details are given.

—D. G. B.

Optimal Bacitracin Concentration for Selective Isolation Media for Haemophilus. Ederer, G. M. and Schurr, M. L. (1971), *Amer. J. med. Technol.*, **37** (7), 304–305.

This technical note discusses the optimum level of bacitracin for use as a selective agent for *Haemophilus*

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All of these examinations can be made in 15-20 minutes. Negative results make emergency treatment unnecessary, and therapy may be cautiously continued with another unit of blood. If a haemolytic process is suspected, emergency treatment should begin at once and not be withheld pending further examinations such as a repeat of group and type. repeat of crossmatches, interdonor crossmatches, serum bilirubin, etc.

Once a reaction has occurred, investigation of its nature and prompt treatment may avert tragedy. Early treatment with mannitol is strongly recommended when haemolysis is suspected.

—J. H.

Automation in Blood Banking. Myhre, B. A. (1971), *J. Ass. Adv. med. Instr.*, 5, 279.

Automation is just beginning to be introduced into immunohaematology. Only two methods of automation are now in use. These are the colorimeter recorder method (agglutinated, sedimented RBCs are separated by means of a decanting fitting, then any RBCs in the supernatant are lysed and the haemoglobin concentration recorded) and the filter paper recording method (any agglutinated, sedimented RBCs are deposited on a moving strip of filter paper). Both methods have the disadvantage of poor sample identification.

Advantages of automation include: sensitivity equal to or greater than manual methods; predictability as to number of tests performed per unit of time; ability to do multiple screenings; automatic quality control, since this must be done to assure that the machine is in adequate running condition; less expensive reagents; a certain amount of quantitation is present.

The disadvantages of automation include, in addition to poor sample identification: anticoagulated blood must be used, and anticoagulants cause complement to become unreactive, thus preventing detection of some 'complement dependent' antibodies; lack of flexibility; the machines are quite fragile and break down easily; need for a new type technologist who can work with machinery; false positive reactions due to factors such as cold agglutinins and 'Autoanalyser specific' RBC antibody, which reacts by the Autoanalyser but not manual methods; the problem of maintenance of machinery; standby equipment must be available in case of breakdown.

—J. H.

Ultrastructure of Hairy Cell Leukaemia. Ghadially, F. N. and Skinnider, L. F. (1972), *Cancer*, 29, 444.

There have been reports of several cases of an unusual type of leukaemia which is characterised by leukaemic cells having a ragged cytoplasm and numerous fine cytoplasmic projections arising from the cell surface. Schrek and Donnelly (1966) have quite aptly named this a hairy cell.

In a case studied by the authors, at one stage the neutrophil count was 92 cu mm, the platelet count 46,000 cu mm and the leukaemic cell count 9,200 cu mm.

A variety of drugs was administered to the patient, and it is speculated that one or a combination of these was responsible for the appearance of the cells.

—J. H.

Quality Control of Routine Haemoglobinometry. I. Cavill (1971), *J. clin. Path.*, 24, 701.

An analysis of haemoglobin estimations in a routine laboratory shows that quality control methods based on mean values are unsatisfactory. The use of a cusum method together with control samples is a sensitive and convenient technique for the early discovery of minor deviations in results and it is not dependent on a stable population of patients.

—M. J. G.

Haemolytic Anaemia Associated with Decreased Concentration of Reduced Glutathione in Red Cells. Lo, S. S., Marti, H. R. and Hitzig, W. H. (1971), *Acta haemat.*, 46, 14.

Three cases of haemolytic anaemia with decreased concentration of reduced glutathione in red cells are described. They all have had signs of hyperhaemolysis from early infancy and two exchange-transfusions had to be performed in one of them. In two of them stomatocytes are present in their blood smears and in one of them high sodium and low potassium in the erythrocytes is observed. Family studies reveal several apparently healthy members with the same deficiency and presenting evidence of mild to moderate hyperhaemolysis.

—M. J. G.

Variation of Specificity of Autoantibodies in Autoimmune Haemolytic Anaemia. Beck, M. L., Dixon, J. and Oberman, H. A. (1971), *Amer. J. clin. Path.*, 56, 475.

A report on a seven-year-old male patient with a severe haemolytic condition with warm autoantibodies. When first diagnosed, antibody tests showed a warm Ig G type autoantibody which reacted equally with all cells tested and was considered nonspecific. Four years later, anti-e was detected and identified and shortly after that, anti-c and anti-f specificities were demonstrated. Treatment given and possible clonal alterations are discussed.

