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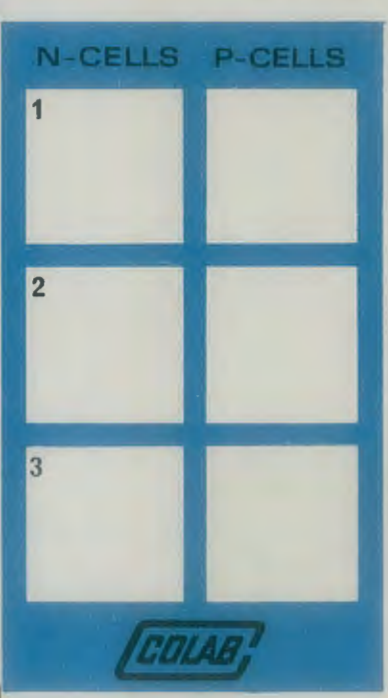

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Electrophoresis of Hb A_2 on Cellulose Acetate

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Received for Publication, March, 1969.

Zone electrophoresis is now accepted as routine practice in clinical laboratories, although its commonest usage is still limited to the separation of serum protein fractions. However, the importance of the electrophoretic separation of haemoglobin into its types is gradually becoming recognised. Within New Zealand, the chances of finding abnormal haemoglobins are becoming greater each year, resulting from increases in visitors and immigrants.

So many variable factors affect ionic movement in electrophoresis that it is often difficult to select a suitable combination. The variables from which a working method must be selected include the following:

(a) *Buffers:* (i) pH and ionic strength
(ii) chemical constitution
(iii) continuous or discontinuous system

(b) *Support medium:* the commonest are starch gel, agar, paper and cellulose acetate

(c) *Strength of electric current*

(d) *Duration of electric current*

(e) *Method of application of sample*

(f) *Position of application of sample*

(g) *Vapour content of surrounding atmosphere*

(h) *Type of tank*

(i) *Method of fraction quantitation.*

The choice depends largely upon exactly which fractions of haemoglobin are of most interest, upon speed and ease in use, and upon reliability under the conditions in a routine clinical laboratory.

It is just over ten years since Kohn[5] first described the use of cellulose acetate for electrophoresis. As a support medium it offers many advantages. A very rapid separation of haemoglobins is achieved with well-defined zones that can be quantitated with remarkable accuracy and precision. This is of great importance for haemoglobin work, especially in detecting the slight increases of Hb A_2 which lead to diagnosis of thalassaemia minor. Kunkel and Wallenius[6] first isolated the normal haemoglobin A_2 on starch block in 1955; three years later Gerald and Diamond[3] suggested that an increase in Hb A_2 was a minimum criterion for the diagnosis of the thalassaemia trait. The method described here has been found reliable in routine use over a period of at least eighteen months and the results obtained in haemoglobin electrophoresis by this method are presented here, together with the normal range of Hb A_2 values established in the Auckland area of New Zealand.

Principle:

In some electrophoresis the ions in solution move as discrete zones under influence of electric current. Under the conditions in this method, using Oxoid brand cellulose acetate, reverse-flow electrophoresis occurs (Afonso)[1]. These conditions exist on the anodic half of the strip because of the strong electro-osmotic flow enhanced by the capillarity flow of the buffer coming from tank to strip to replace loss by evaporation. A water-sealed tank is essential for the maximum humidity needed. Because of the described conditions, the haemoglobin appears to disobey the conventional wisdom that "haemoglobin has a negative charge and migrates towards the positive pole."

The terms "fast" and "slow" fractions are obsolete because of their ambiguity. Two faint carbonic anhydrase bands (Briere *et al.*)[2] are seen nearest the cathode and are not usually eluted. The Hb A_2 fraction is seen between these anhydrases and the major Hb A. The latter is nearest the anode. (See Figure 1.) Hb F is partially "fused" with the Hb A band, although Rosenbaum[8] claims discrete separation.

Preparation of Haemolysate:

A haemolysate is made by the method of Singer *et al.*[9] It is simultaneously standardised to a haemoglobin value of 10g. per cent. by using the mean corpuscular haemoglobin content (MCHC) of the patient's whole blood. After the MCHC has been determined, 1 ml. of packed red cells from EDTA or heparinised blood is washed four times with physiologically normal saline. The formula for establishing the exact number of millilitres of de-ionised water to then add to these washed red cells is: $\frac{\text{M.C.H.C.}}{10} - 1$

After the water add 1 ml. of toluene. Shake for five minutes and then centrifuge the haemolysate for twenty minutes at 3000 rpm (1200 G). The clear haemolysate is then carefully sucked from beneath the top layer of toluene fat and stroma, and is filtered through 5.5 cm. diameter double Whatman No. 42 papers, trimmed to minimal size and very slightly dampened if haemolysate is scarce. For storage the haemolysate is placed in several aliquots in very small screw-capped containers and stored at —20°C.

Reagents:

Buffer for both electrodes (continuous): (Briere *et al.*)[2]. In a litre volumetric flask dissolve TRIS 16.5 g.; disodium EDTA 1.56 g. and boric acid 0.92 g. Make up to one litre with de-ionised water (pH 9.1 — 9.2). Store at 4°C. when not in use.

Stain:— (Graham and Grunbaum)[4]

Within a volume of 250 ml. dissolve 0.5 g. Ponceau S, 7.5 g. trichloracetic acid and 12.5 g. sulphosalicylic acid, in de-ionised water.

Rinsing solution: 5% v/v acetic acid.
For elution: (i) 0.25 N sodium hydroxide
(ii) 40% v/v acetic acid.

Electrophoretic Apparatus:

A Shandon horizontal Universal tank (Mark II) with sloping perspex lid is used, together with a Vokam (type SAE - 2761) power-pack.

Method:

Prepare the electrophoresis tank by filtering 700-800 ml. of the buffer into the four compartments and ensure an equal depth in each. The shoulder gap is set to 10 cm. and new shoulder strips (Whatman chromatography paper No. 3 MM) are fitted daily and moistened with buffer. The moat is half-filled with de-ionised water to provide a seal later when the lid is in place.

Carefully prepare three Oxoid cellulose acetate electrophoresis strips 12 x 5 cm. from a 36 cm. length. Using a soft lead pencil and a suitably constructed template of X-ray film, dot the mid-points and across the strip put a very faint guide line 2.5 cm. from centre and name the patient and a "normal" on each strip. Fingers must on no account touch the area in which electrophoresis occurs.

Float each strip for two minutes in an open dish of buffer to avoid air becoming entrapped in the acetate. Then immerse each strip completely for one minute. Blot strips lightly between fresh filter papers and position each one in the tank across the shoulder pieces so that the centre mark corresponds to the tank centre and the guide line for application is nearer the anode. Put the sloping lid on the tank to seal the atmosphere, so that effective vapour saturation occurs and the membrane surfaces do not overheat.

Set the power pack to provide "constant current"; for the three strips (later totalling six haemolysates) allow 400 volts. At this stage, run current for 15 minutes to establish equilibrium conditions. Switch off and remove lid with extreme care to prevent any moat-water dropping from the edges of the lid on to the strips; this is a common cause of bad "runs" and is hard to detect.

Apply between 1 and 1.5 microlitres of test haemolysate in a straight line 11 mm. long on the anodic pencil mark, ensuring a free 8 mm. from each edge of the strip. Always run a normal control haemolysate in parallel on the same strip as an unknown haemolysate. Use two separate test strips in order to gain sufficient material for elution.

Immediately replace the lid and switch on the current, as above, for one hour. After switching off, again very carefully remove the moisture-laden lid. Dry strips in a hot-air oven at 90°C for ten minutes, hanging them by one end.

Later float each strip on the Ponceau S stain for one minute before immersing completely for ten minutes. Rinse the stained strips in four changes of 5% v/v acetic acid. Dry them in a hot-air oven (90°C) for ten minutes.

Quantitation by Elution:

Duplicate each test so that 2-3 microlitres of haemolysates are electrophoresed and quantitated by pooling the appropriate fractions.

Cut out each stained haemoglobin band carefully into labelled plastic dishes and cut out blank acetate pieces of equal area to each band from the same acetate strip.

Float two Hb A bands on, and then soak in 9 ml. 0.25 N sodium hydroxide in wide 10 ml. beakers. Agitate for a minute to ensure complete elution and then add 0.6 ml. of 40% v/v acetic acid; mix and immediately read Absorbance at 515 mμ (Unicam SP600). It is probably advisable to remove the acetate pieces soon after elution, because sodium hydroxide degrades them and produces cloudiness.

Two Hb A_2 bands are treated in the same manner, but volumes are 3 ml. of 0.25 N sodium hydroxide and 0.2 ml. of 40% v/v acetic acid per band.

All the corresponding blank acetate pieces are similarly treated and their optical density subtracted from that of the Hb bands.

$$\frac{\text{Optical density } A_2}{(3 \times \text{OD of A}) + \text{OD of } A_2} \quad \frac{100}{1} \quad = \quad \%\ \text{Hb } A_2$$

If Gelman brand cellulose acetate is used, proceed as for Oxoid but apply haemolysate on the cathodic side.

Elution and Normal range of Hb A_2 From Oxoid Cellulose Acetate:

Haemolysate from thirty-two staff members were electrophoresed and eluted by the above method on Oxoid brand cellulose acetate. Their Hb A_2 ranged from 0.4 to 3.1% with a mean of 1.7%.

Haemolysates from forty-four adult patients being tested for increases in Hb A_2 and found to be within the normal range, gave results from 1.3 to 3.2% with a mean of 2.2%. Ten babies gave Hb A_2 results from 0 to 1.2% with a mean of 0.6%. This conforms with the findings of Allison *et al.* as cited by Lehman and Huntsman.[7]

The reproducibility of the elution method was examined. (See Table I.) The elution method with Oxoid cellulose acetate

TABLE I

Rob. Hb A_2	Ber. Hb A_2	Cle. Hb A_2	Stu. Hb A_2
5.7%	2.6%	1.9%	2.7%
5.5%	2.5%	2.6%	2.6%
	1.2%	1.3%	1.9%
	2.0%	1.9%	1.9%

Table 1 shows typical results when one haemolysate from each of four patients was repeatedly electrophoresed and Hb A_2 quantitated by elution.

TABLE II

Dilution of 10g.% haemolysate.	Hb.	Hb A_2
3:3 of 10g%	100 µg	5.4%
2:3 of 10g%	66 µg	4.8%
1:2 of 10g%	50 µg	5.2%

Table II illustrates electrophoresis of varying amounts of haemoglobin from one patient; Hb A_2 quantitated by elution. (Same patient as in Table IV).

showed both sensitivity and accuracy. To assess further such day-to-day variables as the amount of haemolysate applied, a 10 g.% haemolysate was diluted 2:3 and 1:2. Both dilutions were electrophoresed together with the undiluted haemolysate: Results are in Table II.

The Scanning of Hb A_2 on Oxoid Cellulose Acetate:

This was carried out by the method of Briere *et al.*[2] using a Joyce Lobel Chromoscan with blue filter, a gear ratio of 1:3 and a slit width of 0.5 mm. with the slit modified to reduce length to suit the strips used. Forty normal haemolysates gave Hb A_2 values of 2.2 - 5.9% with a mean of 3.3%.

The day-to-day reproducibility of the scanning method of quantitation varied with clearing technique, evenness of application of haemolysate and the skill of the technical worker who carried out the scanning procedures. Typical results are shown in Table III.

TABLE III

Sant. Hb A_2	Rob. Hb A_2	Fitz. Hb A_2
10%	13.1%	5.2%
6.6%	14.4%	4.4%
	13.6%	7.5%
		5.7%
		5.9%
		2.8%

Table III shows typical results when one haemolysate from each of 3 patients was repeatedly electrophoresed; Hb A_2 quantitated by scanning.

Sensitivity and accuracy of the scanning procedure were also assessed by electrophoresing and scanning dilutions of the 10g.% haemolysate. The undiluted haemolysate was compared with the 2:3 and 1:2 dilutions. See Table IV.

TABLE IV

Hb.	Dilution	Hb A_2 peak integral	Hb A peak integral	Ratio Hb A_2:Hb A	Hb A_2
100 µg	3:3 of 10g%	18	192	1:10.7	8.6%
66 µg	2:3 of 10g%	9	131	1:15	6.4%
50 µg	1:2 of 10g%	7	97	1:14	6.7%

Table IV illustrates electrophoresis of varying amounts of haemoglobin from one patient; Hb A_2 quantitated by scanning. (Same patient as in Table II.)

Differences in Various Brands of Cellulose Acetate:

Comparisons of Oxoid and Gelman brands of cellulose acetate produce different normal ranges of Hb A_2 and differences in the positions of the Hb A and Hb A_2 bands in relation to the electrodes and positions of application. Figure 1 illustrates this. It will be seen that anodic application of haemolysate on cellulose acetate utilises the reverse flow conditions on Oxoid only and the bands migrate toward the cathodic side whereas the same technique on Gelman shows that Hb A apparently moves toward the anode. Cathodic application on Gelman does give excellent separation of Hb bands, and bands are much more discrete than on Oxoid. Cathodic application on Oxoid is unsatisfactory; note the position of application residue in Figure 1.

Twenty-four normal haemolysates were run on both Oxoid and Gelman. The average Hb A_2 result was higher by 1.4 on Gelman. The normal range from forty-seven staff members' haemolysates, electrophoresed on Gelman and eluted, was 2.3 - 4.3% Hb A_2 It will be noted that the upper limit is higher by 1.2 than the normal range for Oxoid given earlier in this paper.

Cellogel, a preparation of gelatinised cellulose acetate (Chematron, Italy) was also tried with the buffer described in this paper, and the positions of the Hb A_2 and A bands after anodic application are seen in Figure 1.

Elevated Hb A_2 on Oxoid Cellulose Acetate:

Over an initial period of twelve months 60 patients were referred for haemoglobin electrophoresis by the method described at the beginning of this paper. Of these sixteen showed elevated Hb A_2 and led to investigations of two families, from which seven out of eleven relatives showed increased Hb A_2. Therefore, twenty-three increased Hb A_2 fractions were disclosed. These were probably indicative of beta thalassaemia minor. Their Hb A_2 values on Oxoid cellulose acetate ranged from 3.2% to 6.2% and their mean was 4.7%.

Of the twenty-three haemolysates showing increased Hb A_2, nineteen were also tested by the alkali denaturation test of Singer *et al.*[9], and eight were found to have Hb F of 2% or greater, the highest being 3.6%. Eleven had Hb F below 2%, and the other four were not tested.

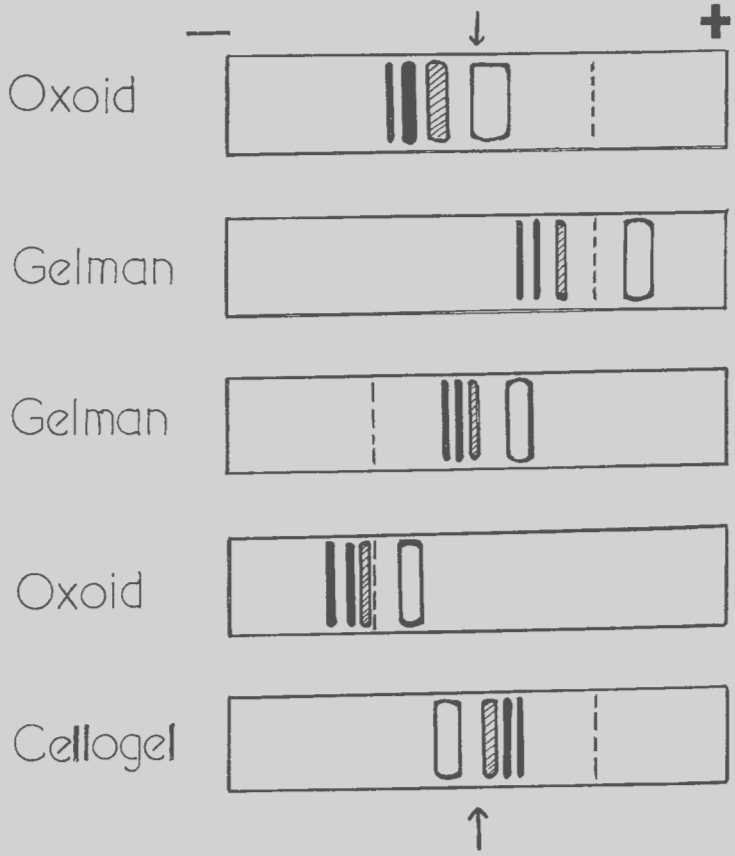

Figure 1: Diagrammatic comparison of electrophoresed haemoglobins applied anodically and cathodically (dotted lines) on several brands of cellulose acetate. Arrows indicate mid-points; Hb A is unshaded: Hb A_2 is striped; the two carbonic anhydrases are blackened and CA_2 is furthermost from Hb A.

