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Index to Advertisers

Errata

N.Z. J. med. Lab. Technol., Volume 23, No. 3 (November, 1969). In the article "Electrophoresis of Hb A_2 on Cellulose Acetate" by M. Jeannette Grey, there is an error on page 81, line 16.

Delete the words "per band" and close the sentence after "v/v acetic acid."

In the article "A Simple, Quick Method for the Estimation of Total Protein and Albumin in Serum" by D. W. Everard is an error on page 114, line 9. Under "Materials and Methods," the weight of dye to make 0.01M bromocresol green is incorrectly given as 0.968g.

The figure should read 0.698g.

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The Indirect Fluorescent Antibody Test for the Detection of Toxoplasma Antibodies

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Received for publication, August, 1969.

Introduction

In spite of the recognition of toxoplasmosis in man many years ago [22] the serological diagnosis of toxoplasma infection is still limited to a few major central laboratories. In a recent article [10] it was stated that only 23 laboratories in the U.S.A. were capable of performing toxoplasma antibody tests, and only 16 of these were using the dye test of Sabin and Feldman.

The dye test [14] has become generally recognised as the standard serological test for toxoplasmosis. Undoubtedly the need for maintaining live cultures of toxoplasma, the use of live organisms in the test and the difficulty in obtaining a constant source of accessory factor have prevented the test from becoming more widespread. Other tests have been introduced, but so far none appear to have gained general acceptance except as supplementary procedures to the dye test. The haemagglutination test [12] has a level of reactivity approximating that of the dye test, but is less sensitive in the early stages of the disease.[11] There is also probably a large variation in the quality of the antigen used. Other sensitized particle techniques [1 15] do not appear to be any better. The complement fixation reaction [21] is a useful supplement to the dye test for demonstrating rise and fall in antibody levels, but its sensitivity is usually too low for diagnostic purposes.

The fluorescent antibody technique was first used with toxoplasma by Goldman.[9] The fluorescent inhibition test that he described for detecting serum antibodies has been superseded by the indirect fluorescent antibody technique, which has now been favourably reported upon by a number of workers.[2 5 8 20] Titres obtained in human infection have closely paralleled those obtained with the dye test. It has also been shown that in experimental infection of pigs, the dye and indirect fluorescent antibody tests are in close agreement in demonstrating time of appearance and rise and fall of antibody levels.[17]

The present paper investigates a number of aspects of the indirect fluorescent antibody test and compares results obtained on human sera with those of the dye test.

Materials and Methods

Fluorescent Antibody Test

Antigen. Peritoneal exudate from white mice infected 3 days previously with the RH strain of *Toxoplasma gondii* was mixed with an equal quantity of 2% formol-saline. After 30 minutes the

exudate was centrifuged for one minute at low speed to remove large cells and debris. The toxoplasma in the supernatant were recovered by centrifuging for 20 minutes at approximately 1000 x 6, and were washed twice in phosphate buffered saline, pH 7.2. The washed organisms were suspended in sufficient phosphate buffered saline so that the final films showed 50-100 organisms per high power field under the microscope. The suspension was spotted on to slides which were air dried and stored at −15°C for up to three months.

Fluorescent anti-human globulin. Anti-human globulin was prepared in rabbits against Cohn fraction 2. The gamma globulin fraction of the rabbit serum was conjugated with 2.5% fluorescein isothiocyanate for 18 hours at 4°C, and unreacted fluorescein was removed with Sephadex G25. The conjugate was absorbed with hog liver powder and bovine bone marrow, and freeze dried. The optimum titre was determined using serial dilutions against known positive and negative sera.

Microscopic equipment. A Leitz Laborlux microscope was used with an HBO 200W ultra-violet lamp, primary fluorescence filter 3 mm BG 12, secondary filter K530, dark ground condenser na 1.2, x54 oil immersion objective na 0.95, binocular phototube and x6 eyepieces.

Technique. Toxoplasma slides were removed from the deep freeze and dried at 37°C. Serial dilutions of test sera in phosphate buffered saline were applied to the antigen spots and the slides were incubated at 37°C for 30 minutes. Slides were rinsed in three changes of phosphate-buffered saline over a period of 10 minutes, covered with fluorescent anti-human globulin in optimal dilution and reincubated for 30 minutes at room temperature. They were then washed as before and mounted under coverslips with glycerol, buffered to pH 9.0 with carbonate buffer. Slides were examined under the U.V. light and the reaction was considered positive when at least 50% of organisms showed peripheral fluorescence completely outlining the organism.

Dye Test

The dye test on all sera was performed at the National Health Institute, Wellington. The method used was that of Manning and Reid.[13]

Test Sera

Sera tested were those sent to the laboratory for routine toxoplasma antibody tests. They were further selected, in that the dye test was not performed on many sera which showed a negative fluorescent antibody test. They were stored at −15°C until testing.

Experimental

1. Several methods of antigen preparation were tried. (a) After fixing in formalin as described toxoplasma were washed only

once. (b) Toxoplasma were not fixed in formalin but were washed three times in phosphate buffered saline. (c) Toxoplasma were not fixed and were washed only once.

2. (a) Prepared toxoplasma slides were fixed in 10% methanol for 10 minutes. (b) Slides were immersed in 0.3N HCl for five minutes and rinsed in phosphate buffered saline for two minutes. (c) Slides were rinsed in phosphate buffered saline for five minutes.

3. Various techniques to reduce non-specific staining were tried. (a) Sera were diluted in 1% bovine albumin. (b) Fluorescent antihuman globulin was diluted in 1% bovine albumin and (c) in 5% Rhodamine bovine albumin. (d) Evans's blue dye, 1:5000, was used as a counterstain after the usual fluorescent staining.

4. Possible interference from antibodies to mouse proteins present in the antigen was investigated. (a) Antibodies to mouse proteins were produced in guinea pigs by inoculation of whole mouse blood. The guinea pig serum was used in the first stage of the fluorescent antibody reaction. An anti-guinea pig globulin, prepared in rabbits and conjugated with fluorescein isothiocyanate was used for the second stage of the reaction. (b) 18 human sera with fluorescent antibody titres of 1/16 to 1/1024 were tested by the fluorescent antibody technique after dilution in 2% rabbit serum, and 2% mouse serum.

5. Interference from rheumatoid factor, due to possible sensitisation of gamma globulin bound to toxoplasma, was investigated. (a) Three sera with low titre fluorescent antibody tests were mixed with an equal quantity of pooled rheumatoid arthritis serum and then tested by the fluorescent antibody technique. (b) Pooled rheumatoid arthritis serum with a fluorescent antibody titre of 1/128, was absorbed three times with sheep cells that were sensitised with rabbit anti-sheep cell serum. No agglutinating ability towards sensitised sheep cells remained. As a control the serum was absorbed with unsensitised sheep cells. The absorbed sera were then tested by the fluorescent antibody technique.

6. Thirteen sera with fluorescent antibody titres of 0 to 1/4096, were tested in serial dilutions using fresh specimens and specimens that had been heated at 56° for 30 minutes.

7. Toxoplasma prepared in the usual way by formalin fixation and washing, were suspended in 5% sucrose solution and freeze-dried in 0.25 ml. amounts. The organisms were reconstituted in distilled water and used in the fluorescent antibody test with the standard technique. A modification of the standard technique using HCl treatment of slides as described in 2 (b) was also used. Using this modification 10 sera with fluorescent antibody titres of 1/16 to 1/4096 were tested with the freeze-dried antigen.

Results and Discussion

Table I shows the results of testing 133 sera by both dye and fluorescent antibody tests.

There was good correlation between the two tests, confirming the conclusions of other workers that the tests are very similar in sensitivity. With the fluorescent antibody test, however, it is im-

		Dye Test Titres										
		-ve	16	32 to 128	256	512	1024	2048	4096	8192	16 384	TOTALS
	-ve	26		2								28
Fluorescent	16	3	1	5								9
antibody	32	2	1	7								
test	64	1	1	12	1							39
t'tres.	128		1	13								
	256		1	13	4	2	1					21
	512			5	4	3	1					13
	1024				2	2	2	1	1			8
	2048					1	2	2				5
	4096						1	1	3	1		6
	8192									3	1	4
TOTALS		32	5	57	11	8	7	4	4	4	1	

TABLE I

Comparison of dye and fluorescent antibody test titres on 133 sera.

portant to take into account several variable factors; namely, the loss of intensity of the U.V. lamp after prolonged use, the optical equipment used, and the quality of the fluorescent anti-human globulin conjugate. It is apparent that the sensitivity of the test can easily be altered by a variation in one or more of these factors. It is therefore most important to use a control serum of known titre with each batch of tests, and the level of sensitivity should initially be determined in relation to the dye test with a number of positive sera. Once the test is standardised results are readily reproducible.[16]

Another factor influencing the sensitivity of the test is the method of preparation of antigen. Most workers have used the formalin fixation method of Goldman.[9] In the present experiments it was found that unfixed toxoplasma, washed only once, reacted much better than those washed three times. Fixed toxoplasma washed only once were not quite as sensitive as those washed twice, and fixed toxoplasma were more sensitive than those not fixed. In an analysis of toxoplasma antigens by agar diffusion methods, Chordi *et al.*[4] mention the loss of an " exotoxin-like antigen " which is important in diagnostic tests, when organisms are washed. The present results confirm that there is a loss of such an antigen when the organisms are washed before fixing, and that the loss is not, as suggested by Jacob,[11] simply due to a decrease in the number of viable organisms after washing. Two other serological tests using

formalin-fixed toxoplasma have been described. These are the direct agglutination test of Fulton and Turk,[7] and the complement fixation reaction of Fulton and Fulton.[6] It would seem probable that these two tests, like the fluorescent antibody test, are largely dependent upon this exotoxin-like antigen, as opposed to the haem-agglutination and complement fixation tests which use compound antigens of cellular extracts. It is therefore possible that these two tests, which so far have had little investigation, may give results more closely allied to the dye test than does the haemagglutination test.

Of the various methods investigated in 2 and 3, that different workers have used to try to eliminate non-specific staining and to help determine endpoints of titres, none was found to be particularly helpful. Non-specific fluorescence is fairly easily distinguishable from the specific peripheral fluorescence. Masking of the non-specific fluorescence with counterstains to facilitate reading is merely a matter of individual preference. Washing slides in phosphate-buffered saline before use reduced the sensitivity slightly.