—M. J. G.

The Effects of Immunosuppressive Drugs and Uraemia on Automated Leucocyte Counts. Luke, R. G., Koepke, J. A. and Siegel, R. R. (1971), *Amer. J. clin. Path.*, 56, 503.

Over a six-month period, 42 patients receiving azathioprine and prednisone had simultaneous leucocyte counts done by Coulter S and by the conventional visual-manual haemocytometer techniques, under carefully controlled conditions. The Coulter S leucocyte counts revealed a significantly lower count in patients on the above immunosuppressive drugs. It is suggested that the Coulter S counting system may destroy abnormally fragile leucocytes.

—M. J. G.

Comparison of Three Methods for Measuring Vitamin B_{12} in Serum: Radioisotopic, *Euglena gracilis* and *Lactobacillus leichmanii*. Raven, J. L., Robson, M. B., Morgan, J. O., and Hoffbrand, A. V. (1972), *Brit. J. Haem.*, 22, 21.

The vitamin B_{12} content of 481 sera was estimated by a radioisotopic assay and 478 of these were also assayed by the *E. gracilus* method and 396 by the *L. leichmanii* method. Normal ranges and means are given for each of the methods. In general the

radioisotope assay gave higher results than the other two methods. The *E. gracilis* assay gave the lowest results. Various aspects and discrepancies are discussed, including assays on some folate-deficient patients. The *E. gracilis* assay gave the clearest distinction between normal subjects and patients with untreated pernicious anaemia.

—M. J. G.

Mechanical Fragility of Erythrocytes in Normals and in Patients with Heart Valve Prostheses. Dale, J. and Myhre, E. (1971), *Acta. med. Scand.* **190,** 127.

This paper draws attention to patients' individual differences in haemolysis following insertion of heart ball-valves. Yet more important is the fact that it is the first paper in English to describe the haemoresistometer of Fleisch and Fleisch. A schematical diagram of this instrument is included. The haemoresistometer may have advantages over the classical glass-bead method for determining mechanical fragility. The time of trauma to red cells is shorter and more similar to the physiological trauma of red cells in the circulation. Normal values are given.

—M. J. G.

A Case of Hereditary Methaemoglobinaemia. Lay, H. and Davies, H. E. (1971), *Aust. paediat. J.* **7,** 214.

A case of methaemoglobinaemia detected in a twelve-day-old baby is described together with laboratory findings. The cause was hereditary deficiency of red cell $NADH_2$-dependent methaemoglobin reductase (diaphorase). Cyanosis first noticed on seventh day and confirmed by laboratory spectrophotometric determinations showing 20 percent methaemoglobin, with normal globin. Assay showed that the above red cell enzyme was virtually absent.

—M. J. G.

Phenacetin-induced Haemolytic Anaemia. Davidson, R. J. L. (1971), *J. Clin. Path.,* **24,** 537.

Haemolytic anaemia, rarely severe, is a common yet often unrecognised complication of the prolonged use or abuse of phenacetin-containing analgesics. Irregularly contracted (pyknocytes) or fragmented erythrocytes (schistocytes) are commonly present in the peripheral blood in this form of anaemia. It is emphasised that their recognition during screening of blood films may reveal patients previously unsuspected of analgesic abuse and at a stage before the development of the more serious complication of nephropathy. Fourteen such patients, detected during the last 18 months, are briefly described and the pathogenesis and laboratory features of the anaemia reviewed.

—M. J. G.

Disorders of Neutrophil and Monocyte Function. Douglas, S. D. (1971), *Brit. J. Haem.* **21,** 493.

An annotation that reviews part of the fast-growing field of quantitative defects of leucocytes. Various defects in phagocytosis are described and most emphasis in this paper is upon chronic granulomatous disease of childhood, but the nature of phagocytosis itself is discussed.

—M. J. G.

Cellular Changes in Chronic Myeloid Leukaemia. Pederson, B. and Mayhoe, F. G. J. (1971), *Brit. J. Haem.,* **21,** 251.

This annotation is a concise summary of current knowledge of ultrastructural, biochemical and physiological defects of polymorphs in Chronic Myeloid Leukaemia. These defects include poor phagocytic power, abnormal granular composition and low alkaline, with high acid, phosphatase activities. A proposed hypothesis suggests these defects are signs of incomplete maturation of cells when released from bone marrow into circulation.