Storage of Haemolysates:

Small sterile screw-capped bottles with rubber washers are suitable for deep-freezing haemolysates. No deterioration of Hb A_2 values was detected in normal haemolysates, nor in one with increased Hb A_2, over a period of months. One haemolysate with Hb A_2 of 5.3% was stable for at least six months at —20°C.

Abnormal Haemoglobins Detected:

Both Hb S and Hb Barts were detectable on Oxoid cellulose acetate by the method described earlier in this paper.

The Hb Barts was separated and quantitated to 11.4% alongside an Hb A_2 of 0.8%. The Hb Barts band was situated on the anodic side of the Hb A and F (Oxoid; anodic application). The identity of the band was confirmed by ultra-violet spectrophotometry; the Hb Barts was also electrophoresed on starch gel at pH 7.7 by the Auckland Blood Transfusion Service. The patient was a child of four weeks; foetal haemoglobin and Hb Barts by alkali denaturation were 53%. The findings were consistent with alpha thalassaemia minor.

In another patient Hb S was clearly separated, situated between Hb A and Hb A_2. It was a heterozygous expression of the S gene and gave a Chromoscan quantitation of 23% Hb S. This haemolysate had been deep-frozen for more than three years prior to electrophoresis on cellulose acetate. Recently a fresh haemolysate from this patient gave an Hb S of 27% by elution.

Discussion:

Experience with cellulose acetate haemoglobin electrophoresis over many months and including many trials and modifications of methods has led to the following observations:

(a) The method presented in this paper, using Oxoid cellulose acetate, is a reliable and reproducible method for routine daily use.

(b) There are marked variations in the quality and properties of different batches within a brand of cellulose acetate.

(c) Although Hb E occupies the site of Hb A_2 in the method described in this paper, it is unlikely to cause confusion of identity because Hb E, even in the E heterozygote, amounts to 30-40% (Lehman)[7] whereas Hb A_2 is usually below 7%.

(d) In our experience in a routine haematology laboratory, quantitation by scanning required much more expertise and care by technical staff than did quantitation by elution.

(e) Hb F is better quantitated by the alkali denaturation test as it is extremely difficult to separate completely as a discrete band, although Rosenbaum[8] claims to have done this without difficulty.

(f) The continuous buffer system used in this paper, as described by Briere *et al.*[2], gave better results than a discontinuous system using buffers of barbital and Tris types. The pH of 8.8 given by Briere *et al.*[2] for the buffer described in this paper is unattainable without adjustment with acid. However, this adjustment is not necessary and at the unadjusted pH of 9.1-9.2 the buffer functions efficiently.

(g) The phenomenon whereby, under certain conditions,

haemoglobins applied anodically on Oxoid cellulose acetate migrate towards the cathode by reverse flow electrophoresis is particularly interesting, as this does not occur on Gelman cellulose acetate when haemolysate is applied anodically under similar conditions. Nor does it occur on Oxoid when application is cathodic. (Figure 1.)

(h) Cathodic application on Oxoid and anodic application on Gelman lead to inaccurate quantitations because the residue often present at the application line is situated and is stained in the middle of the haemoglobin fractions required for accurate elution and quantitation (Figure 1.)

Summary:

1. A modified method of haemoglobin electrophoresis on cellulose acetate is presented which is both reliable and accurate. This method has been used to detect and to quantitate Hb A, Hb A_2, Hb S and Hb Barts as well as the anhydrases CA_1 and CA_2. It is reputed to detect other abnormal haemoglobins.
2. A normal range of Hb A_2 from 76 adults is presented using Oxoid cellulose acetate and elution; the range is 0.4-3.2% of the total Hb.
3. A normal range of Hb A_2 from 40 adults is presented using Oxoid cellulose acetate and scanning; the range is 2.2-5.9% of the total Hb.
4. The range of Hb A_2 from 23 cases of beta thalassaemia minor is presented together with results of 19 of the concomitant Hb F determinations by alkali denaturation.
5. The normal range of Hb A_2 on Gelman cellulose acetate was found to be higher than on Oxoid cellulose acetate.
6. Technical details and phenomena relating to Hb electrophoresis on cellulose acetate are discussed, with particular reference to differences between various brands of acetate.

Acknowledgements:

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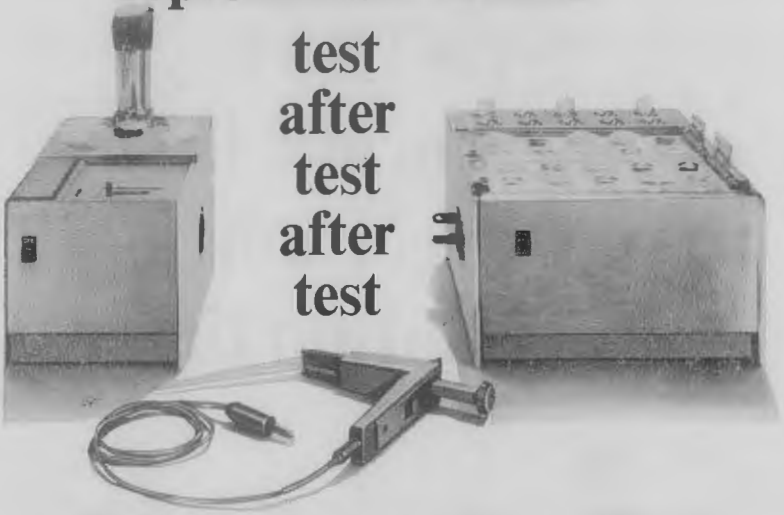

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The Occurrence of *Microsporum nanum* as a Human and Animal Pathogen in New Zealand

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Received for Publication, March, 1969.

The intensive study of keratinophilic fungi which has occurred in recent years has led to the discovery of several previously unrecognised species of *Microsporum*. Of the fourteen species of this genus considered valid by Ajello in 1968[1] only three were in Emmons' 1934 classification[6]: *M. audouinii, M. canis* and *M. gypseum*. Although these three are the most frequently isolated members of the genus from human and animal infections, certain of the other species may occasionally be encountered in the diagnostic laboratory. One of these is *M. nanum*, a fungus first described by Fuentes *et al*[8] as a dwarf form of *M. gypseum* and later given its specific name by Fuentes.[7] It is known as a cause of tinea capitis and tinea corporis in the Americas[2] but has not previously been recorded as a human pathogen in New Zealand. This report concerns the isolation of *M. nanum* from a human infection apparently contracted from infected pigs. These animals are frequent hosts of this fungus[4], [9] and a reservoir of potential human infection which appears to be fairly widespread in New Zealand[10]. We give details of the cultural appearance of this dermatophyte so that it may be recognised in the laboratory should it occur during routine isolations from ringworm material.

Case Reports:

Human

The human infection was of one of us. The lesion on the left forearm was first noticed as a circular, slightly erythematous area, 2 x 1.5 cm. in diameter and 10 cm. above the wrist (Figure 1). There was no associated scaling when first observed but the skin scrapings could be obtained without difficulty by running a scalpel blade over the affected area. Microscopically, in KOH, the skin scrapings contained abundant fungal hyphae but there was no apparent involvement of the hair, although, macroscopically on the skin, some hair follicles had appeared somewhat engorged. There was no fluorescence under Wood's light. (In some cases of tinea capitis caused by this fungus a green fluorescence has been described[5] and an endothrix type of hair invasion may occur[8] [3].) When cultured on Sabouraud dextrose agar containing cycloheximide and chloramphenicol the skin scrapings yielded colonies identified as *M. nanum*.

Scaling of the lesion was first observed on the 5th day and treatment 3 times daily with "Tinaderm" (tolnaftate sulphate)* was commenced The amount of scaling between applications of the Tinaderm, when the skin had become dry, increased over the subsequent 9 days. By this time the centre of the lesion had resolved but the total area involved had not

* Schering Corporation, New Jersey.

increased, there being no peripheral spread. Slight scaling persisted for a further 14 days, when the lesion had almost disappeared.

Porcine

In New Zealand pigs are commonly affected by this dermatophyte. In advanced cases the infection often involves the whole of the body surface. Thick brownish scaling spreads from the initial location which is usually on the upper flanks, the spread being aggravated by the intense scratching of the pig on any available surface. Occasional scab formation occurs, particularly behind the ears (Figure 2). There is no alopecia, the hairs apparently being unaffected by the dermatophyte. In mild infections the scaling may be less obvious and the appearance may be overshadowed by dirt or confused with urine staining.

Figure 1: Lesion of human ringworm from which *M. nanum* was isolated.

Figure 2: Severe *M. nanum* infection of a pig's ear.

Cultural morphology of *M. nanum*

M. nanum shows rapid growth on Sabouraud dextrose agar, reaching a diameter of 25 mm. within 7 days. The colony is flat and at first a white, floccose growth but soon becomes powdery with a light buff coloured surface (Figure 3). The reverse passes from orange to dark red. Some subcultures may maintain a more fluffy appearance, with the entire surface somewhat convoluted. The margins of the colony may be irregular and the reverse pigmentation may remain orange.

Microscopically, macroconidia are numerous and are oval to elliptical, 11-19 μ x 6-9 μ. The outer walls usually have a finely echinulate surface and are thin when compared to the thick walls of *M. canis*. Most macroconidia are one septate, some non-septate or 2-3 septate. (The appearance of the macroconidia may lead to confusion with *Epidermophyton floccosum*.) Microconidia are pear-shaped and sessile on the hyphae, 5 μ x 2 μ (Figure 4).

Figure 3: Cultural appearance of *M. nanum* on Sabouraud dextrose agar.

The fungus forms perforating organs on human hair *in vitro*. *M. nanum* is the imperfect stage of *Nannizzia obtusa*[5] and may be isolated from the soil in piggeries by the hair baiting technique.[2]

Comment on the probable origin of the human infection.

The human lesion was first noticed whilst we were conducting a trial of the effectiveness of Tinaderm in the treatment of the

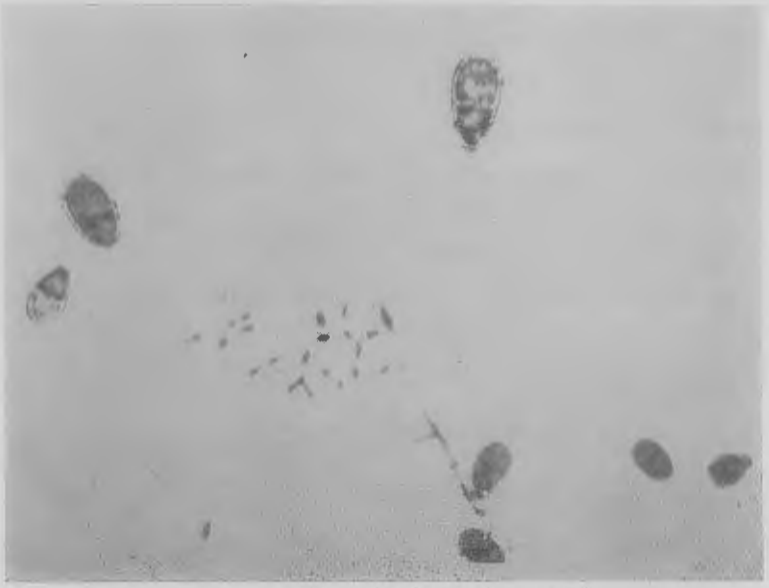

Figure 4: Macroconidia and microconidia of *M. nanum*.

pig infection, in the 2nd week of the experimental period. The Tinaderm had been applied twice daily to one flank of a pig with widespread infection according to the manufacturer's instructions. This involved rubbing the solution well into the skin and in an attempt to prevent a possible contact infection rubber gloves were always worn for this operation. However, an infection was initiated on the left forearm as previously described. This was above the level of the gloves and presumably was derived from material shed during the rubbing process.

Summary

A case of human ringworm caused by *Microsporum nanum* is described which was apparently contracted from infected pigs. As this animal reservoir is common in New Zealand the morphological characters of the fungus are given so that it may be recognised should it be isolated in the diagnostic laboratory.

Acknowledgement

The authors wish to thank Essex Laboratories Pty Ltd for providing the Tinaderm.

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Regional Seminar Report

HAMILTON SEMINAR — JULY 1969

The recently-formed Hamilton Branch of the N.Z.I.M.L.T. held a very successful one day seminar at the Waikato Hospital on July 12 last.

The event was attended by about 120 people, many of them having come from distant laboratories, and the programme consisted of seven half-hour papers and a working trades display. Morning and afternoon teas and lunch were provided by the Branch, with many people taking advantage of a light meal supplied for a small charge to cover costs.

The following papers were presented:

Computers Dr M. R. Fitchett
Radioisotopes in Gastroenterology Mr A. L. Lomas
U.N. Laboratory Work in Turkey Mr T. E. Miller
Mechanism of Action of Antibiotics Dr B. Mandal
Hereditary Spherocytosis Mr D. Haines
Isolation of Mycoplasma Mr J. Holland
Some Aspects of Coagulation Mr C. S. Shepherd

A cabaret evening, held at Fairbank, concluded an interesting and successful day.

J.R.H.

A Simple Method of Hormonal Iodine Assay

I. C. T. LYON, M.Sc., M.A.A.C.B., F.N.Z.I.M.L.T.
C/o Drs Alexander and McCafferty, Lower Hutt*.

Received for Publication, February, 1969.

Introduction:

The evaluation of thyroid status by laboratory investigations has for some time depended on the serum protein bound iodine estimation (PBI) which, though presenting some technical difficulties, has become widely used. Contamination of the specimen both *in vivo* and *in vitro* is well known. and the organic iodine-containing drugs have been a constant source of trouble. Lately the T_3 test[4] and the simpler TBI (thyrobinding index)[7], which are a measure of the unsaturated thyroid binding globulin, have been used by laboratories possessing radio-isotope counting equipment; and these tests have provided a very useful adjunct, drawing attention to the effects of oestrogens, large doses of salicylates, diphenyl hydantoin and the nephrotic syndrome on thyroid function tests. Many authors have endeavoured to improve the PBI estimation to provide greater precision and accuracy, the aim being to determine only the serum thyroxine level. The butanol extractable iodine (BEI) has evolved from the work of Blau[3] to provide for the removal of iodoproteins which are included in the PBI and the iodotyrosines and inorganic iodine, by washing the butanol extract. However, all exogenous organic compounds remain in the final reaction mixture.

An isotope dilution technique in the form "competitive protein binding analysis" has been introduced by Murphy & Jachan[5] and produced commercially[8] but this author has found the method both expensive and lacking in precision.