The guinea pig anti-mouse serum, when used in the fluorescent antibody test, produced diffuse fluorescent staining of red cells, white cells and toxoplasma, indicating the presence of mouse proteins in the antigen preparation. The staining was absent when the guinea pig serum was absorbed with mouse blood. Of the 18 sera tested after dilution in mouse and rabbit serum, none showed any reduction in non-specific polar fluorescence, but one serum which had a titre of 1/64 when diluted in buffered saline and rabbit serum was negative when diluted in mouse serum. Chordi *et al.*[3] have demonstrated inhibition of haemagglutination titres by normal mouse serum in 12% of sera studied, but none of the non-specific titres exceeded 1/100. For a more accurate test, the interference from mouse antibodies can easily be eliminated by diluting test sera in 2% mouse serum. As a rising antibody titre is usually necessary to demonstrate toxoplasma infection it is doubtful if such a procedure is necessary as a routine.

Interference from rheumatoid factor, due to in-vitro sensitisation of gamma globulin on toxoplasma, has not been found to occur under the prescribed conditions of the test. No increase in fluorescent antibody titre was found with the three sera that were mixed with rheumatoid arthritis serum. The rheumatoid arthritis serum from which the rheumatoid factor was absorbed showed no decrease in fluorescent antibody titre over that of the control serum absorbed with sheep cells.

No difference in titres between fresh and inactivated specimens was observed in the 13 sera tested.

The freeze-dried toxoplasma were found to react well with positive sera in the fluorescent antibody test, but the peripheral ring of fluorescence was not distinct, and with negative sera there

was an undesirable amount of fluorescence. Treatment of slides with O.3N HCl as described was found to reduce the non-specific staining, and with positive sera the peripheral fluorescence showed up more clearly. Of the 10 sera that were tested with the HCl-treated antigen, eight had titres the same as when frozen antigen was used, and two had titres one dilution lower. Freeze-dried toxoplasma have been found to be suitably reactive after four months storage at 2—4°C, but after reconstituting they quickly lose their antigenicity and cannot be used for more than a day. Takumi *et al.*[18] described the use of freeze-dried toxoplasma for the fluorescent antibody test and obtained quite good agreement with haemagglutination results. Their method uses the 0.3N HCl slide treatment, as described originally by Voller.[19] In the present study, freeze-dried toxoplasma were not quite as good as those stored frozen on slides. Some improvement could perhaps be made in the suspending medium in which the organisms are dried. For all practical purposes, however, the antigen was quite suitable and would greatly increase the facility with which a number of laboratories could perform the fluorescent antibody test.

Conclusions

The fluorescent antibody test appears to be as specific and as sensitive as the dye test. It agrees well with dye test results obtained on human sera. Antibodies to mouse proteins which may cause low titre false positive results in the fluorescent antibody test can be blocked in the reaction by diluting sera in 2% mouse serum.

The fluorescent antibody test is simpler to perform than the dye test, and does not require the use of live antigen. Preparation of antigen for the fluorescent antibody test is simpler than for haemagglutination, complement fixation or direct agglutination tests. It is not necessary to separate toxoplasma from contaminating host cells, as the reaction of individual organisms is visualised under the microscope. It is important to fix organisms in formalin before washing, to prevent loss of an important endotoxin-like antigen. It is suggested that tests in which formalin-fixed toxoplasma are used have greater comparability with the dye test than tests in which crude cellular extracts are used as antigen.

Variations in microscopic equipment, lamp intensity and fluorescent reagents will cause variations in results of the fluorescent antibody test. The test should initially be standardised against the dye test and control sera used with every batch of tests.

Summary

The indirect fluorescent antibody technique for the detection of toxoplasma antibodies was investigated. A standard technique using formalin-fixed toxoplasma derived from mouse peritoneal exudate, and stored on slides at −15°C, was described. Tests on

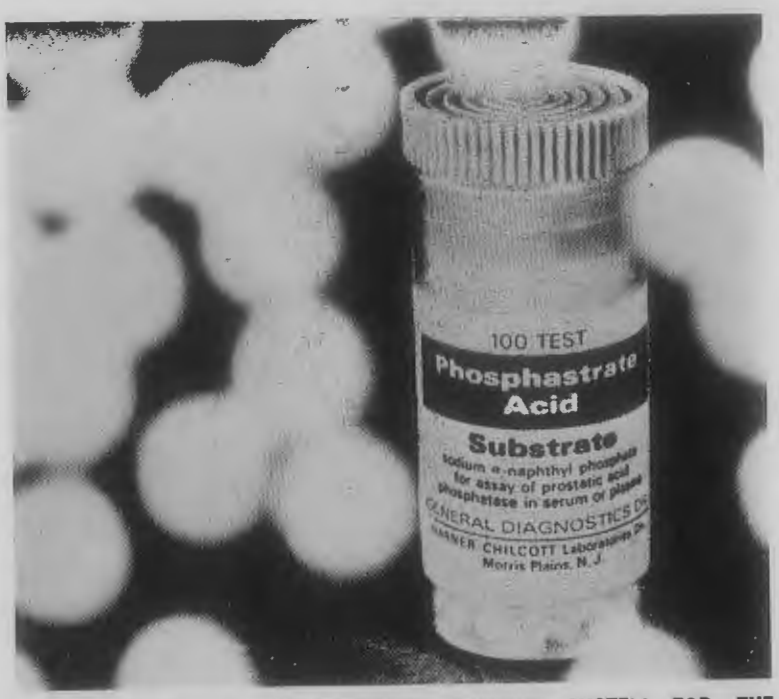
133 human sera showed good comparison with the Sabin-Feldman dye test. Factors affecting the sensitivity and specificity of the test, including the preparation of the antigen and the interference from anti-mouse antibodies, were described. Toxoplasma preserved by freeze-drying were found to be satisfactory for use in the test. The importance of using control sera and standardising the method against the dye test is mentioned. When the method is standardised it is an adequate substitute for the dye test.

Acknowledgments

I am greatly indebted to Dr J. D. Manning, Director, National Health Institute, for the dye test results. I am also indebted to Dr D. E. M. Taylor, Clinical Bacteriologist, Central Laboratory, for initiating this work, and to Mr A. Fischman, Charge Serologist, Central Laboratory, for his valuable advice during this work.

REFERENCES:

1. Bozdech, V., and Jira, J. (1961), *Dtsche Gesundh Wes.,* **16,** 2,397.
2. Camargo, M. E. (1964), *Rev. Inst. Med. Trop. S. Paolo,* **6,** 117.
3. Chordi, A., Walls, K. W., and Kagan, I. G. (1964), *J. Immunol,* **93,** 1024.
4. Chordi, A., Walls, K. W., and Kagan, I. G. (1964), *Ibid.,* **93,** 1034.
5. Fletcher, S. (1965), *J. clin. Path.,* **18,** 193.
6. Fulton, J. D., and Fulton, F. (1965), *Nature* (Lond.), **205,** 776.
7. Fulton, J. D., and Turk, J. L. (1959), *Lancet,* **ii,** 1068.
8. Fulton, J. D. and Voller, A. (1964), *Brit. med. J.,* **ii,** 1173.
9. Goldman, M. (1957) *J. exp. Med.,* **105,** 557.
10. Halling, L. W. (1968), *Amer. J. clin. Path,* **49,** 583.
11. Jacobs, L. (1967), *Advances in Parasitology,* Ed. Dawes, B. Vol. 5, p20. Academic Press, London and New York.
12. Jacobs, L., and Lunde, M. N. (1957), *J. Parasitol.,* **43,** 308.
13. Manning, J. D., and Reid, J. D. (1956), *N.Z. med. J.,* **55,** 441.
14. Sabin, A. B., and Feldman, H. A. (1948). *Science,* **108,** 660.
15. Siim, J. C., and Lind, K. (1960), *Acta. path. microbiol. scand.,* **50,** 445.
16. Sulzer, A. J., and Hall, E. C. (1967), *Amer. J. Epidemiology,* **86,** 401.
17. Suzuki, K., Suto, T., and Fujita, J. (1965), *Nat. Inst. Anim. Hlth Quart.,* **5,** 73.
18. Takumi, K., Takebayashi, I., Takeuchi, H., Ikeda, H., and Toshioka, N. (1966), *Japan. J. Microbiol.,* **10,** 189.
19. Voller, A. (1964), *Bull. Wld Hlth Org.,* **30,** 343.
20. Walton, B. C. Benchoff, B. M., and Brooks, W. H. (1966), *Amer. J. trop. Med and Hyg.,* **15,** 149.
21. Warren, J. and Sabin, A. B. (1942), *Proc. Soc. exp. Biol. Med.,* **51,** 11.
22. Wolf, A., Cowan, D., and Paige, B. (1939), *Science,* **89,** 226.



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The Expression of Blood Acid-base Results

G. R. GEORGE, F.N.Z.I.M.L.T.
Pathology Department, Rotorua Hospital.

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

The term acid-base is in itself inappropriate, since the only measurement which is actually made is one of hydrogen ion concentration when employing routine Astrup technique. These results are expressed as pCO_2 in mm. of mercury, pH in units of a negative logarithm, and bicarbonate ion in milliequivalents per litre. None of these units bear any direct relationship to each other, and do not afford visual evidence of their quantitative inter-relation.

For the purpose of this paper the many differing concepts and physiological interpretations are ignored in an attempt to simplify the situation and to present routine results in a practical manner which can be more easily understood.

The first step is to express all results in the same unit. All pH values are converted to nanoequivalents of hydrogen ion per litre by plotting pH units on a linear axis against nanoequivalents on a logarithmic scale.

Thus the two pH values at known carbon dioxide gas tension and the patient's measured pH are converted to nanoequivalents per litre. These are then plotted against pCO_2 in mm., both on logarithmic scales (See Fig. 1). This yields a straight line on which the point of the patient's pH and pCO_2 are noted at C also the point of intersection of this line with the horizontal 40 mm. line at D.