—M. J. G.

The Biochemical, Genetic and Clinicopathological Aspects of Haptoglobin. Pintera, J. (1971), Series Haematolog. **4,** No. 2.

A whole issue of 140 pages devoted entirely to important aspects of haptoglobin, ranging through isolation and quantitation, typing, complex formation, metabolism, function and diagnostic significance. Six hundred and ninety-seven references to haptoglobins are listed.

—M. J. G.

Laboratory Control of Heparin Therapy. O'Shea, M. J., Flute, P. T. and Pannell, G. M. (1971), *J. clin. Path.,* **24,** 542.

The effect of heparin therapy was followed in 50 patients treated for thromboembolic disease. Individual response to a standard dose of 400,000 units of heparin daily showed a considerable variation and the effect was not constant on subsequent days. Five of the 50 patients developed a serious haemorrhage.

It is proposed that to ensure the adequacy of treatment detectable levels of heparin should be obtained but because of the high risk of bleeding these levels should not be excessive.

The results suggest that control of heparin therapy can be based on the thrombin clotting time. Using this test it is advised that treatment is monitored daily in order to achieve a plasma heparin level of up to 1 mg per 100 ml.

—M. J. G.

Stable Suspension of Erythrocytes suitable for Calibration of the Electronic Counters. Torlontano, G. and Tata, A. (1971), *Acta Haemat.* **45,** 325.

A simple method of preparing stable suspensions of normal erythrocytes is described. The fixative for the cells contains acetic acid, sodium sulphate and sodium chloride; stability of number, shape and volume, without aggregates is assured for at least six months and probably for two years.

—M. J. G.

Gaucher's Cells in Thalassaemia. Zaino, E. C., Rossi, M. B., Pham., T. D. and Azar, H. A. (1971), *Blood,* **38,** 457.

Gaucher or Gaucher-like cells are described in the spleen and bone-marrow of a patient with thalassaemia major. Both light and electron microscopic structure is described. It is suggested that impaired catabolism of erythrocytes may give rise to the increased glucocerebroside that accumulates within the cytoplasm to give the Gaucher cell its characteristic microscopic appearance.

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—D. G. B.

Quantitative Studies on the Salivary Flora. Ross, P. W. (1971), *J. clin. Path.*, **24**, 717–720.

A quantitative survey of the salivary flora of children is presented. The author remarks on the paucity of information available on the salivary micro-flora and stresses the importance of this information in understanding the pathogenesis of oral infections. Of interest is the high incidence of pneumococci in normal saliva (77 percent). Other pathogens found include *Staph. aureus, N. meningitidis* and *H. influenzae.*

—D. G. B.

The Effect on Sepsis Rates of Closing and Cleaning Hospital Wards. Neone, P. and Griffiths, R. J. (1971), *J. clin. Path.*, **24**, 721–725.

The closing of wards in which outbreaks of antibiotic resistant staphylococci infection occur is a common practice. This paper investigates the rationality of such a procedure. The conclusion reached is that sepsis rates are significantly reduced for a considerable period after such closure and cleaning procedures.

—D. G. B.

A Modified Nylon Bridge for a Closed Blood Culture System. Freake, R. and Patten, W. (1971), *Amer. J. med. Technol.*, **37** (6), 221–222.

A modification to the apparatus for blood culture described in 1964 by Freake and Nayman is described. The essential difference is the incorporation of two entry ports into the nylon bridge piece in place of the one port described in the original device. The authors claim that this allows the incorporation of thermolabile substances into the medium after autoclaving leaving a second entry port for injecting the blood sample.

Although this device shows promise its prohibitive price in this country makes it unlikely to gain wide acceptance.

—D. G. B.

The Decline of Antibiotic Resistance of *Staphylococcus aureus* Located in the Community. Bauer, A. W. (1971), *Amer. J. med. Technol.*, **37** (6), 247–253.

This paper discusses the drop in the number of strains of *Staphylococcus aureus* resistant to tetracycline and erythromycin over the five-year period 1964-1969. Resistance to penicillin remained unchanged during the period.

The author also discusses factors influencing the single disk, high potency technique of sensitivity testing.

—D. G. B.

Comparative Immunological Analysis of Enterobactericeae. Garcia, C. F. (1971), *Amer. J. med. Technol.*, **37** (7), 271–276.