This paper deals with modifications to the method of Backer, Postmes and Wiener[1] which originally attracted attention because it offered a "simple and specific method for the determination of iodoamino acids (IAA) and hormonal iodine (HI) in serum." In a later paper, Wiener & Backer[9] suggested the HI level was the most useful, and the presence of many chemically different iodine-containing compounds failed to give falsely elevated HI values. Two groups (Masen[3] and Pileggi and Kessler[6]) have introduced non-incineration techniques for determining the iodine-containing compounds present after their respective isolation techniques, and this type of reaction has been applied by the present author to HI isolated by exchange resin.

***Present address:— c/o Human Genetics Research Unit of the M.R.C., Medical School, Dunedin.**

The test consists of addition of 0.5ml. of serum to a cation column; removal of protein, inorganic and organic iodine. elution of T_3 and T_4 by ammonia; evaporation of the ammonia; release of the iodine; and subsequent estimation of the same.

Equipment:

Chromatography columns consisting of "milk-testing" pipettes 30cm. long and 7mm. internal diameter.

Aluminium block electrically heated and simmerstat controlled, with 40 holes to accommodate 15 x 125 mm. heavy wall Pyrex test tubes (Boiling water bath could be used).

Vortex test tube mixer.

37°C water bath.

Spectrophotometer capable of working at 390 to 420 nM.

Reagents:

Prepared using analytical grade chemicals, deionised water and acid-washed glassware.

1. *Resin:* Bio. Rad* analytical grade cation exchange resin AG (R) 50 W - X2 200-400 mesh H^+ form.
2. *Borate Buffer:* 450 ml. 0.4M boric acid plus 550 ml. 0.1M sodium tetraborate.
3. *Hydrochloric acid* 1N.
4. *Ammonia* 5 N.
5. *Antibumping granules* (BDH)** acid washed.
6. *Sulphuric acid* 3.5N.
7. *Bromine water,* saturated. Ensure a separate phase is present.
8. *Acid bromine solution:* (Prepare fresh) 20 ml. glacial acetic acid, 20 ml. 3.5N sulphuric acid and 2 ml. bromine water.
9. *Arsenious acid reagent:* -Dissolve with heat 1.44g. As_2O_3 in 15 ml. of 0.5N NaOH and add to approximately 850 ml. of deionised water in a 1 litre volumetric flask. Concentrated HCl (28.0ml.) and concentrated H_2SO_4 (45.5 ml.) are added slowly with mixing. After cooling make up to volume with deionised water.
10. *Ceric reagent:*— Slowly add 24.3 ml. concentrated H_2SO_4 to 350 ml. deionised water in a 500 ml. volumetric flask. Ceric ammonium sulphate 10g. is dissolved in the mixture and made up to 500 ml. with deionised water.
11. *Standards.* It is convenient to use a serum of known HI content because thyroxine solutions are relatively unstable. The sera used by the author contains 3.9 and 7.2 μgm/100 ml. of HI respectively.

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* *Bio-Rad Labs,* 32nd and Griffin, Richmond, Calif., U.S.A.

** *British Drug Houses Limited,* Laboratory Chemicals Division, Poole, England.

Method:

Preparation of columns: After Backer *et al.*[1] A small plug of glass wool is placed in the column. A suspension of the resin in water is poured into the column to a depth (when settled) of about 2.5 cm. After washing successively with 5 ml. of 1 N HCl and 5ml. water, the column is ready for use. Regeneration requires washing with 2 ml. water, 5 ml of 1N HCl and 5 ml. water. The same columns have been used some 43 times without apparent loss of usefulness.

Column chromatography: On separate columns, place 0.5 ml. water and 0.25, 0.5 and 0.75 ml. of the respective standards and 0.5 ml. of each unknown serum. Wash each column successively with 2 ml. water, 8 ml. borate buffer, 2 ml. water, 4ml. of 1N HCl and 4 ml. water.

Elution of HI: Add 2 ml. of 5N ammonia and collect each eluate in a 16 x 125 mm. Pyrex test tube containing an anti-bumping granule.

Removal of ammonia: Tubes placed in the aluminium block preheated to 90°C, are allowed to dry at temperatures rising to 125°C. Time required is up to 45 minutes.

Iodine determination: After cooling tubes, 1 ml. of freshly prepared acid bromine solution is added to release iodine from tri-iodothyronine (T_3) and thyroxine (T_4). Mixing on a vortex mixer is followed by placing in a 37°C bath for 6 minutes. The tubes should all possess a yellow bromine colour. Addition of 4 ml. of arsenious acid reagent will discharge the colour and 5 minutes at 37°C are required to equilibrate the temperature of reaction. In sequence at 30 second intervals, 1.0 ml. of ceric reagent is added to each tube which is mixed and incubated for 20 minutes. In the same sequence each tube is read in a spectrophotometer at 395 nM (390-420 nM) using water as reference.

Calculation: A standard curve is plotted of optical density readings of the standards against the μg/100ml. HI, and the results read from the graph.

Development of the Method: Modifications used are basically an increase in washings to include the hydrochloric acid wash indicated by Wiener and Backer[6], as this always removes any residual salts from the buffer wash.

Attempts to acidify the ammonia eluate before the bromination were unsuccessful, as the presence of ammonium ions inhibited the ceric-arsenate reaction. After removal of the ammonia by evaporation, the addition of only 50% acetic acid and saturated bromine water gave results lacking in precision; however, the addition of sulphuric acid solved this problem. The mixing of acetic acid, sulphuric acid and bromine water prior to addition to the

reaction tubes reduces the possible error due to pipette manipulations.

The arsenious reagent is modified to retain the final sulphuric acid concentration of the reaction mixture at the optimum.

Results: In Table I are the results of forty normal PBI, HI[4] and modified HI estimations, with the respective mean values. In each case the TBI was also normal. The correlation co-efficient for 100 tests was 0.89. It is apparent there is no significant difference between the two methods and in fact the mean closely approximates the mean of the normal range of hormonal iodine[1] (4.30 μg/100ml.).

TABLE I

TEST No.	PBI μg/100ml	HI[6] μg/100ml	HI (Author) μg/100ml
1	5.5	4.0	4.6
2	6.3	4.2	4.9
3	5.3	4.3	4.3
4	5.3	4.3	4.4
5	7.6	3.9	5.1
6	4.4	3.1	3.9
7	4.1	3.1	3.0
8	5.9	3.8	4.9
9	6.9	5.7	5.2
10	5.9	4.3	4.7
11	6.5	3.7	3.4
12	6.0	4.3	4.2
13	4.6	4.5	3.9
14	5.2	3.5	4.2
15	6.4	5.7	4.4
16	7.1	4.8	5.1
17	5.6	2.8	3.0
18	5.6	4.6	4.8
19	6.4	4.3	4.8
20	5.4	3.7	3.6
21	6.4	4.4	4.8
22	7.1	4.9	5.7
23	5.0	4.0	4.5
24	5.6	5.0	5.4
25	6.0	5.1	5.4
26	5.4	4.0	3.7
27	4.9	3.7	4.1
28	5.8	4 6	5.4
29	5.8	4.1	4.0
30	5.5	3 3	3.5
31	4.9	3.3	3.3
32	4.8	3 0	3.9
33	4.2	3.7	3.1
34	5.3	3.4	3.4
35	7.4	4 8	3.2
36	4.3	2.9	2.8
37	6 7	3.4	3 6
38	6.8	4.6	4.0
39	3.9	3.0	3.2
40	7.2	5 0	5.5
Mean	5 72	4.07	4.22

The normal range for the present technique obtained using ninety results and probability paper shows the 90% limits to be 2.8 - 5.9 μgm/100ml.

Table II illustrates some interesting results of patient-tests obtained during this preliminary work. In each case the TBI estimation was also carried out and the results noted. The PBI of patient N.H. of 28/11 was suspect since other tests and the clinical state suggested hyperthyroidism. Repeat PBI on 11/12 substantiated this belief.

TABLE II

PATIENT	PBI μg/100ml (Normal= 3.5-8.0)	TBI (Normal= 0.9-1.2)	HI[6] μg/100ml (Normal= 3-6)	HI (Author) μg/100ml (Normal= 2.8-5.9)
N.H. (28/11)	7.5	0.80	—	10.4
N.H. (11/12)	14.2	0.81	9.5	11.8
T.M.	T.H.	1.04	T.H.	5.4
D.S.	8.8	1.07	6.5	6.0
I.W.	7.1	0.80	4.9	5.7
M.G.	9.6	0.99	6.4	6.2
N.S.H.	13.6	0.70	9.0	10.8
J.L.	0.8	1.06	0.0	0.0
J.R.	1.2	1.05	0.0	0.3
M.R.	1.2	1.17	0.5	0.9
M.C.	1.2	1.21	0.0	0.0
C.B.	4.3	0.85	2.9	2.8

In the case of patient T.M. the PBI and digested HI appeared contaminated and it seems the increased washes in the author's method removed this.

Patient D.S. was on thyroid gr. 5 and in this case the TBI is probably a better index of adequacy of therapy.

In some cases the TBI was apparently affected by abnormal protein patterns *i.e.* patients I.W., C.B. and M.G., the last having a marked increase in alpha globulin. However, protein electrophoresis failed to explain the normal TBI in the clinically hypothyroid patients J.L. and J.R.

Patients N.H. and N.S.H. were clinically classical cases of hyperthyroidism.

Discussion:

The most satisfactory method of standardisation was to use serum of known value, for aqueous solutions of thyroxine are very unstable and organic solvents cannot be applied to the column. Monitrol I and II*, which have quoted figures for T_4[6] were found to be most suitable as analysis by the digestion method provided similar answers. The Monitrol I had a value to be most suitable for analysis by the digestion method and provided near identical answers. The Monitrol I had a value of 3.9μg/100ml and Monitrol II 7.2μg/100ml, consequently the use of 0.25, 0.5, 0.75ml. of each of these gave a range from 1.95

* Dade Reagents Inc., Miami, Florida, U.S.A.

to 10.8, with a double check in the mid normal range.

Using a Shimadzu QV.50** with deflection meter accessory set and modified to take a tubular cell sequential sampler, the wave length of 395mμ provided an OD range for the standards of 1.45 to 0.25, which is read with considerable precision using the sensitivity selecting switch set x1, x$\sqrt{10}$, or x10 as required. No real evidence is presented here of the usefulness of this test in the presence of contaminants. One could expect that as the technique of isolation of the HI is virtually that of Backer *et al*[1] and Wiener and Backer[9], and since the latter authors' paper provides evidence of the lack of interference by many organic iodine compounds, little trouble should arise in this connection.

The test correlates well with the digestion method and the PBI, and provides an advantage in that exclusion of incineration or digestion removes the possibility of loss due to vaporisation or cross-contamination in the furnace.

The method is rapid and provides for batches of 33 patient tests with 6 standards and a blank in 3 hours, provided adequate means of evaporation of the ammonium solvent is available.

Summary:

A direct determination of hormonal iodine isolated from serum by cationic exchange resin provides a simple, rapid test of thyroid function. Hormonal iodine is separated by allowing 0.5ml. of serum to run through a column from which, after suitable washing to remove other iodine containing compounds, the hormone is eluted. Removal of solvent is followed by release of the iodine from tri-iodothyronine and thyroxine by bromine. The "free" iodine is then estimated by the ceric-arsenious acid reaction.

Acknowledgement:

The author wishes to thank Dr W. S. Alexander for his continued encouragement to develop improved techniques, and Mesdames Phyllis Hampson and Jan Birt for the TBI and PBI estimations.

** Shimadzu Seikakusho Limited, Kyoto, Japan.

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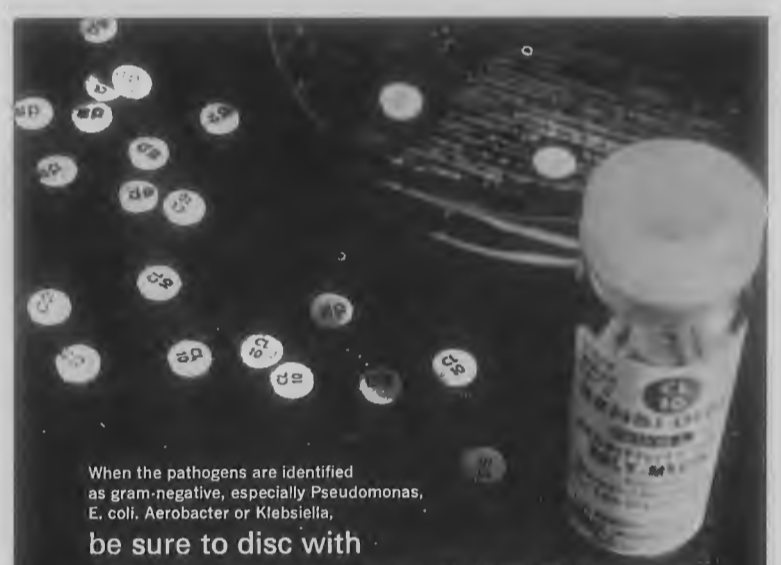

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Patterns of cultures from an audit of 108 Hospitals[1]

Organism	Number of Cultures*	% of Organisms Sensitive to Colistin†
Escherichia coli	2301	90.0
Pseudomonas aeruginosa	772	80.7
Aerobacter aerogenes	721	82.5
Coliform bacteria	286	91.6
Klebsiella-Aerobacter	263	86.3
Paracolobactrum (all)	250	74.4
Escherichia (all others)	124	86.3
Gram-negative rods	103	64.1

* Number of positive cultures tested for colistin sensitivity. † Sensitivity of colistin reported on 100 cultures and over. [1] Audit of Pathology Cultures, Dedham, Mass., R.A. Gosselin and Company, Inc., 1965.

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The T. H. Pullar Memorial Address

Some Perspectives in Medical Technology

STEPHEN E. WILLIAMS, M.B., Ch.B., D.C.P., M.C.P.A., M.C. Path.
School of Medical Laboratory Technology
Auckland Hospital Board

Delivered at the 25th Annual Conference of the New Zealand Institute Medical Laboratory Technology, August, 1969.

I am deeply conscious of the honour you have done me to-day in asking me to give this address to the Annual Conference of our Institute. To this feeling I must add the pleasure and very genuine pride which it gives me, by virtue of my Honorary Membership, to speak to you as a fellow member.

Each year we assemble on this occasion to pay tribute to Dr Thomas H. Pullar, whose memory is still alive in the minds of the senior members present, and whose contribution to both pathology and medical technology in this country have heartened and enriched us all. Last year Dr P. P. Lynch, when giving this address, recorded the important biographical details of Dr Pullar's career and I shall not repeat these to-day, except to remind you that he arrived in New Zealand, a young Scottish graduate, in 1938 and died at the relatively young age of fifty-nine in 1966. In the intervening years, while based at Palmerston North and latterly at Tauranga, he influenced all pathologists, all technologists, many medical students and the officers of our Health Department, in profound and lasting ways.

Tom Pullar was my personal friend. Although we only saw one another at infrequent intervals, usually at conferences and committee meetings, I took many problems to him and received in return the most helpful advice, encouragement and wisdom. On the last occasion on which he came to my home we watched a television programme known to many of you which relates to the Scottish village of Tannochbrae. He mentioned that his youth was spent in just such a setting and that his father, a country medical practitioner, could have doubled for the part of Dr Cameron. In his own way, and in a very different setting, Tom Pullar brought to us this same attitude of professional good sense, warmth, humanity and high principle.

Of the many active contributions which he made, perhaps the most far-reaching and significant was his early recognition of the importance of the role of medical laboratory technology and his subsequent energetic participation in the upgrading of technological education and status in New Zealand. It therefore seemed to me appropriate today to take a brief look at some of the present pressures we are all experiencing in our laboratories,

and to speculate on what the future holds for our profession; to examine, in fact, what our scientific members would refer to as "parameters and extrapolations."