From this the *total hydrogen ion* $\triangle H$, is read counting from C the graph unit spaces to line XY. the vertical 40 nEq. value. This represents the change positive or negative from the mean normal of 40 nEq./l.

The Respiratory parameter R: is read similarly, being the unit distance between C and D. This measures the effect that a change of pCO_2 from the normal of 40 mm., has on the H ion concentration.

$$R = \text{Actual } H^+ \text{ blood} - H^+ \text{ at } pCO_2 \text{ of 40 mm. (nEq.)}$$

The Non-respiratory parameter NR; is measured as the distance between point D and line XY. When the blood has an abnormal hydrogen ion concentration and pCO_2 is 40 mm., there must be an alteration in NR.

$$NR = H^+ \text{ at } pCO_2 \text{ 40 mm.} - 40 \text{ (nEq.)}$$

Both these quantities are related to total hydrogen ion in the following manner:

$$\triangle H = R + NR \text{ (nEq.)}$$

All these quantities may have a positive or negative sign as indicated by the position on the graph.

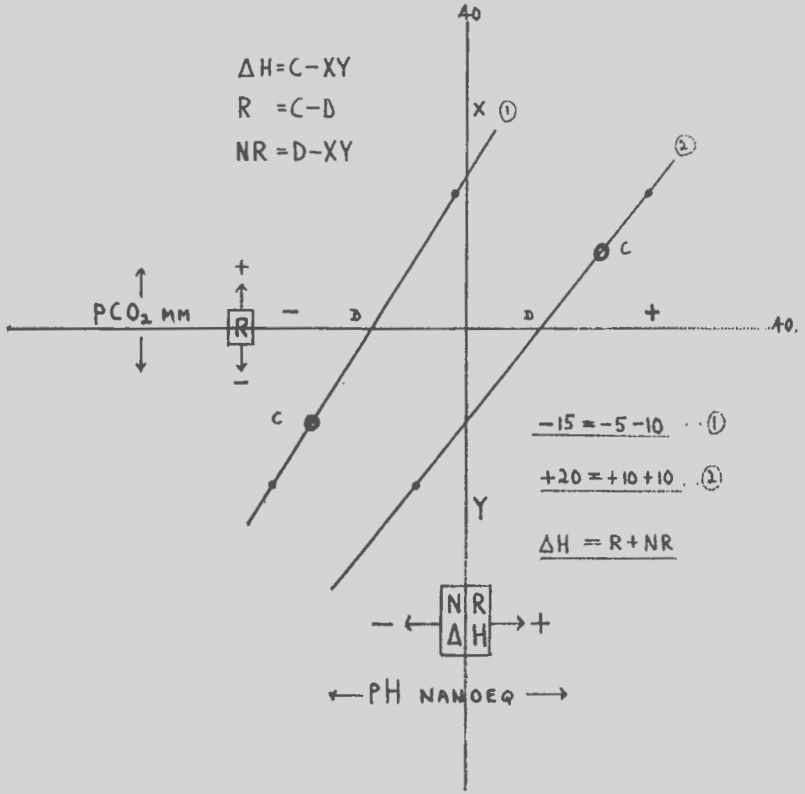

Fig 1: pH in nEq./l. plotted against pCO_2 in mm. to give positive and negative values of Δ H, R and NR.

The normal range for these three quantities is $\pm$ 4 nEq./l.

A further chart is prepared on linear graph paper (See Fig. 2), the central portion being the intersect of the 40 mm. pCO_2 and 40 nEq. hydrogen ion axis. The $\pm$ 4 nEq. limits of normal are drawn in the central portion, with the sides and centre lines projected.

The values for R and NR with the appropriate sign, which have been measured on the logarithmic graph (Fig. 1) are plotted using the linear scales to yield a point on Fig. 2. This represents the status of the patient and can be used for following progress on serial estimations toward the normal zone.

Any point to the right of the projected diagonal lines represents an increase in total hydrogen ion and to the left a decrease, and points between the lines are normal for Δ H. This affords a visual and easily recognisable indication of the state

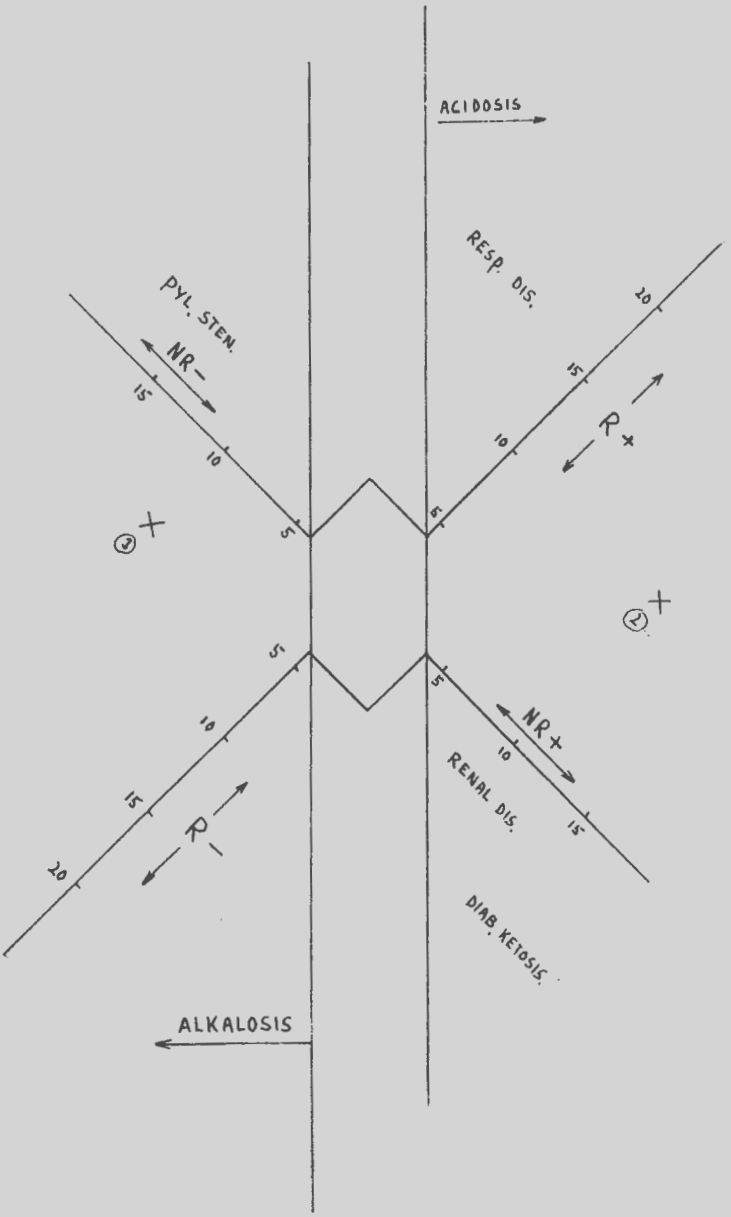

Fig. 2: R plotted against NR positive or negative, on linear scale to give relative △ H point. Values as in Fig. 1. Points in central area are normal. Points between parallel lines are normal for △ H, but compensated by abnormal R and NR values.

of the patient. It will be found that distinct clinical states such as respiratory failure, renal disease and pyloric stenosis produce points consistently in particular areas of this graph. The progress towards the central normal area is readily followed and the effect of treatment on the respiratory and non-respiratory parameter can be immediately apparent.

The determination of a single pH measurement in blood by itself reveals little or nothing, because a normal pH can be achieved by profound and compensatory changes in the extracellular system, which can be regarded as a normal defence mechanism to preserve the constancy of the intracellular hydrogen ion concentration, a condition essential to the continuation of life.

The differential assessment of these changes, which can be grouped as respiratory and non-respiratory, is clearly and quantitatively portrayed in this system of reporting and in a form which can provide most useful clinical information.

Either R or NR can be the primary cause of abnormal hydrogen ion concentration, the remaining quantity then being due to the compensatory effect. Clinical knowledge and an inspection of the result figures can easily decide this in most cases.

For example, in pyloric stenosis a typical result would be $-5 = +10 - 15$. The $+10$ respiratory factor would produce a high pCO_2 result which could erroneously be attributed to a state of respiratory acidosis. Inspection of the equation result shows a decreased total hydrogen ion —5, and therefore a state of alkalosis caused primarily by a decreased (—15) non-respiratory hydrogen ion, due to loss of hydrochloric acid.

This is compensated for by hypoventilation, which raises the respiratory figure and represents a healthy normal response to the condition. If this was treated as a respiratory condition then any assistance to improve respiration would lower the hydrogen ion still further, by reducing R, thus aggravating the alkalosis: *e.g.*, $-10 = +5 -15$

The only parameter being measured and considered is the hydrogen ion concentration. All results should be expressed as this quantity, in the same units of nanoequivalents which bear direct quantitative comparable relationships.

While pH, pCO_2 and MEq bicarbonate represent the functions which tell the whole story of the acidbase status they are conventionally expressed in units which have no direct easily envisaged quantitative relationship to each other. The total hydrogen ion, $\triangle$ H, representing the patient's pH is simply the net result of the activity of R and NR and these are the clinically-important parameters of practical interest. In this system their relationship and quantity is readily apparent, both in establishing the true nature of any abnormality and in reflecting progress or

effect of treatment which is directed at the primary underlying cause, rather than attempting to titrate arbitrarily a transient biochemical inbalance.

This system is an attempt to portray in a simple quantitative fashion the acid-base status which when expressed in the usual terms presents a confused and difficult situation to interpret.

This paper is a summary and interpretation of the reference quoted, which supplies greater detail.

REFERENCE:

1. Whitehead, T. P. (1967), *Advance in Clinical Chemistry.* Ed. H. Sobotka and C. P. Stewart, Vol. 9, 195-225. Acadamic Press, N.Y. and London.

Obituary

Graeme Campbell Taylor Burns, M.B., Ch.B., D.C.P.

Dr Burns died at his home in Christchurch on 22 November, 1969. He was Microbiologist to the North Canterbury Hospital Board from 1948 to 1964, when he retired for health reasons.