This paper describes immunoelectrophoretic precipitation reactions carried out on *Salmonella* and *Citrobacter* species. Although the results are interesting in showing clear antigenic differences between *Citrobacter* and *Salmonella* strains the work appears to be of no value in the diagnostic laboratory.

Full technical details are given.

—D. G. B.

Optimal Bacitracin Concentration for Selective Isolation Media for Haemophilus. Ederer, G. M. and Schurr, M. L. (1971), *Amer. J. med. Technol.*, **37** (7), 304–305.

This technical note discusses the optimum level of bacitracin for use as a selective agent for *Haemophilus*

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Corporation and Diversified Numeric Applications. Prices range from $150,000 to $185,000. Core sizes are roughly comparable and expandable. Most provided tape and disc. Entry is by teletype or console and all can use mark sense cards. There is considerable variety in the type of terminal used, CRT, teletype and many different types of consoles. Lists and labels for venepuncture are now standard. The actual configuration depends on the philosophical approach.

This is not the time or place to attempt comparison, but certain fundamentals seem to emerge such as the need for a dedicated computer, the advantage in time saving of a written programme, disc rather than tape for access to stored data, at least a 300-line printer, mark sense cards and sufficient trained operators. The latter point is subject to the philosophical background of the system.

Chapters are devoted to all the main suppliers, twelve in all. International Business Machines, for example, produce a variety of systems for different situations and require separate programming. This can be a difficult and time-consuming business, as it is the responsibility of the user laboratory. One valuable lesson is deduced, namely, that competent programmers are scarce and in great demand and therefore usually in the employ of a computer firm. Therefore, unless there are some very unusual circumstances laboratories should not employ their own programming staff but should only consider systems whose manufacturers assume complete responsibility.

Some firms such as T. and T. Technology specialise in small modular clinical laboratory data acquisition systems which might well prove to be of more than passing interest to laboratories where the cost of a comprehensive system cannot be reconciled with the work load.

In assessing this book one must consider that in order to be of value it must be up to date, which it is; and it must provide a good cross-section of the data, which it does. There is an immense amount of reported material in this very large book. While it is faithfully reported, the reader must make his own comparisons and reach his own conclusion. The circumstances of its production, which demanded some haste, did not cater for reflection or considered judgment. This may also account for the few spelling errors which came to light such as the second paragraph on the first page of chapter one where 'delivery' is used instead of 'deliver'. On page twenty we have 'Isoteric', and the appendix, first page, refers to 'laborary data'. Other spellings which looked strange and the rather odd phraseology may well be acceptable in the States. The unvarnished paper and the large type are to be commended.

In conclusion it is plain that anyone involved in laboratory computerisation and in particular anyone contemplating the purchase of such a system should certainly invest in this book. The cost is relatively insignificant and diligent study would provide an ample return.

—R. D. A.

Medical Parasitology. Third Edition. Edward K. Markell, PhD, Marietta Voge, MA, PhD, 357 pages. W. B. Saunders Co., Philadelphia. 1971. Price from N. M. Peryer Ltd. $13.75.

In this day and age of rapid transport the problems of parasitology are becoming more and more evident, even in areas where parasites are not usually endemic. Perhaps it is as a reflection of this that more and more texts on parasitology are becoming available. This one is a third edition containing 345 pages, in which the parasites are handled taxonomically instead of by organ systems. Both the physician and the laboratory technologist are amply catered for in this edition, and in fact it is also valuable for the laboratory staff to be able to refer to the clinical material and the sections on pathogenesis and treatment.

The book is divided into 15 chapters, the first three incorporate the introduction, parasites, parasites and host relations, and procedures for examination of stool specimens. The latter of these deals very nicely in a compact way with the methodology related to parasitology, discussing wet preparations, stained films and preparation of smears. Concentration techniques are perhaps only briefly discussed, as are culture methods.

Chapter 4 is only a brief chapter on the examination of blood and other tissues but deals adequately with the methods to be employed.

From Chapter 5 to 11 the various orders of parasites are discussed. The first of these are the human-dwelling protozoa and deals with

the pathogenic types in some detail, including *Isospora* and *Balantidium* as well as the commensals and those protozoa of questionable pathogenicity such as those causing meningoencephalitis (*Naegleria, Hartmanella, Acanthamoeba*). There are a host of diagrams as well as X-ray photographs. The information in this chapter gives a good basis of differentiation of the main species.