In such an exercise it is useful to adopt the technique of historians and to look back over a few years. For those of you who may question the validity of this view I can recommend a study of Thucydides's account of the Peloponnesian War in the year 431 B.C. In a technology of swords and spears you will find here all the problems of our communities today, brilliantly illuminated: the hopes, frustrations, passions, satisfactions and disappointments, the treachery, nobility and the violence of human endeavour. Had Thucydides seen fit to record it, I have no doubt that he could have given us a most vivid description in terms we should all at once recognise, of the reactions of that great medical technologist, Hippocrates, on receiving his statement "original grading determined" from the Athenian Department of Health. My sober point is that in 2,400 years human behaviour has changed very little. But our technology, for better or worse, continues to alter at an ever increasing pace.

Laboratories of the Future

To return, then, to the year 1949. In this year Green Lane Hospital had virtually the same number of beds as it has to-day, and accurate records of its laboratory work are available. We can scan the range and volume of this work to note that in an average month some 1,500 tests were performed by a staff of three. In 1969, 25,000 tests are carried out on the same number of patients by a staff of 70. Analysis of the type of tests done is interesting, for it shows that apart from a diminution in one or two categories, such as red cell counts and the disappearance of a few rather infrequently performed tests, including the Weltman test and that majestically named but very simple measurement of adrenal function known as the Robinson-Keppler-Power test, all the tests done in 1949 are still being requested for patients, but at a greatly increased frequency. More significantly, it is to be noted that many of the tests which make up a large proportion of our work today were not practicable, and were barely conceivable, in 1949. Such categories, for example, as electrolyte estimations, many of the enzyme assays, the steroid tests, electrophoresis and cytology.

A simple projection forward to the year 1990, then, shows us that in this one hospital perhaps 300,000 tests will be done each month by a laboratory staff of 500, and that as well as doing all the tests which we carry out today, much of the work upon which they will be engaged has yet to be developed. Lest any of you should wish to dismiss this view as fantasy, may I record that had the true facts of medical laboratory work today been presented to us in 1919 we should have rejected them out of hand as science fiction of an extravagant type. Contemplate, therefore, your

forward march to the tissue typing service in its six-storeyed Institute, the Department of Electron Microscopy (Routine Service Division), the routine chromosome profile on all admissions, the psychiatric stabilisation absorption test, the laserscope and, of course, the computer. Many of you here will no doubt be able to elaborate the theme in more detailed and descriptive terms of what we know as the "hardware." Such concepts are by no means fanciful and, despite the limits of our present capacity for speculation, advances of this general type are inevitable.

My own interest at this point in time, however, is directed particularly and urgently to those precious components of laboratory practice whom we corporately term "the software." I mean, of course, the human beings who are going to staff these great Institutes. By returning to our two points on the graph, twenty years apart, we can make some deductions in this connection. In the first place, we observe that we are starting to run out of pathologists. I know that some of you may view this development with relative unconcern, feeling with justification, perhaps, that anything in clinical pathology that a pathologist can do a technologist can do better. In all laboratories, however, there is the important interface between the purely medical and the purely scientific aspects of a patient's illness and it is only by a properly regulated and continuous flow across this interface that the laboratory can perform its true functions of medical diagnostic support. The pathologist must place himself in this area and, by virtue of his medical qualification and experience, he must ensure that the vital communications are maintained. It seems probable that in the next twenty years we cannot possibly generate enough pathologists to stand at the proliferated interfaces which we are now establishing, and there is a very real danger that laboratories and laboratory departments could suffer from an increasing degree of professional and spiritual isolation.

Secondly, we note that medical technology, already well founded as a profession twenty years ago, is growing robustly and, like so many other facets of our world today, growing exponentially. The lag phase is over and we may expect to double our size every five or ten years. Without question the medical technologist has emerged at a categorical entity for all the world to note, a well respected paramedical scientist and colleague, the essential structural unit of our laboratory services. Unlike the pathologists, we are not yet experiencing serious shortages of technologists, nor do I feel that their sources of generation are likely to fail, at least in the near future. Perhaps the most significant change in this sector of the staff has been the clear emergence of two further groups of technical workers who have appeared much more recently, in this country only in the last ten years, namely the

hospital scientific officers and the technical assistants. Both these groups are now acting as buttresses for the central structure of medical technologists in such a way that, in the larger laboratories at least, we could not now contemplate operating our departments without them.

From time to time some associates have raised the question of the medical technologist having any place at all in the laboratories of the future, envisaging them being compressed out of existence by the newer categories of scientific officers and technical assistants. Such a view is, of course, quite unrealistic. Laboratories can only operate successfully if we continue to recruit boys and girls of high calibre who from the outset of their training will be exposed to the full range of service pressure of general laboratory practice, whose basis of training will almost certainly become a technical college or university diploma and who will remain an elite of leaders and managers of laboratory groups. This concept must be accepted as fundamental to all future planning of medical laboratory organisation.

Thirdly, we have the phenomenon of a proliferation of hospital laboratories and scientific services other than those which we now incorporate in the routine diagnostic laboratory services, employing in many instances technical staff closely akin to but not actual members of our own group. In this category I would include the medical, surgical and professional units, the nuclear physics, physiology, cardiothoracic, haemodialysis, cancer and other research laboratories.

By applying our method of extrapolation to 1990 we must recognise that in Green Lane Hospital the scientific staff could number around 1,000, working in 40 specialised fields and possibly belonging to 20 different professional organisations. While the medical technologists will remain the core of this group, hospital scientific officers will have increased in number and authority, so that they and the senior technologists will have taken over many of the functions and contributions at present expected from pathologists and other medically qualified scientists.

The Zuckerman Report

I should now like to refer to some of the findings of the Committee set up two years ago by the British Ministry of Health under the chairmanship of Sir Solly Zuckerman to report on Hospital Scientific and Technical Services. For those of you who may not have seen the report of this Committee, I may mention that its terms of reference were "to consider the future organisation and development of hospital scientific and technical services in the National Health Hospitals and the broad pattern of staffing required, and to make recommendations." After examining evidence presented by one hundred and fifty-seven individuals and organisations, the Committee has now made a number of general

recommendations. In a consideration of the present position in the United Kingdom the Report has this to say:—

"In recent years there has been an unco-ordinated growth in the size and nature of scientific services within hospitals. This growth primarily reflects a demand which emanates in the first instance from clinical staff, although some of it has been generated by the scientific staff themselves. Some of the growth has been due to fashion . . . . . .

"The scientific service in hospitals tends to be fragmented; the staff who are classified in a large number of rigid grades (and this brings with it lack of flexibility), sometimes work in isolation. The various disciplines have not been pulled together, and the service, because of its lack of planned organisation, fails to provide good career prospects for all its staff. Furthermore graduate non-medical scientists who work in hospitals do not in general enjoy the opportunities for remuneration comparable to those of their clinical colleagues.

"All this recent growth has been taking place without any clear recognition of the fact that scientists and technicians are in short supply. Unless the growth of the hospital scientific service becomes better organised than it is now, many hospitals will continue to be dissatisfied with the services that can be provided.

"There are considerable variations in the training arrangements from class to class. The basic training of technicians ranges from three years full-time training before entering employment or five years part-time training whilst in employment, to little or no formal training at all. In some specialities the medical profession has played an active part in the promotion of training, while in others it has been left to the initiative of the technicians themselves, through the formation of staff associations which devise courses of training and award their own qualifications . . . The general experience is that the establishment of a suitable training scheme follows long after the technical function for which it is required has developed. Further training and advanced qualifications are available in only a few of the classes, and there are no general arrangements by the hospital service for re-training or re-orientation to meet changing needs."

The Report, after considering all the classes of hospital scientific and technical workers, recommends that 21 of these classes should be associated together to form the basis of a Hospital Scientific Service, which while remaining an integral part of the whole hospital service would be subject to special administrative and organisational arrangements. These arrangements include the establishment of a National Hospital Scientific Council to advise the Ministry of Health, Regional Scientific Advisory Committees to work with each Regional Hospital Board, and a Regional Scientist, with senior administrative status, to be a member of each Board. Amongst many other duties this individual will have administrative responsibilities for the training (including post-graduate training) of scientific and technical staff at all levels.

The future staffing pattern for this Service is visualised as consisting of four categories, very much as we operate the hospital laboratory services in this country today. These are to be Scientific Officers recruited from university graduates, Technical Officers who would include all our qualified technological staff,

Technical Assistants who would include all our trainee staff and Technical Aides, a group somewhat below our own technical assistants, and corresponding to earlier concepts of laboratory assistants. There is nothing very radical in this proposal, but I am interested to note a provision that in addition to entrants to any of these classes being able to make their career within that class, there should also be ample opportunity for advancement to the next higher class, either by obtaining the requisite qualifications or by demonstrating the necessary qualities to the satisfaction of an assessment panel. I interpret this to imply that a senior technologist, for example, could, simply by the determination of a grading committee, be advanced to the status of a senior scientific officer, provided that his capabilities justified this action. The inclusion of this provision seems to me desirable in relation to the very heavy scientific and managerial responsibilities which even today are required of many senior technologists in this country. Certainly it is an objective at which this Institute should aim.

Predictably, the Zuckerman report has received a mixed reception from the medical profession, including a guarded and, in parts, hostile leading article in the *British Medical Journal.* However, subsequent correspondence has indicated that many, if not all, pathologists would give it enthusiastic support. The I.M.L.T. has also been guarded but admits that some process of unification within the ranks of hospital scientific workers is required. I believe that the Report makes a most significant contribution in focusing attention at this time on a confused situation in which we are all more or less deeply involved. Whether its recommendations find acceptance in this country or not, it raises many urgent issues which this Institute cannot ignore. In the period of expansion which we can now clearly see as our destiny an intelligent and realistic administrative basis is quite essential.

Education

However, the linchpin of a Scientific Service is, in my view, the organisation of its educational programme. If this is well constructed, intelligent, appropriate, imaginative and above all flexible, the Service will be secured. If it is badly oriented, rigid or in some respects non-existent, then no matter what efforts are made with rates of pay or conditions of work the wheels of the service will eventually come off. In this country we are fortunate in having a particular agency established by the Department of Health for this purpose, the Medical Laboratory Technologists Board. With this Board, composed jointly of pathologists and technologists, lies the responsibility of translating with speed and insight the changing needs of medical

science into the proper courses of training, examinations and qualifications. In its ten years of existence the Board, despite some acutely evident growing pains, has made notable advances, and is now operating from a position of some competence and confidence. If I have seemed in the past to be impatient with the Board this may be because in Auckland we have encountered quantitative and qualitative problems of service and training which are only now becoming apparent in some other areas. We have adjusted these difficulties in some measure by the formation of our own School and the establishment of special qualifications. More recently the Institute has entered this field with the formation of its Technical Assistants Examination Committee and its qualifications for this group. While this move has been made as the result of strong pressure on a national scale, and is being attended by a fair measure of success, there is a growing awareness that within our professional family it may be sensible to have only one academic agency, which could be either this Institute or the Medical Laboratory Technologists Board.

As you are aware we are now moving, rather late in the day compared with most other countries, to pass some and eventually all of our academic technical training over to the Technical Institutes. This will relieve the Technologists Board of some responsibilities and allow it to develop others. Its successful ventures into the field of "workshop" courses should encourage it to expand this concept not only in relation to educational techniques but into the whole range of post-graduate training. Regular refresher courses for regional and central groups, seminars on specialised topics, workshops for new techniques, the circulation of test slides and specimens, tours by specialist lecturers. brought if necessary from other countries, demonstrations of equipment and the distribution of technical literature should all be accepted by the Board as proper functions of its high office. The continuing education of scientific workers is now regarded as quite essential to the maintenance of proper standards of work, and indeed some countries are proposing a system of formal re-qualification after 20 years in the practice of certain special science fields. The Medical Laboratory Technologists Board is doubtless aware of the problem in this phase of rapid technological expansion and change. It is to be hoped that it will justify its unique position by establishing a sound system of quaternary education for our profession.

With these references to education I turn again to the part played by Dr Pullar, and his contribution to our growth and maturity. The real starting point of the Medical Laboratory Technologists Board was a meeting one Saturday afternoon in

1959 when Mr Whillans and I had travelled to Palmerston North to talk with Dr Pullar and Mr Hutchings. From this meeting came the Joint-Committee of Pathologists and Technologists, which led some years later to the formation of the Board. Dr Pullar's influence in these moves was paramount, as he sought to balance the enthusiasms, apathies and apprehensions of the pathologists, technologists and administrators. With slender resources and often with the most meagre encouragement he purposefully set about the crucial task of fashioning a linchpin for our service.

His conviction that the medical technologist must become a professional colleague of high scientific standing, and his continued support for this principle have placed us deeply in his debt. Unlike the architect or the engineer, Tom Pullar leaves no physical edifice to mark his contribution. His building materials were the hearts and minds of his fellow men, and the dimensions and design of his construction are to be gauged by the solidarity of our own ranks. Your presence here today in such numbers, strength and spirit, is, to me, the best evidence of the success with which he built. In all his endeavours he would surely have echoed the cry of that great Athenian Nicias:

"It is men, not walls, that make the city."

25th Annual Conference, 1969

(Right) Delegates to the 25th (Silver Jubilee) Conference at Auckland, August, 1969.

Photo by: **Barry McKay Industrial Photography Ltd.**, P.O Box 5162, Auckland, from whom prints may be obtained at 85 cents each. (Quote: 69921—B).

A Changing Scene in Medical Laboratory Technology

J. R. SAAL,
Queensland Institute of Technology, Brisbane.

A paper read to the 25th Annual Conference of the N.Z.I.M.L.T., August, 1969.

The Industrial Revolution created the need for tradesmen to use, maintain or manufacture the equipment necessary for industry. From this need there emerged a pattern of education now called technical education, whereby tradesmen were trained on the job in conjunction with studies at night in technical colleges or similar institutions. Along with this development there came a gradual raising of entrance and course standards in order for the tradesmen to cope with the increasing complexity of their trades. This trend is continuing still. In these times the entrance standards have risen to the tenth year of schooling, and the courses are conducted not only by night but also by release from industry during the day.

With the impetus of two world wars and space exploration today, we are living in a technological revolution. Modern industrial needs of skilled manpower include, in addition to scientists and tradesmen, technologists and technicians. Unfortunately, just as in the previous century, educational programmes have followed rather than kept pace with industrial needs. The reason lies in the types of educational establishments available.

Outside the usual school system, the two traditional areas of training have been the universities and the technical colleges. Both have been found to be inadequate for the training of technologists.

The universities, to discharge their function, orientate courses to training students to seek knowledge for knowledge's sake. It should not be inferred that all courses in universities are of this type. Exceptions in most universities include engineering and medicine.

Technical colleges by virtue of staffing, facilities, and for other reasons, have not and cannot reach standards of technological excellence, although there have been exceptional technical colleges which have achieved high standards in technological courses.

On the European and North American continents new types of educational establishments have been developed over the past fifty years. They are variously described as Colleges of Advanced Education, Polytechnics, and Institutes of Technology. Development of such institutions has been rapid in a few countries, slower in some countries, and non-existent in other countries. Sir Ian

Wark, Chairman of the Commonwealth of Australia Advisory Committee on Advanced Education, in a recent address, stated that, on the one hand, New Zealand had six universities and insufficiently developed technical institutes, while on the other hand Russia had ten times the number of institutes to universities, and ninety per cent. of the tertiary students were enrolled in these institutes.