Educated at Christ's College and Otago University he graduated M.B. Ch.B. in 1938. His house surgeon years were spent at Christchurch Hospital and on the declaration of war he volunteered for overseas service and was commissioned as a captain in the Medical Corps, serving in the Pacific and the Middle East, and being mentioned in dispatches. He spent three and a half years as a prisoner of war following capture at Sidi Rezegh and during this time his health was affected.

At the end of the war Dr Burns went to London and studied at the Post Graduate Medical School, Hammersmith, gaining a Diploma in Clinical Pathology. He then worked at St Mary's Hospital, Paddington, before returning to New Zealand in 1948.

His main interests in microbiology were antibiotic sensitivity methods, syphilis and hydatid serology, mycobacteriology and, in later years, virology. In these fields and other aspects of microbiology he was always interested in the preparation and standardisation of reagents as described in original papers.

For a number of years Dr Burns was honorary librarian to the Canterbury Medical Library. He was also keenly interested in New Zealand literature and history, particularly that of Canterbury. Many will remember his ability as a raconteur by his colourful descriptions of local current and historical events. His wide knowledge of microbiological and medical literature was always readily passed on — in a concise form — to the technologists in his unit, and he always had a ready practical appreciation of methods used on the laboratory bench.

One of his outside interests before the war was as an active member of the New Zealand Alpine Club; he took part in climbs in the Rakaia and Rangitata headwaters.

Dr Burns is survived by his wife, daughter and two sons to whom we extend our sincerest sympathy.

G.R.R.

The Cusum Technique in Quality Control

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Received for publication, October, 1969.

Introduction:

Quality control techniques have been reviewed by Melton (1967) [1]. The disadvantages of these methods are that the standard deviation, which may vary with time and change of technique, must be known before a plot can be meaningfully interpreted. The calculations involved are time consuming and liable to error. The cusum technique requires the simplest of arithmetic and is sensitive to small changes in the mean. A complete account is given in *Cumulative Sum Techniques* [2] which is based on industrial quality control, and much of the information is of little use to the medical technologist. However, this book is invaluable for the description of the various forms of decision-making beyond the scope of the present paper.

Method

1. Choose a constant which is near the average for the test under examination.
2. Subtract this constant from the first control value, the difference will either be negative, zero or positive.
3. Repeat the subtraction of the constant with successive control values and add to the previous differences, these are the CUSUMS which are plotted against time.

Notes:

If the current mean has the same value as the constant, the differences will tend to cancel one another and the plot line will be parallel to the time axis. If the current mean is lower than the constant, the differences tend to be negative and the plot line will branch downward. The reverse is true if the current mean is higher than the constant. The angle of the plot line is an indicator of the current mean and changes in this mean are indicated by a change of direction of the line. It is the detection of these changes of direction with which the method is primarily concerned. Expanding the scale of the cusum axis will exaggerate such changes, while compressing it will tend to diminish them. A suitable scale is readily found with practice. The distance of the plot line from the time axis is of no significance and may be restarted from a more convenient point at any time. The constant may also be changed.

Typical Results:

Figure 1 is a plot of cholesterol values on the same serum over a period of three months and illustrates the difficulty of setting standard deviation limits. It might be concluded from this diagram that a steady deterioration has been taking place. Figure 2 shows the same data as a cusum plot which has been recommenced at the breakaway points (indicated by arrows) using a different constant. Three periods of relative stability are indicated making the steady deterioration theory less tenable.

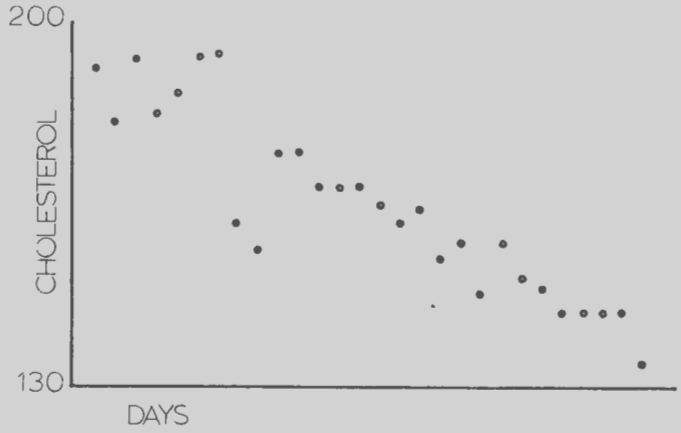

Fig. 1: Plot of cholesterol quality control values against time.

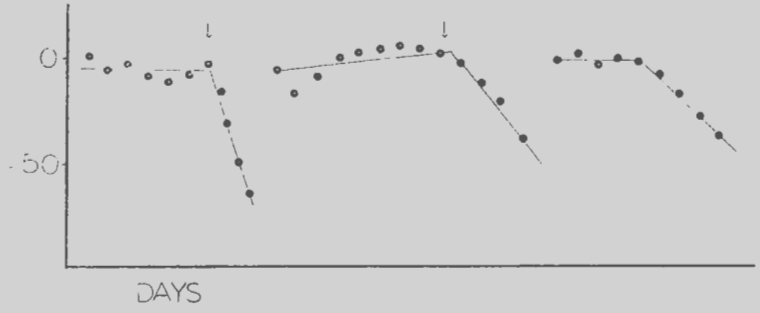

Fig. 2: Cusum plots of the cholesterol values in figure 1.

Figure 3 is of the total protein control values which are more typical of the variations encountered in practice. The upper plot is a cusum, and the lower a plot of untransformed values. The two standard deviations limits on the lower chart were derived from the preceding data which had been shown to be stable. While the cusum plot shows a change of mean value, none of the subsequent points on the lower chart appear outside two standard

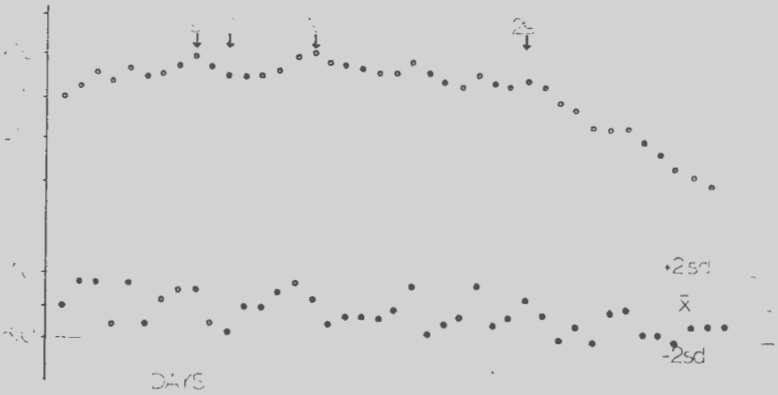

Fig. 3 — Lower: Plot of total protein quality control values against time with mean and two standard deviation limits. Upper: Cusum plots of the same values.

deviations. A rule "11 consecutive points on one side of the mean" has to be invoked before this change is seen. It is obvious that the mean and standard deviation will have to be recalculated on data subsequent to the breakaway point, otherwise the limits will be further increased making the sensitivity of this method even lower.

Interpretation:

Interpretation of the cusum chart (Fig. 3) is best appreciated if the plots are sequentially uncovered. A change of direction is indicated at day 9 but shortly reverts to the previous direction on day 11, so that lines from day 0-9 and 11-16 are parallel. Such a jump could indicate an error in the test or temporary change of operator. From day 16 the cusum decreases with small fluctuations until day 29 when a larger decrease is initiated, remaining consistent to the end of the series. In summary, small changes in trend start on days 11, 17 and 29. Changes in operator, reagents or instrumentation recorded on the chart facilitate interpretation. It has been noted that the quality control changes were sometimes recorded several days after a change had occurred in the general trend of routine results suggesting operator bias in "holding on" to previous control figures.

Conclusions and Summary:

The cusum technique is a simple statistical quality control method with the minimum of mathematics. It provides earlier detection of all deviations in trend attributable to staff changes or reagent deterioration than other methods of analysis.

REFERENCES:

1. Melton, Lynne M. (1967). *N.Z. J. med. Lab. Technol.*, **21**, 66.
2. Woodward, R. H. and Goldsmith, R. L. (1964), *Cumulative Sum Techniques*, Oliver and Boyd, London.

Hormone Assessment by Cytological Study of Urinary Tract Cells

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Abridged from a thesis submitted for the examination of Qualified Technical Officer in October, 1968.

Introduction

Cytological studies of cells exfoliated in urine specimens were originally stimulated by the finding that carcinomas occurring in the urinary tract could be detected by the presence of tumour cells in the urinary sediment. In the course of this development, Papanicolaou, while examining the urine of men with prostate carcinoma receiving oestrogen therapy, noted changes in the cytology of the urinary tract similar to those which occur in the vagina after oestrogen stimulation. Essentially the changes were found to consist of an increase in superficial cells, eosinophilia, cytoplasmic granules and vacuolation due to increased glycogen content[3].

Since the urethral epithelium is considered by many observers [1-6] to develop embryologically in common with the vaginal mucosa from a ridge formed by a proliferation of the mesoderm, it is not surprising that exfoliated cells from these areas are similar in appearance and reflect hormonal changes.

During the past 20 years a number of investigations have been made into the hormone changes observed in the urinary sediment cells, including studies by Lencioni [2], Castellanos [1] and O'Morchoe [4], which have confirmed the usefulness of the method for assessing the results of normal and abnormal endocrine influences. The present study was undertaken with the object of establishing base lines on normal individuals prior to introducing the technique as a routine procedure in this laboratory.

Procedure

Urine Specimens

A total of 420 early morning specimens of voided urine was obtained from a group of 11 volunteers consisting of three post menopausal women, six women with normal menstrual cycles and two men. Details of the group are given in Table I.

Subject	Sex	Age	Parity	Gravida	Menarche	Menstruation
B	Female	46	1	2	14	Post menopausal
E	Female	59	3	5	13	Post menopausal
K	Female	54	3	3	13	Post menopausal
A	Female	17	0	0	10	Regular
C	Female	22	0	0	11	Regular
F	Female	36	0	0	13	Regular
G	Female	19	0	0	12	Regular
H	Female	35	0	0	12	Regular
J	Female	20	0	0	13	Regular
D	Male	22	—	—	—	—
I	Male	35	—	—	—	—

TABLE I
Composition of group providing urine specimens

The six women with normal menstrual cycles were requested to provide daily urine specimens over a period of two months. Since it was felt that prior knowledge of their menstrual dates might influence cell counts, commencement of the collections was not related to a particular point of the cycle, and subsequent charting of the cycle was based solely on cytological evaluations. In some instances specimens could not be procured for one or two days at the weekends and counts for these days were omitted. It was, however, possible to obtain full cytological information on 11 complete cycles.