Malaria is the topic of Chapter 6; this is a fairly extensive one giving very ample coverage of the main types of *Plasmodium*. Each discussion on *Plasmodium* carries with it a full colour page showing the asexual cycle in man. As a source of reference on malaria this is excellent.

As malaria obviously warrants a chapter of its own, so do the remaining protozoans, *Trypanosoma, Lieshmania, Toxoplasma* and related sporozoa. This chapter defines the different trypanosomes with great clarity and some very nice photographs are also included. The section on *Lieshmania* is also endowed with good photographs of lesions and parasites in host cells. The section on *Toxoplasma* is obviously up to date discussing the life cycle in the intestine of the cat and also demonstrates the life cycle with lovely photographs. It is unfortunate that the laboratory analysis section is somewhat lacking in information, especially regarding specific titre levels and fluorescent antibody techniques.

In New Zealand the trematodes do not pose much of a problem and are very rarely encountered; however, with soldiers returning from overseas and an influx of Asian visitors and migrants, this is very much a potential problem. Chapter 8 deals with this fairly extensively and is a big chapter. Intestinal flukes, liver flukes, blood flukes and lung flukes are all detailed with a great deal of authority. They are well illustrated with good diagrams of their life cycles and actual photographs of the adult flukes. This is a comprehensive chapter with X-ray photographs and good illustrations of trematode eggs, and is one of the best texts on the trematodes I have yet seen.

Chapter 9 deals with the cestodes or tapeworms and, as with Chapter 8, very commendably. All the significant tapeworms infecting man from *Diphyllobothrium* to *Hymenolepsis*, are documented, again enhanced by photographs and good diagrams.

Apart from the acceptable intestinal nematodes, such as *Necator, Ankylostoma, Ascaris, Trichuris, Strongyloids* and *Enterobius,* Chapter 10 documents Philippine capillariasis, *Trichostrongylus* and aniskiasis (nematode acquired from fish). One again this chapter is up to the high standard of the previous ones discussed.

Blood and tissue-dwelling nematodes are dealt with in Chapter 11. A majority of the chapter discusses the micro-filariae and the three main types, *bancrofti, loa loa* and *malayi,* are joined by *Dipetalonema perstans* and *Mansonella ozzardi,* two filarial parasites of man which produce little or no damage. The microfilariae of *Onchocerca volvulus* found in dermis and subcutaneous tissue is discussed at some length and clearly demonstrates the symptomatology and pathogenesis of this unusual filariae. *Trichinella* and the Guinea worm are also mentioned in this chapter along with *Toxocara,* which I feel really warrants more extensive handling than it has. However, it is nice to see that *Angiostrongylus cantonensis,* the causative agent of eosinophilic meningoencephalitis rates a segment of this chapter.

Chapter 12 discusses the remaining aspects of parasites, *Sarcoptes, Pediculus,* chigae flea and even myiasis in some detail with good photographs.

Chapter 13 is a chapter of extreme use, especially to the technologist, as it deals very thoroughly with the signs and symptoms of parasitic disease.

Misidentification is a real problem in investigating for parasites and Chapter 14, although only a short chapter, deals neatly with some of the pitfalls in parasitology.

Special procedures, stains and fixatives are the topic in Chapter 15. These discuss animal inoculation, biopsy and aspiration, specimens and culture methods, as well as other techniques. Serological methods discussed here are only briefly mentioned and information on some of the more recent fluorescent antibody techniques for diagnosing parasite infection are left out.

In conclusion, this textbook is an exceptionally fine text on parasites and I would not hesitate to recommend this book as part of the reference library of any laboratory. Perhaps it has a few shortcomings with regard to the laboratory aspects and is more medically orientated, but this does not detract from the

fact that this is a very suitable parasitology text.

N.B.: Available from the publishers with this text are 100 illustrations in a set of two 35 mm colour filmstrips. I am still awaiting the cost of these and further information on them and will make the information available when it comes to hand.

—B. M. C.

Procedures in Clinical Chemistry. Anthony Palma. 109 pages. 1971. Publishers, J. B. Lippincott Company. Price US$4.95.

This little book set out to provide a series of standard methods stripped for action; in other words a bench-book or worksheet approach. The methods are mostly old ones and in fact the author in the preface refers to the 'Classic time-tested standard methods'. Age is a good criterion for spirituous liquors but laboratory techniques have advanced over the last twenty years. Scarcely any of the references given break through into the sixties. Certainly some of the methods such as Clarke Collip and Biuret could be regarded as classical; however the original Folin and Wu and Nessler complete with gum ghatti are a little hard to support nowadays. Curiously enough there is a little note which states that Somogyi gives a true blood sugar. This is not strictly true but it certainly gives a truer blood sugar, so why not use it? It is no surprise that SI Units find no favour here.