In Australia, the development of a second tertiary area of education followed the publication of the Martin report in 1964, and has continued under the leadership of Sir Ian Wark's Committee. This has resulted in the establishment of systems of Colleges of Advanced Education in each State. Certain of these colleges offer courses for both technologist and technician, others for the technologist solely. A range of awards are given from Certificate courses, commencing from the tenth year of schooling, to Associate Diploma courses with full professional acceptance equivalent to university degrees, commencing at the end of the twelfth year of schooling. The Colleges are awaiting implementation of two reports recently submitted to the Hon. Mr Malcolm Fraser, Commonwealth Minister of Education and Science: The Wiltshire report, dealing with the types of awards issued by Colleges, including degrees; and the Sweeney report, dealing with a salary structure for colleges uniform throughout Australia and sufficiently competitive with university salaries to attract staff of high calibre.

In 1966, in this atmosphere of change, the profession of medical laboratory technology in Queensland entrusted educational programmes to the Queensland Institute of Technology, Brisbane. Prior to this, the course had been conducted on a formal basis, by the first two years of basic science being in the technological section of the Central Technical College, and the latter three years being conducted in university departments and major hospitals. The old course was controlled by a Council consisting of representatives of the AIMLT, the college of Pathologists of Australia, and the Department of Education. This council designed the new course and continues to advise the Queensland Institute of Technology in this field.

In designing a suitable course of the requisite standard a number of factors had to be determined. These included:

1. The standard of the course: technician or technologist.
2. Whether the course would be aimed at producing a graduate as a specialist or a general practitioner.
3. Whether the course would be offered as a full time course or a part time course.

As the previous course had been of a professional standard and successful—the course introduced was also one at professional level, an Associate Diploma. Because Queensland is a sparsely

populated large area, with small hospitals distributed widely, the course was designed to include the major areas of medical laboratory technology. Specialisation was to be the subject of post-graduate training. The course was designed to be completed either in three years full time training or in six years part time training. Cognisance was taken of the grave disadvantage of part time training, in that the intake of such courses is based on today's needs while their output should be geared to the needs of six years hence. Thus, part-time courses never satisfy the needs of industry. Consequently a full time course was also offered.

In order to reach professional standards, to train students adequately in today's technology and to provide a graduate who could cope with tomorrow's technology, the new course contained heavy components of basic and applied science.

The academic year is of 32-33 weeks, each week consisting of 32 hours tuition. The first year is devoted to the basic disciplines of chemistry, physics, biology, and mathematics.

The second year contains both basic science and applied science. To cope with the restrictions of three year full time courses as opposed to four year courses, the subjects of first and second year are orientated towards medical laboratory technology by, wherever possible, using illustrations of scientific principles from the field of medical laboratory technology instead of using classical academic examples. The standard of the first two years may be appreciated by stating that the levels reached are equivalent to the same or similar subjects within universities.

The third year deviates from what is usually seen in universities. The subjects taught form the major areas of medical laboratory technology. Although emphasis is placed on routine practices, here again the lectures are directed towards underlying principles rather than a cookery book approach to tests used in routine work. The practical work of all years, but in particular of the third year, consists of the performance of the standard and advanced techniques of each subject.

To offset the lack of practical experience, the full time students are given additional practical training in routine testing in the second and third year, and vacational jobs in diagnostic laboratories are arranged at the end of first and second year.

The part-time students are given less practical work than full-time students, in recognition of their day-time experience. This experience is guaranteed by a regulation which demands that the student be employed in an approved capacity in a laboratory approved on the basis of its staff, workload, equipment and training programmes.

In briefly describing this course, I must also outline the organisation which carries out the programme. The course is one of two Diploma courses conducted in the Department of Para-

medical Studies in the School of Applied Science of the Brisbane Institute.

The students are taught in a building which contains large lecture theatres, special practical laboratories of biochemistry, haematology, microbiology, and stain technology, as well as general chemical laboratories.

Equipment ranges from an ultracentrifuge and auto-analyser to microtomes, pH meters and microscopes.

The staff consists of a blend of highly qualified university graduates and medical laboratory technologists. A large part-time staff of specialists is also employed from the University of Queensland, C.S.I.R.O., Research laboratories and diagnostic laboratories.

I have briefly described the course as it has been conducted for the past four years. In the light of this experience a number of changes, mainly to the full time course, will be implemented next year. No changes will be made in the academic content of the course, but formal practical work will be reduced. This reduction will allow in first and second year greater utilisation of library and assignment work, and will permit a longer period of time spent by the student on projects. The latter should develop in the students a greater independence of thought than was possible under the previous structure. No doubt in the near future further changes will be found necessary.

A laboratory service which is dependent entirely on the use of technologists and technologists in training is one which is not efficiently utilising labour. At the present time a certificate course is available for those who wish to enter the ranks of the technicians of the hospital diagnostic service. This certificate is a broadly based one, covering a range of basic subjects and basic biological techniques. It is not directed at the techniques of medicine, although many are covered. Thought is now being given to a course orientated more directly to the needs of medicine.

You, in New Zealand, are also changing your educational needs to meet the demands of the ever-widening requirements of our profession. May I sound a warning to your considerations. Do not look at your present system to seek where the course may be modified. If you do you may perpetuate old ills and concepts. Start afresh. Begin by defining objectives in terms of responsibilities of the graduates, taking into account that the graduates will have some forty years of everchanging responsibilities. Once the level of responsibilities has been set, the type of course may be determined. At the technologist level, provide a substantial core of basic and applied Science. Around this core, set the subjects which impart the necessary skills. Above all remember that industry has a role to play in the training of graduates. It cannot expect fully trained graduates from formal courses of any

kind. It must be prepared to give additional training in the form of supervised experience.

During your deliberations, think carefully on the advantages of full-time courses. I have already drawn attentien to the failure of part-time courses to provide sufficient graduates. Another and most important factor is that full-time training requires full-time lecturers. Tertiary education is a demanding vocation for those who elect to work in it. Not only have the lecturing staff to be highly qualified in their professional field, and have had a wealth of experience, but they must also be prepared to inform themselves, either formally or informally, on the theory and practices of education. The training of professional men and women is a task not to be taken by the amateur educator, even though he may be of high standing in his own profession.

Pictured during the celebrations at Smith-Biolab Ltd. are (from left): Mr D. R. Miller (C.S.L., Melbourne), Mr P. W. Maxwell (Manager, Scientific Division, Smith-Biolab Ltd.), Mr I. Cole (Principal Technologist, Green Lane Hospital), Mr J. R. Saal (the Institute's guest from Brisbane) Mrs D. G. Till, Mr D. G. Till (Chief Technologist, National Health Institute), Mr D. W. Hill (Managing Director, Smith-Biolab Ltd.) and Mr B. M. Prendergast (Director, Smith-Biolab Ltd.).

What's New

INDIVIDUAL DEIONISER FOR EACH HOSPITAL LABORATORY

A new wall-mounted deioniser has been introduced by *Elga Products Ltd.*, of Lane End, Bucks., so that purified water conforming to International Pharmacopoeia standards is available from the mains water supply, merely by turning the tap on. The laboratory can thus have a local source of purified water which does not depend on a central distillation or deionisation plant.

The deioniser, known as the Elgacan B 114, employs a disposable canister containing ion exchange resins. Water from a potable supply is passed through the mix of cation and anion resins, and emerges with a purity of 1-2 megohm cm. This is typically 5-10 times better than that obtained from distillation. The total of dissolved solids in the effluent is less than one part per million and a pH value of 6.6-7.0 is claimed. Flow rate is 0.5 litres per minute. The total capacity of the ion exchange canister is never less than 25 litres and often as high as 300 litres; this depends on the quality of the tap water.

When the purity meter shows that the ion exchange resins are exhausted, the canister is unscrewed and thrown away; a new canister is fitted within seconds.

Further information obtainable from: *Elga Products. Ltd.*, Lane End, Bucks., England.

PRECISION METAL FOILS

Goodfellow Metals Ltd. announce the availability of ultra-thin metal foils offering high levels of purity.

Part of the firm's organisation is now being devoted to meeting the demand for special foils needed in relatively small quantity and concerning the supply of which there is often difficulty because the item required is so special that it is not a production job.

Precision metal foils are available in a range of thickness from 0.001mm to 0.15mm, to a 99.9% purity standard and in sheets usually up to a maximum of 12″ x 12″.

Further details and samples may be obtained on application to: *Goodfellow Metals Ltd.*, Ruxley Towers, Claygate-Esher, Surrey, England.

CLINICAL ASSAY SETS

As an extension to the range of reagents for clinical analysis produced in kit form by *BDH Chemicals Ltd.*, the company has, during the past few months, introduced three new clinical assay sets:—

G-6-PD Set — for the determination of D-glucose-6-phosphate: NADP oxidoreductase; each set provides for 25 tests.

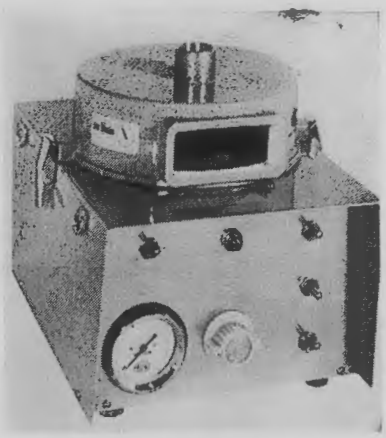
Amylase Set — for the determination of serum-1, 4-glucan-4-glucanohydrolase; each set provides for 48 tests.

5'-Nucleotidase Set — for the determination of 5'-nucleotidase by the method of Persijn and Van der Slik; the set comprises 5 units, each containing reagents for 6 single or 3 duplicate tests.

The three new clinical assay sets, in common with the others in the range, are supplied complete with full details of the test procedure.

Further details from *BDH Chemicals Ltd.*, Poole, England.

SAMPLER FOR AIRBORNE BACTERIA



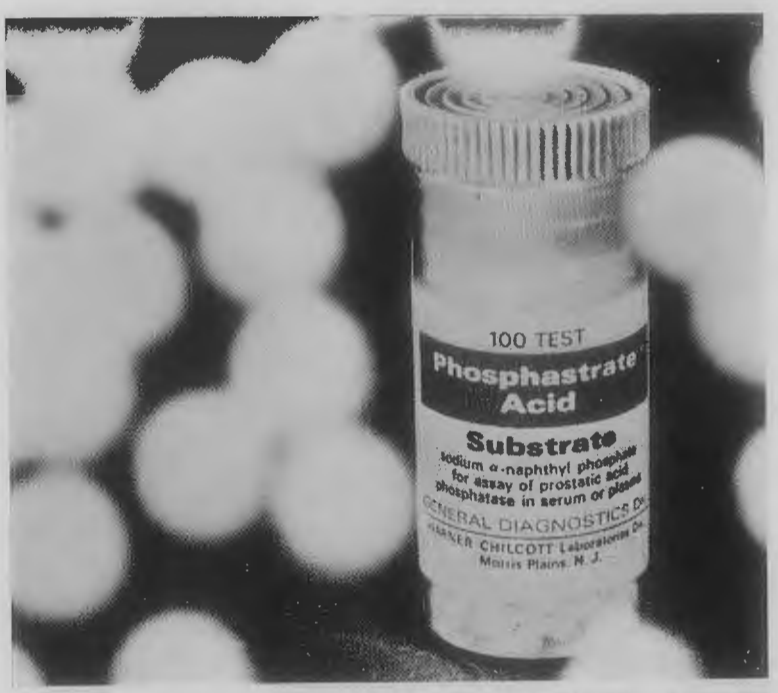
A new instrument for assessing the concentration of airborne bacteria is announced by *C. F. Casella and Co. Ltd.*, Britannia Walk, London N.1. It works, in principle, by passing a known volume of air at high speed through a slit on to a slowly rotating culture-plate containing agar jelly. Airborne bacteria are caught on the plate, which is then incubated for 24 hours at 37°C. This causes each bacterium to form a small colony visible to the naked eye. The colonies can then be counted with the aid of a ruled screen, different species being distinguished microscopically if necessary.

The instrument's main applications are to assist in the control of the airborne (especially respiratory) diseases by measuring bacterial concentration in confined spaces where people congregate, such as canteens, schoolrooms and underground trains; to study exposure to cross-infections in hospital wards and operating theatres; and to monitor the highly sterile conditions required for the manufacture of antibiotics and certain dairy and food products. In addition, if the culture-plate is allowed to rotate only once, the variation of bacteria concentration with time can be studied. The "killing rates" of different bactericides can be compared, for example. Another application is qualitative sampling of bacteria deposited on clothes, blankets and other fabrics.

The sampler has been developed from one originally designed at the *Medical Research Council*, Hampstead, London. It consists of a basic turntable and control unit which can be used with two interchangeable sampling heads. One head covers concentrations from 1 to 30,000 organisms per 100 litres, as found in most public rooms; the other covers relatively sterile conditions with concentrations from 0.033 to 5,000 organisms per 100 litres. Either head, or both, can be ordered, together with suitable pumps if required: the required aspiration rates are 30 litres/minute and 700 litres/minute respectively.

The instrument complies with the safety code of the British *Ministry of Health* for use in areas containing explosive anaesthetics. It has three turntable speeds (one revolution in ½, 2 or 5 minutes), and the turntable and pump can be set either to run until switched off or to stop automatically after one revolution. A marker device enables the timing of particular events to be noted on the plate.

Further information from the N.Z. agents: *Watson Victor Ltd.*, P.O. Box 1180, Wellington.



NEW AN IMPROVED REAGENT SYSTEM FOR THE QUANTITATIVE COLORIMETRIC ASSAY OF ACID PHOSPHATASE IN SERUM OR PLASMA.

Highly specific. The α-naphthyl phosphate substrate for this reagent system shows marked specificity for prostatic acid phosphatase, but is unaffected by red-cell phosphatase. Unlike other methods, Phosphatase Acid does not require L-tartrate inhibition.

Greater sensitivity. Adopting a new diazonium salt as colour developer, Phosphastrate Acid introduces a higher degree of sensitivity to the assay of serum acid phosphatase. The procedure does not depend on a final inorganic phosphate assay, thus eliminating errors due to phosphates already present in the serum.

Simple and rapid. Just incubate a mixture of substrate and serum for 30 minutes; add colour developer and 0.1N NaOH; read at 580-600mμ in a 12mm cuvette.

Colour stable for one hour. An important practical advance, facilitating the performance of a large number of tests.

Insnred accuracy and precision. For consistently - dependable results, the entire system should be standardised and controlled with Versatol-E and Versatol-E-N.

William R. WARNER and Co. Ltd.
AUCKLAND

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A Simple, Quick Method for the Estimation of Total Protein and Albumin in Serum

D. W. EVERARD, A.N.Z.I.M.L.T., F.I.M.L.T.
Department of Chemical Pathology,
Memorial Hospital, Hastings.

Received for Publication, May, 1969.

Introduction

The method outlined uses the " albumin error " of certain dyes to quantitate albumin, and the conventional biuret reaction for total protein.

The albumin method is that of Bartholomew and Delaney (1966)[1], which is not affected by abnormal concentrations of globulins, bilirubin, haemoglobin, salicylates or heparins. The reagents are cheap, easy to make up and keep well at 4°C.

In setting up this method the author found it necessary to alter the procedure for making up the buffered dye solution, as when made up according to formula the graph was linear only to a concentration of 2 g./100 ml. of albumin.

It was found possible to rectify this by the addition of more dye to the buffer solution until the range became linear up to a concentration of 5 g./100 ml. of albumin. As it was felt that a higher top limit would be useful and it was not considered desirable to increase the optical density of the blank, more buffered dye solution was employed in the test, thus increasing the ratio of dye to albumin without further increasing the optical density of the blank.

The effect of protein on certain dyes in solution has been known for a number of years and was originally called the " protein error," but as this change can only be attributed to albumin, the term " albumin error " has been substituted.