The purpose of including the post menopausal women and the male volunteers was to examine the day to day variations in cells not affected by ovarian hormones. Specimens were collected for varying periods of six to eight weeks, although with one subject, B, specimens were obtained for one week only. Occasionally several days elapsed without the collection of some specimens.

Specimens were processed immediately on arrival at the laboratory, or were refrigerated for short periods of time. Specimens collected at weekends were stored in refrigerators until they were brought to the laboratory.

Preparation of Smears

A preliminary evaluation of several methods of preparing smears showed that Lencioni's technique [2] was the most suitable and this was adopted throughout the investigation. An aliquot of 50 ml. of urine was centrifuged at 1500rpm for 10 minutes, the supernatant decanted and the deposit resuspended in 20ml. of Ringer's solution. This was again centrifuged for 5 minutes at 1000rpm, and the deposit transferred to glass slides with a pipette. The smears were evenly spread, allowed to dry at room temperature, and finally coated with a spray fixative *(Cytofix)* for storage.

Staining

After a number of trials with various staining techniques it was decided to use a combination of haematoxylin and Shorr's polychrome stain in the following method:—

Slides were placed in Harris' haematoxylin for eight minutes, rinsed and immersed in a solution of 70% alcohol containing 3% of ammonia for one minute. They were then transferred through graded alcohols containing 0.25% of glacial acetic acid until dehydrated, placed in Shorr III stain for 10 minutes, rinsed again in acid alcohols and absolute alcohol, cleared and mounted.

Cell Counts

Classification of the squamous cells followed the accepted definitions laid down in the 1958 Opinion Poll [5], and the indices used were those defined in the 1968 Symposium of Hormonal Cytology [7] for the maturation, karyopyknotic and eosinophilic indices.

Standard counts of 300 cells were made from each smear, using brightfield illumination for determining the maturation and eosinophilic indices, and phase contrast illumination for the pyknotic index. Squamous cells constituted the majority of the cell population, with a small proportion of transitional epithelial cells and occasional leucocytes. Mucus was frequently seen, appearing as a blue-green background. Calcium oxalate crystals and amorphous urates were present in some smears, and almost all included bacteria.

The staining method produced a sharp clear colouration of the cells, the cytoplasm of the transitional, parabasal, intermediate and a few superficial cells being blue-green, and a bright pink in the majority of the superficial cells. Nuclei of all cells stained varying shades of purple or dark red, while the pyknotic nuclei appeared almost black.

Results

Observation in Males

A total of 64 smears from subjects D and I was studied. The most striking feature was the paucity of epithelial cells in the smears as compared with any of their female counterparts. Mucus and spermatozoa were not uncommon and leucocytes were seen occasionally. The predominant staining reaction was cyanophilic. Cell counts were not determined on any smears from these subjects as squamous cells were too few in number and the general cytological picture showed little variation.

Observations in Post Menopausal Females

A total of 62 specimens were examined from the post menopausal subjects B, E and K. In general the smears consisted of an even spread of predominantly intermediate cells with little

clumping. Parabasal cells were seen in all these smears but in surprisingly low numbers, averaging 12%.

The maximum number of superficial cells seen in any of the smears was 6%. Non-nucleated cells, as described by Castellanos and Sturgis, were identified in many smears. Transitional cells appeared infrequently while mucus, bacteria and leucocytes in small numbers were often present. Crystals and spermatozoa were occasionally noted. Although the staining reaction was predominantly cyanophilic, occasional mature eosinophilic squamous cells and orange-stained non-nucleated cells were present in most smears.

When graphed serially, the maturation indices of the post menopausal subjects showed no more than erratic variations which lacked any specific pattern.

Observations in Normally Menstruating Females

A total of 294 smears was studied from the six subjects A, C, F, G, H and J. Smears from these subjects all contained ample cellular material which consisted almost entirely of intermediate and superficial squamous cells. Transitional cells in small groups were occasionally noted while numerous crystals and amorphous deposits were more frequently seen.

The menstrual phase was characterised by the relatively few cells present. Intermediate cells in small clumps predominated the smears but occasional parabasal and superficial cells were also present. Mucus was common in this phase and red blood cells were identified in some smears. The overall staining reaction was cyanophilic.

As the follicular phase advanced, cellularity was increased. There was a gradual rise in the proportion of superficial cells with flat, transparent cytoplasm, many of which contained cytoplasmic granules. The cells were more evenly spread and appeared singly or in loose groups. Eosinophilia accompanied this increase in superficial cells until a peak, presumed to denote ovulation, was reached. Immediately after this peak a sharp drop in superficial cells occurred and staining reaction became again predominantly cyanophilic.

The luteal phase, under the influence of progesterone, was characterised by an increase of intermediate cells. Many had translucent, wrinkled or folded and sometimes elongated cytoplasm, thickened cell borders and small, oval eccentric nuclei. These particular forms of intermediate cells are known as navicular cells, and were first described by Papanicolaou. Their presence, together with increased clumps of intermediate cells, in either urinary sediment or vaginal smears, is indicative of progesterone effect.

As the premenstrual phase approached an increased desquamation of cells was observed. Superficial cells were still seen but were out-numbered by groups and clusters of intermediate

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When represented graphically the serial karyopyknotic and eosinophilic indices showed a standard pattern for the members of this group, characterised by a slow build-up of superficial cells to an ovulatory peak between the 12th and 18th days of their cycle, followed by a sharp drop. A typical record, which includes two complete cycles is shown in Figure 1.

Relatively low peaks were seen in subjects A and G, but it was assumed that these were true ovulatory cycles because of the marked progesterone effect subsequently observed in both cases. Subjects C, H and J showed higher peaks and both C and J demonstrated classical oestrogen and progesterone responses with sharp, high peaks. Subject F showed no definite peaks but cellular changes indicated that these might have occurred on days when specimens were not collected.

Discussion

The pattern of changes observed in the squamous epithelial cells exfoliated in urine specimens is found to follow very closely that described for vaginal epithelium, and even though the menstrual dates were not known for the volunteers in this study, it was possible to identify each phase of the cycle.

It is of interest to note that in several normal 26-28 day menstrual cycles the karyopyknotic peaks appeared as late as the 18th day of the cycle. While this is probably caused by delayed tissue reaction to oestrogen produced in the ovary, it could also be that these are not true ovulatory peaks. However, to clarify this point it would be necessary to study further serial urine specimens in conjunction with quantitative chemical estimations.

Of the three cell indices used, the eosinophilic index appears to be the most uncertain as it relies on cytoplasmic colour alone. With varying pH of the urine specimens the affinity of the cells for staining reaction is thus altered, causing fluctuating eosinophilic percentages.

Sediment of voided urine was found to contain exfoliated cells from any part of the urinary tract and in the female, cells from the vagina and vulva, washed down in the urine stream, were also present. Erythrocytes seen in a few smears correlated with days of menstrual flow, spermatozoa were occasionally noted and the bacteria present in many smears morphologically resembled the vaginal flora.

The method of preparation, although time consuming, was simple, reproducible, involved the use of standard laboratory equipment only and achieved good results in both smearing and staining. The cells did not become distorted in smearing as many scrape or swab deposits do, because they were in suspension when spread on the slides and even after air drying, all constituents of the urinary sediment stained well with the Shorr III stain.

cells. Mucus and occasional leucocytes appeared in most smears during this phase and were present throughout the menstrual phase. With the onset of menstruation, cellularity rapidly decreased.

The difference in karyopyknotic peaks between all six subjects varied by 40%, but each subject showed fairly constant individual patterns of cell maturation during the cycles studied. The full range of variation in these individuals is shown in Table II.

Subject	Percentage of Superficial cells	
	Maximum	Minimum
A	23 16	1 1
C	33 52	6 4
F	29 23	1 8
H	37 40	9 5
J	56 46	2 1
G	24	3

TABLE II
Variation in proportion of superficial cells in normal menstrual cycles

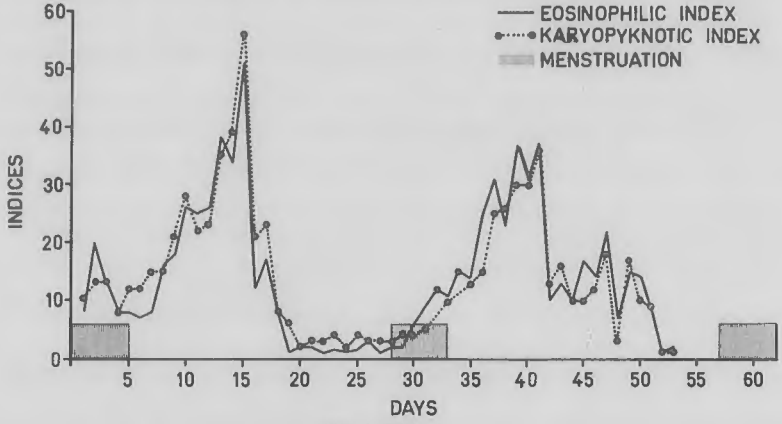

FIGURE 1
Serial karyopyknotic and eosinophilic indices, Subject J.

In view of these findings it would appear that epithelial cells from serial voided urine specimens provide a hormonal index equivalent to that obtained from serial vaginal smears. The technique has the added advantage, particularly in children and adolescents, that specimens can be obtained without vaginal manipulation and emotional trauma. This feature coupled with uniform cellularity of specimens and the fact that specimens can be stored for a short time, supports the view that urinary sediment smears could be the method of choice for the cytological assessment of many endocrine problems requiring sequential investigation.

Summary

Serial studies of cells in urine samples obtained from six normal females showed cyclic changes which reflect the hormone influences on menstruation.

It is considered that the procedure is a valid and useful cytological approach to the investigation of endocrine disorders, and offers a number of advantages over the vaginal scrape method.