The details of the methods given seem accurate enough as are the brief notes on the significance of the tests. There is a pitfall in the uncritical acceptance of stark details such as definite values for commercial control sera.

This book with its explanations, could be of use to laboratory aides doing a small range of routine tests in an un-automated laboratory; provided that the arbitrary selection of tests were acceptable.

—R. D. A.

Fundamentals of Clinical Haematology. Third Edition. By Byrd S. Leavell, MD and Oscar A. Thorup, Jr, MD. 659 pages. $18.00.

This book judging by the preface has as its aim the presentation of a readable, concise, yet comprehensive volume on clinical haematology. In the main it is successful in these objectives although in parts the standard is uneven. It has always been difficult for me to imagine a natural dividing line between the clinical and laboratory aspects of haematology. This edition in order to provide space for new material, has omitted descriptions of laboratory procedures and in many cases, has also omitted the theory behind certain of these tests. Surely the more common tests such as Ham's acid test, the determination of red cell mass and the tournique test to name but a few, require some explanation more than just a passing mention.

The subject of disseminated intravascular coagulation is treated superficially, and is in general unhelpful to the practitioner confronted with this increasingly recognised problem. We are not informed of the conditions in which this may occur nor are we given a rational approach to therapy. The chapters on acute leukaemia and agranulocytosis deal little with the problem and management of infection, except to mention that in various circumstances (not mentioned) antibiotics are useful. This may be concise, but is less than precise.

Throughout the text are helpful classification tables, illustrative cases of various haematological problems, discussions on differential diagnosis, and a liberal sprinkling of references backed by an extensive bibliography. Mention is also made of photomicrographs illustrating cell morphology and obtainable separately at an undisclosed price from the publishers. These are to be used as a teaching adjunct. As these were not supplied to me I cannot comment on their quality.

The emphasis then of this book, is mainly clinical, but aetiology and pathogenesis have not been by-passed. It has much to offer as a bridge between the necessarily incomplete descriptions of haematology found in general medical texts, and the larger more comprehensive reference books and monographs.

—L. A. B.

Microbiology. Philip L. Carpenter, Third Edition, 1972. Contains 494 pages with numerous photographs, tables and four colour plates. W. B. Saunders Co., Philadelphia. Price $9.50.

The 26 chapters which make up this volume are grouped into five sections dealing with individual aspects of microbiology—micro-organisms and their study; biology of the lower

protists (bacteria and viruses); higher protists (protozoa, algae, moulds and yeasts); ecology of infectious diseases; environmental and applied microbiology. Each chapter is followed by a list of standard textbooks or articles for supplementary reading. There is a useful glossary.

This book is written for students who are following a course in microbiology as part of their study of general biology or as an introductory course for those who intend, at a later stage, to specialise in a branch of microbiology. As a result the contents are somewhat patchy and those parts dealing with micro-organisms in relation to human disease, food and dairy products and the microbiology of soil, water and air are of an elementary and abbreviated nature.

The first two sections, constituting 260 pages, deals very fully with basic microbiology including history, classification, method of study, the structure and function of microbial cells, metabolism, genetics and the growth and death of micro-organisms. These accounts are well written and are illustrated by carefully chosen and well-reproduced photographs, diagrams and charts, mostly taken from authoritative reference sources. The general presentation of these sections is such that the letterpress and illustrations blend together to make factual assimilation interesting and easy.

The section on the ecology of infectious diseases contains good material on the normal and pathogenic body flora and on resistance and immunity.

Students studying medical microbiology would find the sections on basic microbiology most useful but they will need to consult textbooks devoted entirely to medical microbiology to obtain a wider knowledge of this subject.

—N. P. M.

Directory of New Zealand Electronics 1971. Published by Magazine Press Ltd. One to four copies, $1.30; five to nine copies, $1.15; 10 and over, $1.00.

The publishers, in co-operation with the National Electronics Development Association, are to be complimented in producing a most comprehensive directory of distributors serving the electronic industry in both the supply and manufacturing fields.

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—W. N. M.

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Illustrations

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

Nomenclature and Units

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

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