In effect, when albumin is added to buffered dye solutions it appears that a substantial change of pH has taken place, but in fact this is not the case, as only a very minor pH change has occurred.

Many methods using this albumin error have been tried, but most failed in conditions of abnormal albumin globulin ratios or were too expensive.

Bromocresol green was found to give linear results in relation to albumin concentration, and is unaffected by abnormal albumin globulin ratios, bilirubin in excess of 40 mg/100 ml., haemoglobin up to 750 mg/100 ml. and salicylates.

Materials and Methods

Reagents:

(1) *M. Sodium Citrate:* dissolve 147 g. of sodium citrate ($Na_3C_6H_5O_7$-$2H_2O$) in water and make up to 500 ml. Store in the refrigerator at 4°C.

(2) *M. Citric Acid:* dissolve 105 g. of citric acid in water and make up to 500 ml. Store in refrigerator at 4°C.

(3) 0.01M. bromocresol green: add 9.8 ml. of 0.1N NaOH to 0.968 g. of dye, dissolve as much as possible, then make up to 100 ml. with water. Store in a dark bottle at room temperature.

(4) Buffered indicator solution: to about 400 ml. of water add 8.6 ml. of M. sodium citrate, 16.3 ml. of M. citric acid, and 3-6 ml. of dye solution*. Check pH 3.8 and store in refrigerator at 4°C.

(5) *Biuret reagent.*

Solution A.	Potassium Sodium tartrate	— 45 g.
	Potassium iodide	— 5 g.
	Cupric Sulphate	— 15 g.
	Sodium hydroxide	— 8 g.
	Water to make	1000 ml.
Solution B.	Sodium hydroxide	— 80 g.
	Potassium iodide	— 50 g.
	Water to make	1000 ml.

For use take 100 ml. of Solution A. and 40 ml. of Solution B., then make up 500 ml. with water.

(6) 0.9% *sodium chloride* in water.

(7) *Standard protein solution:* dilute concentrated human albumin approximately 25 g% to give 7 g%. Check against commercial dried sera by the Biuret method.

Methods

Total Protein

To 1.9 ml. of 0.9% saline add 0.1 ml. of serum, then 8 ml. of Biuret reagent. At the same time put up a reagent blank of 2 ml. of saline and 8 ml. of Biuret reagent, and a standard treated as the test.

Place all tubes in a 37° C water bath for 10 minutes, then measure optical densities of tests and standard against reagent blank, at 540 mμ. Calculate results from standard or from a previously constructed calibration graph.

Albumin

Due to the variation in batches of dye it is necessary to standardise the buffered dye solution. Make up the buffered bromocresol green reagent and measure the optical density of the

* The amount of dye solution needed changes from batch to batch of dye.
See calibration of dye reagent.

solution against water at 635 mμ. Keep a note of the value (on the instrument employed, and using 6 ml. of dye solution per 500 ml. of buffer, this gave a reading of 0.230).

Prepare a series of albumin dilutions, ranging from 1 g% to 7 g% and treat them as for tests. Plot the optical densities against albumin concentration. This should produce a straight line graph. If the line curves at the higher concentrations more dye should be added to the indicator solution, (e.g. 0.5 to 1 ml. should be sufficient, but this will depend on original batch of dye and the amount of dye solution added to the reagent).

To 5 ml. of buffered indicator solution add 0.02 ml. of serum; treat a standard albumin solution in the same manner and measure their optical densities against a reagent blank at 635 mμ.

The colour may be read immediately or any time within three hours. Calculate results against a known standard or from a previously calibrated graph.

The method has been used in the author's laboratory for the last few months and found to be both quick and reliable. Day-to-day variations using commercial freeze-dried quality control sera are ± 0.2 g/100 ml.

REFERENCE:

1. Bartholomew, R.J. and Delaney, A.M. (1966), *Proc. Aust. Assoc. clin. Biochem.*, 7, 214.

Technical Communications

Simple Colorimetric Estimation of Serum Calcium

Sir,

The following method for estimating serum calcium, which is that of Bellinger and Campbell[1] with minor modifications, may be of interest to readers.

Method:

1. Place 2.0ml. water in duplicate tubes for test (T) and standard (S) and 2.1 ml. into a fifth tube for a blank.
2. Into (T) pipette 0.1 ml. serum and into (S) pipette 0.1 ml. of working calcium standard (10mg./100ml.) and mix.
3. To all tubes add 1.0ml. of G.B.H.A. reagent (see under "Reagents") and mix, then add 0.2ml. of colour developer. Mix well, place in centrifuge and bring to about 3,000 r.p.m. then switch off and allow to coast to a gentle stop.

4. Add 5.0ml. chloroform to all tubes, stopper with polyethylene stoppers then shake vigorously for about 15 seconds.

5. Again centrifuge tubes at about 3,000 r.p.m. for 2-3 minutes to separate the chloroform layer containing the violet calcium complex.

6. Immediately read optical density of the chloroform layer at 535 mμ against the blank.

Due to turbidity which begins to develop, particularly in T soon after the centrifuge has stopped, reading of optical densities should be made immediately.

Reagents:

1. G.B.H.A. reagent — 0.4% Glyoxal bis — (2-hydroxyanil) (Hopkin and Williams) in absolute ethanol. (I find this keeps at least a week in the refrigerator, in contrast to most authors who state that it must be prepared fresh daily).

2. Colour developer — 10% NaOH and 0.5% Na_2CO_3 in distilled water.

3. Stock calcium standard (1mg./ml.) [Modified] — Dry $CaCl_2$ overnight at 105°C and cool in a desiccator. 2.768g are weighed and transferred to a litre flask. 200 ml. of distilled water and 50ml. 1.0N HCl are added, shake to dissolve and dilute to the mark with water. Keeps well at room temperature.

4. Working calcium standard (10mg./100ml.) — Dilute stock standard 1 in 10 with distilled water. Keep in refrigerator.

5. Chloroform — B.P. or reagent grade is satisfactory.

Discussion:

The above method depends on the ability of calcium in alkaline solution to react with glyoxal bis — (2-hydroxanil)[2] to form a red complex which is extractable into chloroform. Certain other metal ions (e.g. cobalt and nickel) will also react, but the colours produced do not extract into chloroform.

In this laboratory optical densities are read on a Coleman Jr. spectrophotometer and although this type of instrument does not have a narrow band-width, it was found that Beer's law is obeyed over the range 5-15 mgm Ca^{++}/100ml. Because of the turbidity which develops in the chloroform extract, and because of the difficulty of pipetting this extract so as to obtain a clear solution free from tiny water globules or protein particles, it is recommended that cuvettes or precalibrated test tubes be used for the entire procedure. In this way colorimetry can be performed immediately after removal of tubes from the centrifuge without the need for additional manipulation. We have found a set of specially selected 4 x 5-8″ test tubes to be most convenient. Because of the sensitivity of the method it is essential that all glassware be scrupulously clean.

The standard is a modification of the original and uses calcium chloride instead of calcium carbonate, the reason being that

this particular standard solution was already in use in the laboratory and was found to be perfectly satisfactory for this method, too.

The precision of the method was checked in this laboratory by pooling fresh serum and deep freezing it in aliquots sufficient for one test in duplicate. At intervals over the next seven weeks calcium estimations were performed, the total number of tests being twenty-one. Results ranged from 9.4 to 10.1mgm/100ml. although most values were between 9.7 and 9.9 The mean was 9.8 and the standard deviation 0.17 (see table I).

Table I.

	Number of Estimations	Mean	Range of results	Standard Deviation
Pooled serum	21	9.8	9.4-10.1	0.17
Versatol (Value 10.2mg./100ml)	10	9.9	9.6-10.5	0.25
Versatol A (Value 6.9mg./100ml)	10	6.8	6.6-7.0	0.14

The above method has now been in routine use in this laboratory for over a year and no problems have been encountered.

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REFERENCES:

1. Bellinger, J. F. and Campbell, R. A. (1966). *Clin. Chem.*, 12, 90-94.
2. Johnson, W. C. (1964). *Organic reagents for metals*. Vol. 2, pp 48-50. Hopkin and Williams. London.

Sterilisation Caps

Sir,

In the past few years, short supplies of non-absorbent cotton wool has been a headache to many microbiology laboratories. Cotton wool is used extensively in plugging test tubes and media flasks prior to sterilisation, and acts as an ideal air filter and cap.

There is on the market a selection of rigid aluminium caps which also have their use in tube and flask sterilisation. However, the expense, availability and selection of sizes obtainable make them of limited value in the laboratory.

As an alternative to both rigid aluminium caps and cotton wool plugs, we have found that pleated aluminium foil, wire bottle caps are an excellent substitute. They are cheap, will fit any size flask neck up to 6 cm. in diameter, come in a wide range of colours

and, if gold coloured tops are available, they have the added advantage of showing a detectable colour change upon sterilisation.

The caps are obtainable from most wine wholesalers or from Murie and Co. Ltd., P.O. Box 1250, Wellington.

R. D. PEARSON
Department of Animal Health
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Explosion Hazard in Laboratory Refrigerators

Department of Health Circular Letter 1969/101 dated September 4, 1969, refers to an explosion in a hospital laboratory refrigerator due to ignition of flammable vapour, and suggests measures necessary to prevent a recurrence of the incident.

In a recent communication to the official journal of the N.Z. Institute of Chemistry, D. Bradwell[1], of the Chemistry Division of the D.S.I.R., Auckland, has reported an explosion in an upright deep freeze unit which resulted from the ignition of ether vapour by a spark from the thermostatic control of the unit which was sited inside the cabinet. The quantity of ether involved was not great being that contained in the carcasses of some rats which had been anaesthetised with ether. The accompanying photograph (block by courtesy of *Chemistry in New Zealand)* shows the extent of the damage that resulted.

The Circular Letter suggests that in most cases it is not necessary to store potentially explosive materials in refrigerators, but that when it is necessary the material must be enclosed in a vapour-tight container. In addition it is recommended that:—

1. Refrigerators and deep freeze units should be converted to external thermostatic control and have their internal lights and switches disconnected from the power source.
2. All new refrigerators and deep freeze units for all laboratories should comply with these requirements.
3. In a few laboratories where storage of anaesthetised small animal carcasses presents a problem, the installation of a commercial Wastemaster could be considered.
4. A cool-room associated with a laboratory may require modification as recommended for refrigerators, but each cool-room should be considered individually, as other possible sources of ignition (e.g. electric motors of fans) may also require attention.
5. All laboratory staff should be made aware of the hazard.

REFERENCE:

1. Bradwell, D. (1969), *Chem. N.Z.*, **33**, 122.

Selected Abstracts

BLOOD BANKING

A New Method for Detection of Red Blood Cell Antibodies. Lalezari, P. (1968), *Transfusion, Philad.*, 8, 372.

Suitable for use in automated procedures, this technique employs Polybrene, a positively charged polymer, to produce agglutination of red cells. This agglutination is reversible by the addition of hypertonic salt solution but persists in the presence of antibodies.

The method has proved to be highly sensitive and with a wide spectrum of usefulness for the detection of both complete and incomplete antibodies, including those which are normally inhibited by the use of enzymes.

Anti-C as a Naturally-Occurring Antibody. Beck, M., Dixon, J., Lawson, N. S. and Oberman, H. A. (1968), *Transfusion, Philad.*, 8, 387.

Anti-C was detected in the serum of a 49-year-old man who had no transfusion history. The facts that the antibody was characterised as an IgM globulin, and with a low thermal range of activity, were consistent with the behaviour of a naturally occurring antibody.

Transmission of Syphilis by Fresh Blood Components. Chambers, R. W., Foley, H. T. and Schmidt, P. J. (1969), *Transfusion, Philad.*, 9, 32.

This is the report of a case of secondary syphilis resulting from the transfusion of fresh platelet-rich plasma from donors who were all serologically negative for syphilis.

Agglutinins for "Null" Red Blood Cells. McGinnis, M. H., Kaplan, H. S., Bowen, A. B. and Schmidt, P. J. (1969), *Transfusion, Philad.*, 9, 40.

Sera from two anaemic patients gave a pattern of agglutination which was the reverse of that described for antibodies of Rh-like specificity associated with autoimmune haemolytic anaemia. They reacted not only with Rh_{null} cells but also with U-negative, Oh and other cells lacking usual agglutinogens. They did not react with cells possessing the usual blood types and it is suggested that the sera contain antibodies directed against antigen sites which are normally either absent or concealed.

Transfusion Reaction: A Reappraisal of Surgical Incidence and Significance. Baker, R. J., Moinichen, S. L. and Nyhus, L. M. (1969). *Ann. Surg.*, 169, 684.

A review of the incidence of immediate reactions to transfusion of 116,273 units of blood and blood products was undertaken. These were administered to 42,331 recipients. A reaction was recorded following 2,569 transfusions (2.21%). These reactions were seen in 2,293 recipients (5.42%), with the following distribution of types: allergic, 45.6%, febrile, 43.5%, haemolytic, 9.9% and miscellaneous, 1.0%.

Serious, symptomatic haemolytic reactions occurred in 1 in 3,322 units infused or 1 in 1,209 recipients. Fourteen (40%) of these 35 recipients died. J.H.

CHEMICAL PATHOLOGY

Serum Triglyceride Levels of Normal Subjects. Kudo, H. (1969), *Tohoku J. exp. Med.*, 97, 35.

Using the method of Van Handel and Zilversmit, with modifications, fasting serum triglyceride levels of 263 normal subjects, 2 to 79 years of age, were determined. The overall mean level was 70 ± 1.3mg./100ml., with a range of 30-110 mg./100ml. It was concluded that levels over 120 mg./100 ml. may be regarded as indicating hypertriglyceridaemia. J.H.

Observations on Diagnostic Kits for the Determination of Urea Nitrogen. Logan, J. E., Renton, H. M. and Eby, P. W. (1969), *Clin. Biochem.,* 2, 189.

Fourteen diagnostic kits or reagent sets from 12 manufacturers were assessed for adequacy of literature supplied, reproducibility, accuracy compared to an automated diacetyl monoxime method, recovery of added urea and stability of reagents throughout the test period.

Three kits failed to show sufficient precision, i.e., within ±10 per cent.

Although the precision was adequate, 3 kits yielded values significantly higher than those of the reference method, while 3 gave lower results.

While slight improvement has been shown recently in the adequacy of the literature enclosed with the kits, much more complete data could be given in many cases.

J.H.

A simple Method of Estimating Plasma Salicylate Levels. Burston, G. R. (1969), *Scot. med. J.,* 14, 55.

On addition of heparinised plasma to a colour reagent (ferric nitrate-mercuric chloride-hydrochloric acid), a precipitate forms. The tube is centrifuged and the colour of the supernatant compared visually with a set of comparatively permanent standards. The concentration can be estimated to within ±5 mg. per cent. J.H.

Normal Values for Cerebrospinal Fluid Protein Concentration in Children: What is the Upper Limit of Normal? Abramowicz, M. (1969), *Clin. Pediat.,* 8, 300.

Standard textbooks of paediatrics set the normal range of cerebrospinal fluid protein concentration in children at 15-40 mg. per cent. However, investigations by the author and studies by others suggest that the upper range should be set at 27 or at most 31 mg. per cent. Other workers have found that values obtained from infants less than 6 months of age were higher. J.H.

A Comparison Between Albustix, Hema-Combistix, Labstix, the Sulphosalicylic-Acid Test, Heller's Nitric-Acid Test, and a Biuret Method: Diagnosis of Proteinuria. Thysell, H. (1969), *Acta med. scand.,* 185, 401.

This comparison of the various tests for proteinuria showed unexpectedly great discrepancies. There is a great need for simple, rapid and reliable screening methods for detecting proteinuria.