Acknowledgments

The author wishes to thank Mr M. J. Churchouse and Miss J. E. Taylor for suggestions and advice during the course of this work, and Dr S. E. Williams for assistance in the preparation of this paper.

REFERENCES:

1. Castellanos, H., and Sturgis, S. H., (1958), *J. clin. Endocrin. Metab.*, 18, 1369.
2. Lencioni, L. J., and Staffieri, I. J., (1954), *Acta Endocrin.*, 16, 270.
3. McCallin, P. F., Taylor, E. S. and Whitehead, R. W., (1950), *Amer. J. Obst. Gynec.*, 60, 64.
4. O'Morchoe, P. J. and O'Morchoe, C. C., (1967), *Acta Cytol.*, 11, 145.
5. Opinion Poll on Cytological Definitions, (1958), *Ibid.*, 2, 1.
6. Solomon, C. P., Panagotopoulos, P., and Oppenheim, A., (1958), *Amer. J. Obst. Gynec.*, 76, 56.
7. Symposium on Hormone Cytology, (1968). *Acta Cytol.*, 12, 88.

Biographical Sketch

Benedict's Solution.

Who was Benedict?

Stanley Rossiter Benedict was born in Cincinnati on 17 March, 1884, the son of a Professor of Psychology and Philosophy at Cincinnati University. He graduated from Cincinnati and Yale Universities and, after teaching at Syracuse and Columbia Universities, took a position at Cornell University Medical College, where he taught biological chemistry until his death.

Benedict's claims to distinction are numerous; apart from being a teacher of wide repute his skill as an analyst was considerable. His friendly rivalry with Folin led to many important discoveries in biological chemistry. Not the least of these was the introduction of the reagent which bears his name, for the detection of urinary reducing substances.

Benedict's work in the field of metabolism covered an extensive area. Many investigations were carried out on glycosurias of various types, the creatine/creatinine problem, purine metabolism and his work on the formation of ammonia by the kidneys upset many of the accepted theories of that time.

He was a member of the National Academy of Sciences, a past President of the Society of Biological Chemistry, and at the time of his death, on 2 December, 1936, he was Managing Editor of the *Journal of Biological Chemistry.*

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Sample preparation
1. Resin extraction
2. Proceed to ashing

BUTANOL EXTRACTABLE IODINE (BEI)
Sample preparation
1. Resin extraction
2. Three Butanol Extracts (Supernatant)
3. Drying
4. Proceed to ashing

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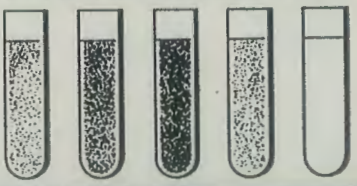

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Laboratories doing controlled routine work should expect to obtain values for individual constituents within the range of allowable variation when methods listed are used.

CONSTITUENT	MONI-TROL® I		MONI-TROL® II	
	per 100 ml	meq/liter	per 100 ml	meq/liter
Protein	± 0.2 gm		± 0.2 gm	
Glucose	± 6.0 mg		± 10.0 mg	
NPN	± 2.0 mg		± 4.0 mg	
BUN	± 1.0 mg		± 2.0 mg	
Creatinine	± 0.2 mg		± 0.3 mg	
Chlorides (as NaCl)	± 8.0 mg	± 1.4	± 10.0 mg	± 1.7
Phosphorus	± 0.2 mg	± 0.1	± 0.2 mg	± 0.1
Calcium	± 0.3 mg	± 0.15	± 0.3 mg	± 0.15
Sodium	± 5.0 mg	± 2.0	± 5.0 mg	± 2.0
Potassium	± 1.0 mg	± 0.25	± 1.0 mg	± 0.25
Magnesium	± 0.3 mg	± 0.25	± 0.5 mg	± 0.4
Iron	± 8.0 mcg		± 8.0 mcg	
TIBC	± 25.0 mcg			
Bilirubin	± 0.1 mg		± 0.3 mg	
Uric Acid	± 0.3 mg		± 0.5 mg	
PBI	± 0.3 mcg		± 0.5 mcg	
T3	± 2		± 2	
Cholesterol	± 10.0 mg		± 10.0 mg	
Enzymes	± 10%		± 10%	

ADDITIONAL VALUES*

Constituents	per 100 ml	Normal Adult Range per 100 ml		Method
THYROID PROFILE:				
Butanol Extractable Iodine	3.5 mcg	3.2-6.2	mcg	OADE
Thyroxine (T_4-Isotope f = 78.1)	7.3 mcg	4-11	mcg	Murphy-Pattee
Thyroxine Iodine (T_4 – column)	3.6 mcg	3.2-6.4	mcg	DADE
Thyroxine Binding Globulin	13.8 mcg	14-21	mcg	Beirwaltes-Robbins
Protein Bound Iodine	5.7 mcg			AutoAnalyzer† (N-56)
LIPID FRACTIONATION:				
Total Lipids	600 mg	400-1000	mg	Pernokis, et al.**
Phospholipids (as Phosphorus)	8.1 mg	7.2-12.2	mg	Dryer-Tammes-Routh, Mod.
Phospholipids (as Lecithin)	202 mg	180-305	mg	Factor***
Triglycerides	68 mg	0-150	mg	Carlson-Wadstrom, Mod.
Fatty Acids	285 mg	250-460	mg.	Stern-Shapiro
ENZYMES:				
Phosphatase, Alkaline	1.2 mM units/liter	0.8-2.9	mM units/liter	Bessey-Lowry-Brock
Phosphatase, Alkaline	4.4 units/100 ml	3-13	units/100 ml	King-Armstrong
Phosphatase, Alkaline	5.3 units/100 ml	3-13	units/100 ml (K-A)	AutoAnalyzer (N-6)
Phosphatase, Acid	0.08 mM units/liter	0.04-0.7	mM units/liter	Bessey-Lowry-Brock
Phosphatase, Acid	0.8 units/100 ml	0-4.0	units/100 ml	King-Armstrong
Phosphatase, Acid	1.0 units/100 ml	0-4.0	units/100 ml (K-A)	AutoAnalyzer (N-7)
Leucine Aminopeptidase	126 units	70-200	units	Rutenburgh-Goldberg-Pineda
Cholinesterase	0.93 units	0.41-1.65	Delta pH units	Michel
Aldolase	2.4 units	up to 6.0	milli-units/ml	Boehringer
Creatine Phosphokinase	31 units	up to 35	I.U.	Nuttall-Wedin, Mod.
MISCELLANEOUS:				
Phenylalanine	2.0 mg/100 ml	1-4	mg/100 ml	McCaman-Robins
Osmolality, milli-osmoles per kg serum water	267	300-320		
Tyrosine	1.22 mg/100 ml	0.82-1.30	mg/100 ml	Wong-O'Flynn-Inouye
THYMOL TURBIDITY:				
Coleman, Jr.	4.2 units	0-5.5	units	Buffer – Tris, pH 7.55 Units – Shank-Hoagland
Coleman II	3.4 units	0-4.5	units	
Leitz	3.3 units	0-4.5	units	
Klett	3.5 units	0-4.5	units	

*Averages of replicate determinations, the numbers of which are insufficient to produce Assay Values.

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***Factor 25 x Phospholipids (as Phosphorus)

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CONSTITUENTS AND ASSAY METHODS	per 100 ml	Conversion Factor mg/100 ml × Factor = meq/liter	meq/liter
TOTAL PROTEIN — Macro Kjeldahl and Improved Biuret	7.0 gm		
ALBUMIN — Improved Biuret	4.6 gm		
GLOBULIN — Improved Biuret	2.4 gm		
A/G RATIO	1.88		
GLUCOSE — Folin-Wu	99 mg		
Nelson-Somogyi	89 mg		
AutoAnalyzert (N-2a)	94 mg		
NON-PROTEIN NITROGEN — Koch-McMeekin	24.2 mg		
BLOOD UREA NITROGEN — Gentzkow-Masen	14.0 mg		
Kawerau (Monoxime)	13.7 mg		
AutoAnalyzer (N-1a)	15.3 mg		
CREATININE — Folin-Wu	1.1 mg		
AutoAnalyzer (N-11b)	1.0 mg		
CHOLESTEROL — DIRECT METHOD: Ferro-Ham	189 mg		
EXTRACTION or SAPONIFICATION METHOD:			
Abell (Anderson-Keys Modification)	172 mg		
AutoAnalyzer (N-24a)	166 mg		
CHOLESTEROL ESTERS	136 mg		
CHLORIDES (as NaCl) — Schales & Schales	595 mg.	0.171	101.7
Chloridometer††	596 mg	0.171	102.0
AutoAnalyzer (N-5a)	594 mg	0.171	101.6
PHOSPHORUS — Inorganic — Fiske-SubbaRow	3.6 mg	0.580	2.1
AutoAnalyzer (N-4a)	3.4 mg	0.580	2.0
CALCIUM - Total — Clark-Collip, Ferro-Ham	10.0 mg	0.499	4.99
Flame Photometer	10.2 mg	0.499	5.09
AutoAnalyzer (N-3)	10.0 mg.	0.499	4.99
Atomic Absorption	10.1 mg	0.499	5.05
SODIUM — Flame Photometer	315 mg	0.435	137
AutoAnalyzer (N-20a)	315 mg	0.435	137
POTASSIUM — Flame Photometer	16.8 mg	0.256	4.3
AutoAnalyzer (N-20a)	16.4 mg	0.256	4.2
MAGNESIUM — Orange-Rhein (Modified)	2.3 mg	0.823	1.9
Atomic Absorption	2.2 mg	0.823	1.8
IRON — Peters (Modified)	105 mcg		
IRON BINDING CAPACITY — Total - Peters-Giovanniello	340 mcg		
BILIRUBIN — Malloy-Evelyn	0.5 mg		
Jendrassik-Gróf-AutoAnalyzer (N-12b-P)	0.4 mg		
Malloy-Evelyn-AutoAnalyzer (N-12)	0.6 mg		
URIC ACID — Folin	4.9 mg		
Kern-Stransky	5.5 mg		
Caraway	5.2 mg		
AutoAnalyzer (N-13a)	5.4 mg		
PROTEIN BOUND IODINE — Hycel Dry Ash	4.9 mcg		
Barker — DADE	5.2 mcg		
T3 (Resin Sponge Uptake 25 C)	31.6%		
TBI (Thyro Binding Index)	0.99		
TRANSAMINASE-SGOT — Reitman-Frankel	27 units/ml		
TRANSAMINASE-SGPT — Reitman-Frankel	24 units/ml		
LACTIC DEHYDROGENASE — Cabaud-Wroblewski	285 units/ml		
AutoAnalyzer — DADE	295 units/ml		
DADE TETRA-FORM™	104 units/ml		
LD_5 — Wroblewski-Gregory	105 units/ml		
LDH ISOENZYMES — DADE ISO-FORM™	LD_5 (Heart) 17.8%; LD_1 (Liver) 12.6% LD_4 35.3%; LD_3 21.9%; LD_2 12.4%		
HBD — Rosalki	215 units/ml		
LIPASE — Cherry-Crandell	0.7 units/ml		
Tietz-Borden-Stepleton	0.4 units/ml		
AMYLASE — Somogyi-Saccharogenic	86 units/100 ml		
Somogyi-Amyloclastic (Dade)	62 units/100 ml		
Dade-Iodometric	73 units/100 ml		

SERUM PROTEIN FRACTIONS: Paper Strip Electrophoresis

	gm per 100 ml	Percent of Total Protein	Beckman, Microzone‡‡ Percent of Total Protein	Gelman (Cellulose Acetate) HR Buffer 0.05 Ionic Concn. Percent of Total Protein
Total Protein	6.90			
Albumin	4.49	65.0	63.0	64.0
Total Globulin	2.41	35.0	37.0	36.0
Alpha$_1$ Globulin	0.23	3.3	3.5	4.0
Alpha$_2$ Globulin	0.52	7.6	8.0	8.0
Beta Globulin	0.71	10.3	11.0	9.5
Gamma Globulin	0.95	13.8	14.5	14.5
A/G RATIO	1.86			

†Reg. T.M., Technicon Corporation, Tarrytown, New York.
††Reg. T.M., Buchler Instruments Inc., Fort Lee, New Jersey
‡‡Occasionally, using the Microzone technic, an additional peak appears between the beta and gamma zones. Divide by extending the Gaussian curves of the areas, and placing the perpendicular at the intersect.

For complete details of Assay Methods, see Dade Manual of Clinical Chemistry Procedures.
When methods other than those listed are used, different results may be obtained.

It was noted during experiments with the serum that the antibody produced the best antiglobulin results with a locally-made goat antiglobulin reagent which is normally found to be inferior to most commercial broad-spectrum reagents. This observation prompted an investigation into the sex of the eleven donors who had been screened out as compatible for the patient, and it was found that eight were male, while there was a distinct female preponderance amongst the donors rejected as unsuitable.

The numbers were scarcely great enough to be significant, but with high hopes, though with no optimistic expectation, a fresh blood sample was obtained from the patient and was sent to Dr Ruth Sanger at the Blood Group Research Unit in London, as the Dunedin panel had not been typed for Xg^a. There, it was tested by Dr Patricia Tippett, who identified the antibody as anti-Xg^a.

It would appear that this represents a second example of a naturally occurring anti-Xg^a, which is unique in that it presented as a room-temperature, saline-reactive antibody that became stronger and active by the indirect antiglobulin technique as a result of the transfusion of one unit of Xg^a positive blood.

By the time the sample was taken off for dispatch to London the antibody had lost its antiglobulin activity, but it remained easily detected in saline and was, as Dr Tippett confirmed, more avid at room temperature than at 37°C. or at 4°C.

Acknowledgments

The author's thanks are due to Dr Patricia Tippett for identifying the antibody and to Dr Ruth Sanger for her exultant enthusiasm at the find.

REFERENCES:

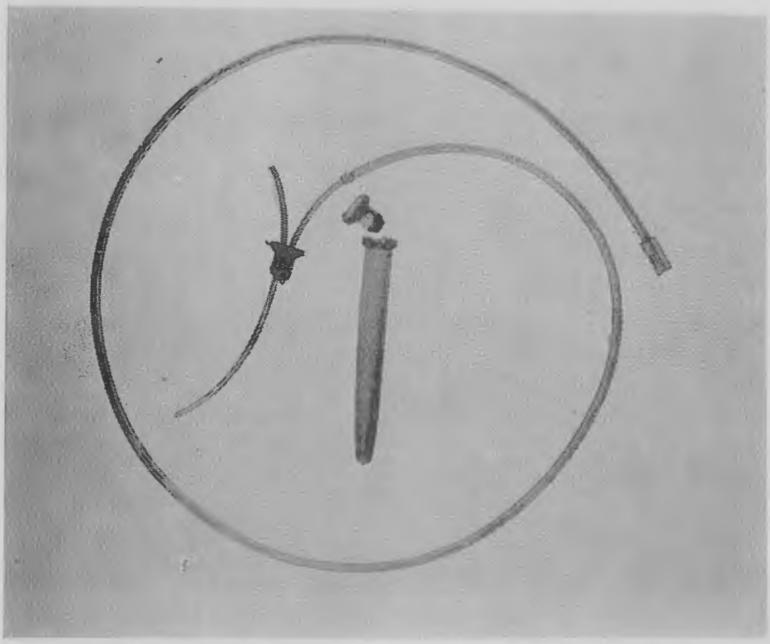
1. Allen, F. H. (1967) cited by Race, R. R. and Sanger, Ruth (1968) *Blood Groups in Man,* Fifth edition, p.532. Blackwell, Oxford.
2. Cook, I. A., Polley, Margaret J. and Mollison, P. L. (1963), *Lancet,* i, 857
3. Fisher, Nathalie (1965) cited by Race, R. R. and Sanger, Ruth (1968), *Blood Groups in Man,* Fifth edition, p.532. Blackwell, Oxford.
4. Mann, J. D., Cahan, A., Gelb, A. G., Fisher, Nathalie; Hamper, Jean; Tippett, Patricia; Sanger, Ruth and Race, R. R. (1962), *Lancet,* i, 8.
5. Sausais, Laima; Krevans, J. R. and Townes, A. S. (1964), *Transfusion, Philad.,* 4, 312.
6. Shepherd, L. P.; Feingold, Elaine and Shanbrom, E. (1969), *Vox. Sang, Basel,* 16, 157.
7. Yokoyama, M., Eith, D. T. and Bowman, M. (1967), *Ibid.,* 12, 138.

Technical Communication

Collection of Capillary Blood Samples

Sir,

The sucker illustrated was made to facilitate collection of capillary blood from babies.



Rigid polyethylene catheter tubing was inserted through two small holes bored in the stopper of a Beckman Spinco plastic centrifuge tube and the short arm was extended by sleeving into a piece of flexible plastic tubing of suitable bore. The long arm through which the blood is sucked must extend to the bottom of the tube to avoid air locks and regurgitation, and the short one should only project slightly below the stopper.

As always, free flowing drops are required, so that the blood can be drawn in before clotting interferes. To rinse the sucker, fill a plastic centrifuge tube with water, insert sucker and blow through.

Three tubes hold about 400 microlitres and are spun down in the Beckman Spinco microcentrifuge. The serum is transferred with a plastic pipette to a fresh plastic tube for storage. To facilitate access to the serum the tubes can be shortened by cutting with a scalpel.

R. D. ALLAN.

Selected Abstracts

Contributors: D. G. Bolitho, Alison Buchanan, B. M. Cornere, M. Jeannette Grey, J. Hannan, D. Tingle.

BLOOD BANKING

The Rapid Papainisation of Red Cells for the Detection of Rh Antibodies. Izatt, Marian, M., Blue, A. M., Muir, W., Sheridan, R. M. and Morrison, C. J. (1969), *Vox Sang., Basel,* 17, 157-160.

Washed, packed cells are mixed with cysteine-activated 1% papain for 10 seconds at room temperature in the proportion of one volume of cells to four of papain, then centrifuged through forty volumes of saline at 37°C for 3 minutes. After two further washes in warm saline the cells are ready for use.

A Simultaneous Modification of A Agglutinogen of Erythrocytes and Loss of Specific Platelet Antigens in a Patient with Gall Bladder Carcinoma. Majsky, A. and Jakoubkova, J. (1969), *Neoplasma,* 16, 297.

A simultaneous modification of RBC group antigens (two populations of erythrocytes, 76% A_2 and 24% A^m) and of specific platelet antigens (a transitory loss of two platelet antigens) in a patient of blood group A, D positive, is described. There is a discussion of possible mechanisms involved.

J.H.

CHEMICAL PATHOLOGY

Estimation of Faecal Fat Excretion using Cuprous Thiocyanate as a Continuous Marker. Lee, M. F., Temperley, J. M., and Dick, M. (1969), *Gut* 10, 754.

A method for the estimation of faecal fat excretion using cuprous thiocyanate as a continuous marker is described. Results obtained from a single stool sample are in close agreement with those from a three-day collection using this technique. The method is simpler and more accurate than collections done without a marker.

A.B.

On the Determination of Serum 5' — Nucleotidase Activity in the Presence of B-Glycerophosphate. Persijn, J. P., Van Der Slik, W. and Timmer, C. J. (1969), *Clin. Biochem.* 2, 335.

It was reported that the addition of 20m mol/L of glycerophosphate to the reaction mixture evaluated only "true" 5-Nucleotidase (5 Nu). The authors found that irrespective of its alkaline phosphatase (AP) activity the decrease in 5 Nu activity, measured by the colorimetric determination of adenosine liberated from adenosinemonophosphate, was directly proportional to the glycerophosphate concentration. Only in the range 1-8m mol/L of glycerophosphate was the 5 Nu not affected — being about 95% of the activity without glycerophosphate.

A.B.