It was concluded that none of the applied tests satisfied the demands that should be made on such screening tests. Until more reliable and objective methods are available, the older methods should be used, preferably the sulphosalicylic acid test and Heller's nitric acid test. It should be possible to screen out some of the false-positive results if the tests are repeated. However, the high frequency of false-positive results is a factor to be noted. J.H.

HAEMATOLOGY

A Comparison of Two Broad-Spectrum Coagulation Tests. Wolfe, W. T., Hunter, D. T. and Allred, L. (1969). *Sth. med. J., Nashville,* 62, 490.

There are two major differences between the two tests studied, the partial thromboplastin time (PTT) and the plasma recalcification test (PRT). In the former a synthetic platelet Factor 3 is added, while in the latter, the patient's own platelets contribute this factor. Hence, the PRT gives an evaluation of platelet number and function, while the PTT completely omits this vital parameter. Since Factor VII is intrinsic in the artificial PTT reagent, assay of this factor is also omitted in the PTT.

It was concluded that the PRT more nearly fulfils screening test criteria.

Evaluation Studies of Peripheral Blood Leukocyte Changes in Malignancy. Malmgren, R. A., Bennett, J. M., Del Vecchio, P. R., DeWitt, Sara H., Feld, Minna, Kazam, E. and Schneiderman, M. A. (1969), *Acta cytol.*, **13**, 149.

A study of "malignancy-related" changes in peripheral blood cells is described. These changes consisted of thin (or fine) excrescences in the polymorphonuclear leucocytes and halos in mononuclear cells (monocytes and lymphocytes).

There was a significant difference between the average percentage of cells showing characteristic morphologic changes in 11 cancer patients as compared to 11 normal individuals. However, the overlap in the counts of positive cells, and the variability in counts within each group were too large to make the procedure useful either for the study of tumour-host interaction or as a cancer detection method. J.H.

Laboratory Control of Heparin Therapy with the Activated Partial Thromboplastin Time Test. Degnan, T. J., Karasik, S. and Lenahan, J. (1969), *Curr. ther. Res.*, **11**, 390.

To clarify the equivocal literature reports, the authors have studied patients during the course of long-term heparin therapy. Their results suggest that the activated partial thromboplastin time offers a reproducible, practicable and sensitive guide for the administration of heparin. J.H.

Leucocyte Death in Generalised Virus Infection. Smith, H. (1969), *Aust. paediat. J.*, **5**, 56.

Dead leucocytes may be detected in increased numbers in blood films from patients with a variety of disorders, but in adults at least, gross increases are unusual. This paper describes a neonate with a generalised infection with a virus of herpes simplex type whose blood contained dead leucocytes in striking numbers.

Cells were regarded as necrobiotic only when there were obvious changes in the nucleus, such as loss of internal structure, conversion to a homogenous mass or masses, and shrinkage, fragmentation and dispersal of the nuclear substance. Increase in staining intensity, to a deep red-brown or almost black, usually accompanied these changes, but an increase in staining intensity alone, without structural alteration, was not accepted as evidence of cell death. Frequently there was a change to a globular or approaching a globular shape, and often, in multilobed cells such as neutrophils, interlobar connexions had disappeared.

Changes in the cytoplasm alone were not accepted as evidence of cell death although they were common in cells in which the nuclei appeared to be non-viable.

The infant died at 11 days of age. J.H.

Platelet Adhesion: A Comparison of Four Methods. Sjogren, A., Bottiger, L. E. and Biorck, G. (1969). *Acta med. scand.*, **185**, 127.

A comparison of Wright's, Hellem's whole-blood and platelet-rich plasma/adenosine diphosphate and Salzman's methods showed good correlation between Hellem's and Salzman's whole-blood methods, a weaker, yet significant correlation between Wright's and Salzman's methods, but no correlation between any of the three whole-blood methods and Hallem's platelet-rich plasma/adenosine diphosphate method. J.H.

MICROBIOLOGY

A Comparative Study of the Pathogenic Criteria of Staphylococci and Pathogenicity of Coagulase Negative Staphylococci. Panda, G. K., Mohanty, D., Nanda, B. K. and Naik, U. P. (1969), *Indian J. med. Sci.*, **23**, 7.

Of 262 strains of staphylococci isolated from various clinical materials,

37 were found to be coagulase negative. Six of these 37 strains were suspected of being pathogenic since they were isolated on repeated occasions. Two were isolated from post-operative wounds, 1 from otorrhoea and 3 from urine. Strains isolated from urine were in significant numbers (>10,000/ml.) and pus cells were also found in the urine in these cases. Further support of pathogenicity was obtained from the clinical course of the disease following drugs given according to the sensitivity pattern. Mannitol fermentation, gelatin liquefaction and urea hydrolysis are taken as evidence of pathogenicity and these tests were positive in these 6 strains. J.H.

Herellea vaginicola Dalton, H. P., Allison. M. J. and Escobar, M. R. (1969), *Va med. Mon.,* **96,** 412.

Herellea vaginicola is one member of a poorly-defined group of bacteria, the tribe Mimae. Clinical laboratories are increasingly isolating these agents and this necessitates some knowledge as to how these organisms might be contributing to the infectious state.

During the last 9 months of 1967. 222 isolations were made in the authors' laboratory: 119 from wounds, 65 from the respiratory tract, 35 from urine and 3 from blood. These results are in agreement with the literature which points to the fact that these organisms demonstrate a low degree of invasiveness and are often associated with underlying conditions such as wounds, burns, malignancy, and indwelling catheters; infections sometimes occur after prolonged antimicrobial or steroid therapy.

Neomycin and colymycin appear to be effective against the majority of strains. Tetracycline and kanamycin also show activity against some strains. J.H.

Recent Developments in the Laboratory Diagnosis of Gonococcal Infections. Reyn, Alice (1969), *Bull. Wld Hlth Org.,* **40,** 245.

The most significant new developments are the use (*a*) of fluorescent antibody techniques and (*b*) of selective media, particularly that of Thayer & Martin. for the isolation and identification of *Neisseria gonorrhoeae.* The sensitivity and specificity of culture methods and of the direct "delayed" fluorescent antibody technique (FAT) are very similar, but the FAT allows more rapid reporting. In the "delayed" technique, specimens from patients are preincubated on a nutrient medium before smears of the culture are treated with fluorescent antibody.

Other new achievements are a complete, chemically defined, protein-free liquid medium for the culture of fastidious *Neisseriae;* a commercially available, chemically defined enrichment supplement: and a new specimen-transport kit using Stuart's medium. J.H.

Identification of *Pseudomonas aeruginosa* in the Clinical Laboratory. Phillips, I. (1969), *J. med. Microbiol.,* **2,** 9.

The literature concerning the identification of *Pseudomonas aeruginosa* is large and increasing. Because of this, it is difficult to choose a series of tests that. while technically simple and suitable for use in a hospital diagnostic laboratory, will yield the most useful information in the least time. The purpose of this paper is to report an assessment of some of the methods in a hospital laboratory, and to suggest a simple scheme of identification. J.H.

Control of Microbiological Hazards in the Laboratory. Phillips, G. B. (1969), *Amer. ind. Hyg. Ass. J.,* **30,** 170.

Through the years, several hundred publications have mentioned approximately 6,000 laboratory infections, but reports and surveys do not adequately illustrate the safety problem because only a fraction of the laboratory-acquired infections are ever reported in the literature. However, where infectious disease agents are handled to an appreciable extent a frequency rate between 1.0 and 5.0 per million man-hours would be typical; unsafe acts or conditions are difficult to identify in approximately 80 per cent. of the cases. The estimated case fatality rate for laboratory infections is 4.0 per cent.

The causes of laboratory infection are discussed as are approaches to hazard control. J.H.

Flotation Hydrocarbon Technique in Isolation of Tubercle Bacilli from Cerebrospinal Fluid in Tuberculosis Meningitis. Thapar, R. K., Atal, P. R. and Dayal, R. S. (1969), *Indian Pediat.,* 6, 172.

Equal parts of cerebrospinal fluid (CSF) and chloroform were mixed and centrifuged. A thick scum formed at the junction of chloroform and CSF which may be used for smear and/or culture.

By this technique, 50 per cent. of cases in early stages, 73 per cent. in the intermediate stage and 92 per cent. in the late stage showed tubercle bacilli in smears.

Other authors have demonstrated that non-acid-fast bacilli in the water phase of a water-hydrocarbon emulsion, on reaching the interphase either remain in the interphase or return to water but never pass into the hydrocarbon phase. They suggested that the difference of behaviour is due to the presence in acid-fast organisms of a mixture of fatty substances with many non-polar groups, which prevent their being wetted by water, but allow them to be wetted by non-polar liquids such as chloroform, xylol, petroleum and oils. J.H.

Diagnosis of Pyelonephritis. Chaterjee, A. M. (1969), *Antiseptic,* 66, 330.

Kidney infection is remarkable for its silent course, often difficult to recognise. Kleeman (1960) noted in his survey that only 20 per cent. could be diagnosed during life. Similarly, Brod (1962) reported that of his 78 cases of pyelonephritis, only 24 (32 per cent.) were diagnosed during the illness. Pathologists claim pyelonephritis to be the commonest cause of death from uraemia. Brod (1962) noted that 36 per cent. of deaths from uraemia were due to chronic pyelonephritis. Such a silent and relentless progression of the disease necessitates early diagnosis.

Various laboratory tests to aid in the diagnosis are reveiwed, keeping in view the problems of the general physician. Simple tests are described in some detail. J.H.

MYCOLOGY

Rapid Contrast Stain as a Diagnostic Aid for Fungous Infections. Swartz, J. H. and Medrek, T. F. (1969), *Archs Derm.,* 99, 494.

Ink Blue PP and Rose Bengal are among the ingredients of this stain. preparation of which might be somewhat time-consuming for the average laboratory; the stain is, however. available ready for use.

The suspected material (skin scales, nail scrapings, hairs, sputa, pus, exudate or a teased representative portion of a grown colony is placed in a drop of stain on a slide. A coverslip is added and the slide warmed.

The staining procedure does not replace cultural and other studies for the identification of species. J.H.

Modified Sabouraud Medium Containing Neomycin and Polymyxin. Cohen, S. N. (1969), *Appl. Microbiol.,* 17, 486.

Addition of a neomycin-polymyxin B mixture to Sabouraud dextrose agar was found to be a useful adjunct in the isolation of fungi and a definite improvement over earlier modifications containing penicillin and streptomycin or chloramphenicol, since there was decreased overgrowth by the Gram-negative flora frequently found in the sputum of hospitalised patients. It is suggested that a similar modification of cycloheximide-containing Sabouraud agar would be expected to improve the performance of that medium. J.H.

UNCLASSIFIED

Icteric Serum: A Helpful Diagnostic Clue in Comatose Patients. Whang, R., Saiki, J. H., Mansour, E. J. and Gehred, G. A. (1969), *Sth. med. J., Nashville,* 62, 361.

Because of either the inability of the comatose patient to provide a

history or a paucity of overt clinical manifestations of liver disease, the diagnosis of hepatic coma may be delayed. This article documents experiences with two patients having no physical signs of hepatocellular disease, and in whom detection of icteric serum led to prompt initiation of appropriate therapy.

In Case 1, although numerous laboratory examinations were performed, icteric serum was not noted until the physicians examined the serum.

In Case 2, many admission laboratory tests were done but the presence of icteric serum was not reported until it was noted the following day during a toxicological survey. J.H.

Book Reviews

Chromatographic and Electrophoretic Techniques: Volume 1, **Chromatography.** Third Edition. Edited by I. Smith, B.Sc., Ph.D., F.R.I.C., M.I. Biol. 1080 pages with illustrations, 10 in colour. William Heinemann, London. (1959), U.K. price 130s 0d.

A new edition of "Ivor Smith" is a noteworthy event for the practising chromatographist, for one is assured of finding the practical details and tricks of the trade which are necessary when applying new techniques. The first edition (1957), one third of the current size, involved ten authors. Thirty-seven authors were required for this third edition, indicating the expansion of the subject.

There are many new topics of wide interest, one group related to the structure of proteins. This describes sequence analysis through phenylthiohydantoin degradation and subsequent identification of lansyl amino acid compounds. Another deals similarly with (^{32}P) labelled ribonucleotides and the last chapter, which can be obtained as a separate pamphlet, describes a system of plastic macro-molecules called "Bio Bits."

Specialist chapters include a spark chamber technique for evaluating radio-chromatograms, ion exchange celluloses, preparative layer chromatography handling up to 100g. quantities, automated quantitative analysis, column chromatography, inorganic ion chromatography and steroid analysis. Incidentally, the only typographical error I detected was a heading on page 602. "Specific 'Steroid' Problems."

The chapters on amino acids are of particular interest to the clinical chemistry laboratory in regard to aminoaciduria and inborn errors of metabolism. All of these chapters, and indeed all of the book, has been extensively revised.

One finds corraboration of the advantages of paper over thin layer in certain instances; for example, the single run screening techniques for urine and serum without deproteinising. The chapter by the late Mary Efron on pathological aminoaciduria contains useful tables classifying disease, abnormal enzyme, amino acid, clinical feature and treatment. There is also an extensive classified list of references. How delightful it would have been to see colour plates of the urine amino acids of the same quality as those depicting pigments in inks! Without doubt meticulous technique is required to produce uniform and comparable results and considerable experience needed to interpret chromatograms. The comment that seventeen amino acids are present on the fingers is a warning against undue handling!

One of the later chapters outlines a rational approach to the investigation of new compounds and gives examples of the techniques employed.

This book now contains such a wealth of diverse material one wonders whether it might not with advantage be made available as separate parts. In any event, those involved in chromatography can scarcely do without it.

R.D.A.

Comprehensive Review for Medical Laboratory Technologists. F. E. Dolan Ph.D. Editor, B. L. Garrick M.T. (ASCP) B.S., S. P. Gottfried Ph.D., C. F. Spiltoir Ph.D., D. P. Stiff M.D., F.C.A.P. 181 Pages, C. V. Mosby Co. St. Louis (1968) U.S. price $6.95.

This slim volume in an attractive strong hard cover presents the information in a somewhat unusual manner.

Each section is introduced by a posed question heading, and the text which follows presents the relevant facts woven into a practical explanation and answer. The emphasis is on the interpretation and relevance of information to an understanding of the practical significance and usefulness of knowledge; so that reasons, principles and evaluation form a good deal of the subject matter.

The level of knowledge is primarily directed to the first few years of training, but the fairly broad subject coverage could serve as useful recall material for more advanced students to enlarge further in detail.

There are ten chapters, the short first one gives some good sound advice to examination candidates. The chapter on Anatomy and Physiology (of 60 pages) presents useful material in palatable form, although some diagrams could help. However, a comprehensive review must necessarily omit much minor and specific detail which can be consulted elsewhere.

The chapter on general chemistry is brief and includes very elementary basic material, but is none-the-less pertinent. One would like to see this expanded with some specific applications of physico-chemical properties to biological states, such as osmolarity.

The three page chapter on "special chemical tests" seems hardly worthy of separate identity, but could warrant its place if many of the newer tests available were included; for example, chromatography and various "function" tests which fill the gap between this book's arbitrary division between "Urine Chemistry" and "Blood Chemistry." This latter chapter is reasonably well done and includes most of the basic major areas in some twenty pages.

Two further chapters of the same length are devoted to Blood Transfusion Serology and Haemotology, plus one of half this length on haemostasis. These all appear to have more detail, coupled with tables and diagrams, than the other sections. Most of the important subject detail appears to be well covered and up to date. One notable omission could be any reference to the international haemoglobin standard. These three chapters, together with that on Anatomy and Physiology, are the highlights of this book. Microbiology, the final chapter, seemed rather skimpy and not in keeping with other sections, but in a wide subject with many specialised details, inclusion of these would be difficult. However, one would expect more on fundamental principles such as growth requirements and environment as it affects the cultivation and isolation of organisms. Nevertheless there is some good basic but very first year stuff in this.

This is not a reference book full of detailed facts and procedures, but presents the more important aspect of the way in which factual knowledge is employed to build up understanding and evaluation. Today's facts may change, but the manner of their use and application will not. The authors would appear to be successful in conveying this idea, in contrast to the assimilation of a mass of knowledge followed by meaningless regurgitation.

This book should prove stimulating and useful to new trainees and serve as good revision material for others to part I level. G.R.G.

A Guide to Practical Histochemistry. First Edition. J. Chayen Ph.D. D.Sc., Lucille Bitensky M.B., B.Ch., Ph.D. M.C. Path., R. G. Butcher B.Sc., L. W. Poulter F.R.M.S. 261 pages. Oliver and Boyd, Edinburgh 1969. Price in U.K. 63s 0d.

This is a good book to place in the hands of junior technicians to enable them to appreciate the "art" in histochemical procedures. Advanced students of histology will find many of the techniques described in great detail with the rationale and biochemical data included where relevant. The references quoted are well selected and provide adequate coverage.

Chapter V is of particular interest in that it presents the problem of analysis of chemical components of cells and tissues in a "sequence of tests" analogous to "group separation" familiar to students of chemistry. It is a fascinating and highly commendable approach and should prove useful in creating an atmosphere of analytical approach to students of histochemistry.

The growing importance of enzyme histochemistry is well emphasised, as this subject utilises half the book. It is stimulating to read the discussion on aspects dealing with the enzyme-substrate complex formation, their demonstration and interpretations. The usefulness of procedures for positive and negative controls, substrate control and inhibitor control does not appear to have been stressed by the authors. They must have had good reasons for this. Since false reactions and artefacts can cause a great amount of confusion, descriptive and relevant photomicrographs (in colour where necessary) would have considerably enhanced the value of the book as a reference source. Even a companion volume of photographs would demonstrate the results outlined in the book, especially in view of the elusiveness of enzyme histochemical reactions.

The guide is concise and comprehensive in its coverage of the rapid studies made in applied histochemistry, and is a very valuable addition to any laboratory bookshelf.

Students of histochemistry in all walks of biology would find it extremely rewarding from the point of view of practical application and as a springboard to further interest in the subject.

S.N.R.

An Introduction to Virology. C. R. Goodheart. 432 pages, illustrated. W. B. Saunders Co., Philadelphia (1969). Obtainable from N. M. Peryer Ltd. at $10.00.

This new book provides an excellent introduction to the study of viruses without regard to the hosts in which they replicate or the effects which they have on their hosts. After an introductory chapter and a description of the size and morphology of viruses, the author deals with the thorny problem of virus classification. This is a question which has exercised the minds of virologists for a number of years and is still far from being answered unanimously. Dr Goodheart states his preference clearly, while conceding that it is not universally accepted. There are several chapters on the general characteristics of viruses, covering the assay of virus suspensions, the action of physical and chemical agents on viruses and virus serology.

The book then embarks on its main theme, with a series of chapters dealing with the replication of viruses and the associated molecular biology of infected cells. Viruses are considered in turn according to the type of nucleic acid which they contain, first double-stranded and single-stranded DNA and then the RNA viruses.

Later chapters deal with the interrelationships between viruses. Here we have the "satellite" or "associated" viruses which can only replicate in cells already infected with another virus; and interference, where the growth of one virus can inhibit the growth of another.

The two final topics are viral genetics, dealt with under the headings of mutation and recombination, and tumour viruses.

The book is clearly and concisely written with relevant data drawn from recent articles used to illustrate the various points. Of necessity most of the conclusions are based on the results of experiments using

bacterial and animal viruses because these are the groups in which reactions between virus and cell can be studied *in vitro*. However with the recent advances in insect tissue culture it is certain that the next few years will produce a lot of fascinating information about the replication and molecular biology of insect viruses.

The book is well documented, with over 1,400 references—many of them from the last two years, and the illustrations are clear and relevant. This is not a technical manual; it makes no claim to be. It is simply an excellent introductory work to the discipline of basic virology.

F.J.A.

Medical Laboratory Technology and Clinical Pathology. Second Edition. M. J. Lynch, M.D., F.R.C.P., F.C. Path., F.C.A.P., M.R.C.P.; S. S. Raphael, M.B., F.R.C.P., F.C. Path.; Leslie D. Mellor, L.C.S.L.T., F.I.M.L.T.; P. D. Spare, F.I.M.L.T., M.R.S.H., A.C.I.C. and M. J. H. Inwood, B.Sc., L.C.S.L.T., F.I.M.L.T. 1,359 pages illustrated. W. B. Saunders Co., Philadelphia (1969). N.Z. price (from N. M. Peryer Ltd., P.O. Box 833, Christchurch) $23.00.

This second edition of "Lynch" has been considerably expanded and, as will be seen from the title the emphasis on the underlying clinical pathology stressed.

The author's stated intention is to produce a textbook in one volume, covering all aspects of clinical laboratory work.

Although the book collects into one volume the relevant information on most routine clinical laboratory work the doubling in its size since the first edition in 1963 shows that it will be difficult to keep to one volume in future editions. It is apparent that, in spite of the expansion of the text, severe limitations are apparent in parts, particularly microbiology. It is difficult to see who the book is intended for. It is in some aspects too advanced, and in others too sketchy for the student technologist, and its high price of $23 makes it unattractive. For the trained technologist single subject textbooks covering the ground in greater depth are available. I think this book will be most useful, to the tutor technologist, particularly for its wedding of theory to practice.

This edition, as mentioned above, places great emphasis on the theoretical background to laboratory work. To this end eight new chapters have been introduced; three of them in biochemistry, embracing immunoglobulins, enzymology and amino acid studies. The haematology section has been extensively revised and a new chapter on bone marrow and plasma studies added. Histopathology has been extensively revised and now includes cryostat processing and a greatly expanded chapter on cytological diagnosis.

Entirely new sections have been added on cell organisation and structure, virus and rickettsial diseases and cytogenetics.

The first section of the book, that on cell organisation and function, is a concise and lucid exposition of the current state of knowledge in this field. Chapter two contains an excellent section on laboratory safety, as did the first edition; however the discussion of laboratory records is inadequate.

The chapters covering biochemistry as mentioned above have been greatly expanded. They are of a uniformly high standard and this is one of the few textbooks of clinical biochemistry which gives adequate coverage of the underlying theory of the tests being used. The "Methods of Analysis" chapter has been revised, updated and enlarged by bringing together items which in the previous edition were scattered throughout the text. In addition, a short section on automated chemistry has been added. Although far from comprehensible this chapter is a useful source of data for the student.

Haematology is given good coverage, particularly non-spherocytic haemolytic anaemias. The description of the haemoglobinopathies is also

very sound. As in the first edition the description of blood coagulation is the most lucid generally available and is excellent for students. Two criticisms of the haematology section are the poor coverage of acquired auto-immune haemolytic anaemias and the advocacy of the use of Thoma pipettes for cell counting.

Blood Bank Serology is adequately covered although some of the techniques advocated are controversial, the reduction of the time of incubation of a routine compatibility test to 30 minutes for instance. The advocacy of the albumin replacement rather than displacement technique is also surprising. No mention is made of screening recipients for antibodies before transfusion and, although a quality control system in the blood bank is suggested, no concrete suggestions as to how to implement such a system are made. On the credit side the discussion of basic blood bank reagents is very good and fully covers the requirements of the non-reference, hospital laboratory.

The Microbiology section is undoubtedly the weakest in the book. Culture media and sterilisation are very sketchily covered. It is refreshing, however, to come across a textbook dealing with clinical microbiology which uses up to date nomeclature. The section on mycobacteria has been updated and includes a classification of the anonymous mycobacteria. Mycoplasma are very cursorily dealt with. The chapter on virus and rickettsial diseases is a good introduction to the subject.

The histology section gives good coverage and background information on all methods likely to be used in most laboratories. It includes a section on cryostat technique and a good description of cytological diagnosis. As only monochrome illustrations are used throughout the book however, the illustrations in this section are not as valuable as they could be.

The final chapter on cytogenetics I found most instructive to a non-specialist such as myself.

The printing and paper quality of this edition have been improved; however, some annoying transpositions and mis-spellings were evident in the review copy. D.G.B.

Textbook of Medical Virology. A. Cohen, M.A., D.M. (Oxon.), M.C.Path. 550pp., 98 illustrations. Blackwell, Oxford. U.K. price, 60s 0d.

A manifestation of increasing interest in viruses and viral diseases is the recent publication of several new textbooks dealing with the subject aimed primarily at the non-virologist.

This book is based on the author's lectures at University College Hospital, London; and although intended mainly for students Dr Cohen has covered his subject in sufficient detail to enable use during study for higher qualifications.

The text is grouped into three parts: General Virology, Virus Infections, and Rickettsial Infections.

The General Virology is well done as Part 1 covering techniques, structure, nomenclature, infection, replication and immunity. The recommendations of the Provisional Committee for Virus Nomenclature have been followed throughout with the use of the more familiar colloquial names as alternatives.

Part 2 covers Virus Infections which are arranged in broad clinical groups, for example—Respiratory Infections which covers Myxoviruses, Adenoviruses and Rhinovirus.

Each agent discussed is dealt with from the aspects of Morphology, Biological properties, Antigenic composition, Cultivation, Clinical features, Pathology, Epidemiology and Immunology. Excellent chapters on Antibiotics and Chemotherapy of Virus Infections and Oncogenic (tumour inducing) Viruses conclude Part 2.

The Rickettsial Diseases are covered in Part 3.

One criticism of Dr Cohen's book is that after having defined the Psittacosis-Lymphogranuloma-Trachoma group of organisms as being closely related to the Rickettsia and therefore not viruses; he has arranged the chapters dealing with these organisms in Part 2 between the Arboviruses and Miscellaneous infections, rather than with the Rickettsiales.

The illustrations include photographs and line diagrams. Many of the latter, particularly those drawn especially for the book, are excellent.

The photographs are, in general, good but the omission of a scale would in some cases be confusing to a non-virologist.

"Feulgen" has been mis-spelled three times in the section on adenoviruses, elsewhere it is spelled correctly.

These minor criticisms aside, Dr Cohen has produced an excellent introduction to the subject which more than fulfils his aims. This is an excellent introduction to the subject for a newcomer to the field or to anyone wishing to bring themselves up to date with broader aspects of current knowledge in the subject, be they medical student, technologist, physician or trainee virologist.

An adequate bibliography is given at the end of the book for those who would like to read further. Detailed references have rightly been excluded as this book is not primarily intended for the professional virologist.

R.H.J.

Book Received

Fundamentals of Mycology. J.H. Burnett, M.A., D. Phil. 546 pages, illustrated. Edward Arnold, London (1969). U.K. price £6 10s. (Also available in paperback at 65s 0d.)

Papers Read at the 25th Annual Conference

CHEMICAL PATHOLOGY FORUM (Chairman: D. A. McArthur)

Keynote Address — Dr R. O. Farrelly.

Simultaneous Automated Method for S.G.O.T. and Alkaline Phosphatase — G. R. McLaren

Clinical Enzyme Units — An Unresolved Problem — G. R. McLaren

A Comparison of 5' Nucleotidase and Alkaline Phosphatase Levels in Disease of Hepatic and Osseous Origin — E. K. Fletcher

A study of the Heat Inactivation of Serum Alkaline Phosphatase by an Automated Technique — C. W. Small

Some Observations on Spectrophotometric Methods for the Iodometric Estimation of Serum Amylase, with Reference to the Method of King and Wootton — Miss A. Buchanan

Automation of a Method for the Estimation of Pepsin — G. C. Rimmer

Analysis of Quality Control Results — K. G. Couchman

The Expression of Blood Acid-Base Results — G. R. George

A Case Study Showing Clinical and Laboratory Management of Acid-Base Regulation — R. MacKenzie

Earthquakes — A Source of Error — H. T. G. Olive

Demonstration of Analgesic Agents in Serum by Thin Layer Chromatography — W. J. Sloan

Molecular Sieve Chromatography — S. H. Drumgoole

HAEMATOLOGY FORUM (Chairman: J. Marr)

Some Rare Blood Group Findings of Considerable Clinical Importance — J. W. Stinear

Standardisation of Results for the Laboratory Control of Oral Anticoagulant Therapy. — E. J. Montgomery

Haemoglobin Standardisation — K. Wilding

Classification of Leukaemia — Dr J. Buchanan

The Laboratory Diagnosis of Haemolytic Anaemia — A. D. Nixon

Two Case Reports of Phenacetin Induced Haemolytic Anaemia C. S. Shepherd
Mycosis Fungoides — Sezary Syndrome J. Rees
Haemoglobin Electrophoressis on Cellulose Acetate M. Jeannette Grey
Some Interesting Findings Associated with Hereditary Elliptocytosis M. S. G. Clist
The First New Zealand Example of a Rare Blood Group Antibody J. Case
Some Aspects of Laboratory Investigations in Haemolytic Disease of the Newborn
Miss M. Beatie, Mrs B. Montgomery, Mrs M. McDonald, R. J. Coleman
Blood Volume Estimation in Pregnancy R. J. Coleman
The Composition of Romanowsky Stains and the Role the Components Play in the Romanowsky Effect J. R. Saal

MICROBIOLOGY FORUM (Chairman: Mr J. T. Holland)

Disc Sensitivity Testing — Its Control and Errors B. M. Cornere
Some Aspects of Sulphonamide Sensitivity Testing R. J. Holloway
Serum Antibiotic Assays G. L. Cameron
Clinical and Laboratory Aspects of Transferable Drug Resistance H. C. W. Shott
Antibiotic Susceptibility of Urinary Tract Pathogens Determined by Serial Tube Dilution and Single Disc Tests T. E. Miller
The Determination of Penicillinase Production by Staphylococci G. L. Cameron
Staphylococcal Deoxyribonuclease Production and Pathogenicity G. Thorne
Brucellosis Miss A. Robinson
Lancefield Grouping of Streptococci Using an Autoclave Method for Antigen Extraction Mrs M. Hilbourne
Some Recent Developments in Tuberculosis Laboratory Methods Miss G. R. Bott
Haemophilus J. C. Beattie
Listeria monocytogenes — *A Review* R. C. Sowden
A Case of Blastocystis hominis *in Urine* G. Hill

HISTOLOGY FORUM (Chairman: Dr S. E. Williams)

Tissue Reaction to Surgical Glove Powder B. Glynn-Jones
Special Stains for the Identification of Connective Tissue Components Dr G. Hitchcock
The Alcian Green-Saffron Stain in Lung Carcinoma P. Hickey
Immunofluorescence Techniques in Histopathology K. G. Couchman
Discussion on Future Development in Histology and Cytology introduced by J. R. Saal
Cytology in New Zealand Dr S. E. Williams
The PAS Stain in Cytological Diagnosis J. Taylor
Undecalcified Bone Sections Dr R. Palmer
Histological Staining of Islet Cell Tumours R. J. Patterson
Rapid Tissue Processing and Cone Biopsies in a Gynaecological Laboratory Dr M. McLean
Identification of Lipofuscin with Particular Reference to the Dubin-Johnson Syndrome B. Glynn-Jones
The Place of Histochemistry in the General Laboratory E. Manns
Histological Observation of Wound Healing Dr M. Flint

PLENARY SESSION

Technical Variation in Cytogenetics seen in Britain and the U.S.A. H. E. Hutchings
Oral Examinations R. T. Kennedy
Report on a Proposed New Zealand Certificate in Science Course for Medical Laboratory Technologists D. J. Philip
Changing Scene in Medical Laboratory Technology J. R. Saal

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