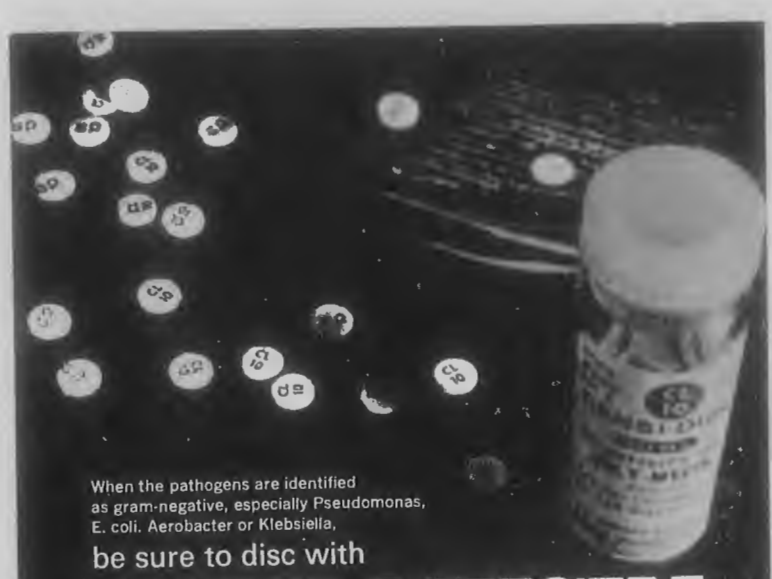
Enzyme Diagnosis in Hepatobiliary Disease. Coodley, Eugene L. (1969), *Amer. J. Gastro.* 52, 189.

Enzyme profiles have proved useful in separating the various causes of hepatobiliary disease. The uses of glutamic-oxaloacetic and glutamic-pyruvic transaminase, lactate dehydrogenase, guanase, isocitric dehydrogenase, sorbital dehydrogenase, leucine amino peptidase, alkaline phosphatase, 5-nucleotidase, ornithine carbamyl transferase, aldolase and alcohol dehydrogenase are discussed.

A.B.

Atypical Lactate Dehydrogenase Isoenzyme Pattern Caused by Immunoglobulin G Interaction. Kindmark C.-O. (1969), *Scand. J. clin. Lab. Invest.,* 24, 49.

A case of abnormal electrophoretic pattern of the serum lactate dehydrogenase isoenzymes is described. It is shown that this abnormality



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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	86.5
Gramnegative rods	103	64.1

* Number of positive cultures tested for colistin sensitivity. † Sensitivity at 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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was caused by an interaction between lactate dehydrogenase and immunoglobulin G. The complex dissociated continuously during electrophoresis; the "antibody" was therefore presumably of low avidity.

A.B.

The Fluorimetric Estimation of Cortisol in Human Plasma. Purves, H. D. and Sirett, Nancy E. (1969), *Aust. J. exp. Biol. med. Sci* **47**, 589.

A method similar to that of Mattingly (1962) is described. The effects of reagent composition, temperature of reaction and reaction time on the specificity of fluorescence measurements have been explored. Technical modifications have been made which include extraction of cortisol from plasma by vigorous agitation with methylene chloride followed by centrifugation, automatic pipetting of methylene chloride extracts and the use of tubes with fused on curvettes for the reaction with ethenol-sulphuric acid reagent.

A.B.

Serum Chromatography as a Screening Procedure for Inborn Errors of Amino Acid Metabolism. O'Kell, R. T. and Elliott, J. R. (1969), *Missouri Med.*, **66**, 661.

One dimensional paper chromatography of serum proved to be a simple and reliable method, extending far beyond the isolated parameter of the Guthrie test. It is simple enough to be performed daily in most hospital or large clinic laboratories. It is emphasised, however, that this and similar procedures are screening tests and that positive results require definitive confirmation.

In argininosuccinic aciduria, cystathioninuria and, possibly, homocystinuria, the renal clearance of the substances are so high that testing the urine is preferable.

J.H.

Disc Electrophoresis of Cerebrospinal Fluid Proteins on Polyacrylamide Gel. Cudny, D. and Wald, I. (1969), *Pol. med. J.*, **8**, 456.

The authers present a method, with separation of the proteins of non-concentrated cerebrospinal fluid of 82 patients being done. Electropherograms showed usually 16 protein bands with distinctly marked prealbumin, albumin and β-globulin bands. Quantitative determinations were carried out using a densitometer and on photographic films after taking photographs of the gel.

The diagnostic value of this method is still difficult to evaluate. Its diagnostic possibilities in cases of "blood-brain" barrier disturbances should be studied.

J.H.

An Improved Microcuvette for Photometric Measurements. Schneider, W. and Greger, R. (1969), *Pflugers Arch.*, **311**, 268.

The curvette is basically a 10mm long silver capillary tube closed on both ends by plates of quartz glass, the filling volume being 0.5 μl. The unit is held in place by a plastic adapter to assure reproducible positioning of the curvette.

J.H.

A New, Sensitive Determination of Phosphate. Eibl, H. and Lands, W. E. M. (1969), *Analyt. Biochem.*, **30**, 51.

This method uses Triton X-100 instead of reducing agents. Organic phosphate esters do not appear to interfere. All reagents are stable at room temperature and the absorbance is measured at convenient wavelengths.

J.H.

On the Quantitation and Analysis of Bile Pigments in Amniotic Fluid. Heirwegh, K. P. M., Meuwissen, J. A. T. P. and Jansen, F. H. (1969), *Biologia Neonat.*, **14**, 74.

Accurate determination of bile pigments in amniotic fluid is ham-

pered by several factors. The pigments are generally present in very small amounts and their nature and composition are still poorly understood. High turbidity due, among other things, to vernix caseosa and the presence of a variety of coloured compounds further complicate analysis.

The authors review some trends in the quantitation and analysis of amniotic bile pigments. The merits and pitfalls of currently used methods are examined, ways being indicated to increase the sensitivity and selectivity of some procedures. Finally, it is suggested that determination of conjugated bilirubin in amniotic fluid samples may become of interest.

J.H.

A Simple Thin Layer Chromatographic Screening Test for the Detection of Hyperaminoacidaemias. Kraffczyk, F., Helger, R. and Lang, H. (1969). *Z. klin. Chem. u. klin. Biochem.*, **7**, 521.

Plasma is chromatographed on precoated cellulose plates using the elulant butanol/acetone/acetic acid/water (35:35:10:20, v/v) with important types of pathological rises being detected by comparison with a standard chromatographed on the same plate. Histidinaemia is detected by chromatography with tertiary butanol/ethylmethylketone/25% ammonia/diethylamine/water (50:30:10:0.4:20, v/v). With the aid of a reagent set, this method can also be used in laboratories which do not have experience in thin layer chromatography.

J.H.

The Examination of Amniotic Fluid in the Management of Haemolytic Disease of the Newborn. Cussen, G. H., Sister St. Colum, and Magner, J. W. (1969). *J. Ir. med. Ass.*, **62**, 352.

For measuring bilirubin, a chemical method appears to be better than a spectrophotometric one now that a satisfactory technique is available and it is also more reliable in the presence of haemolysis, should this occur in the taking of the specimen.

J.H.

Calcium Binding Capacity of Plasma Proteins in Certain Diseases. Adamski, A. and Smarz, C. (1969), *Pol. med. J.*, **8**, 304.

An increase in calcium binding capacity of plasma proteins can reduce the concentration of ionised calcium to an extremely low level thus leading to tetany, despite marked hypercalcaemia. Another author has observed some disturbances in blood coagulation resulting from a deficiency of ionised calcium.

J.H.

The Simple Clinical Evaluation of Renal Function. Vidt, D. G. (1969). *Penn. Med.*, **72**, 77.

None of the five tests discussed in this paper meet the specifications of the ideal renal function test, yet when used singly or in combination, these tests provide valuable information in regard to the overall adequacy of renal function: urinalysis, serum creatinine concentration, endogenous creatinine clearance, PSP, and urinary concentration test.

It is the author's view that the urine is best examined by the physician responsible for the patient. While it is common to rely on the BUN as an indicator of renal function, unfortunately the BUN is neither specific nor sensitive and does not become elevated until the glomerular filtration rate has declined by as much as 60%. Much more specific than the BUN is the serum creatinine concentration but, as with the BUN, it provides no indication of renal impairment until the renal clearance has been substantially reduced. The endogenous creatinine clearance becomes nearest to being the ideal test.

In early renal impairment, the 15 minute PSP excretion may be reduced, whereas when time for repeated recirculation is allowed, the 2 hour excretion may be normal. Measurement of the specific gravity

of the urine after a period of dehydration provides on extremely simple, yet reasonably accurate assessment of tubular function. Impairment in the ability to concentrate is an early feature of most generalised renal diseases which may be detected even when results of other tests of renal function still are normal. A specific gravity of more than 1.023 obtained on any random specimen or after overnight dehydration indicates normal tubular function.

It may be said that a carefully performed urinalysis will provide invaluable information regarding the presence of renal disease, while the remaining 4 tests provide an adequate clinical assessment of renal excretory function. Specific diagnosis will depend upon other studies, such as intravenous urography, pyelography, selective renal angiography, or renal biopsy.

J.H.

HAEMATOLOGY

Multichannel Continuous Flow Analysis on the SMA-4/-7A. Nelson, M. G. (1969). *J. clin. Path.*, **22,** suppl. (Coll. Path.) **3,** 20.

This paper on automation in Haematology is part of an excellent issue on automation and data processing in pathology, edited by Prof. T. P. Whitehead. In Nelson's paper, the use of the SMA-4/-7A is discussed. The haematocrit by electrical conductivity is evaluated together with the quality control, cost, speed, precision and accuracy of the SMA-4/-7A.

M.J.G.

Inheritance of an Increased Sodium Pump in Human Red Cells. Wiley J. S. (1969), *Nature,* (Lond.) **221,** 1222.

This paper discusses a possible explanation of the appearance of both more sodium pump and a greater sodium leak in red cells of patients with hereditary spherocytosis. A mutation at a control gene site is suggested as the cause. Nevertheless, abnormalities in membrane structure in hereditary spherocytes have not been excluded.

M.J.G.

Regulation of Iron Entry into Reticulocytes. 1. Feedback Inhibitory Effect of Heme on Iron Entry into Reticulocytes and on Heme Synthesis. Ponka. P., and Neuwirt. J. (1969). *Blood,* **33,** 690.

Experiments are described in which the authors followed iron incorporation into reticulocytes during haem synthesis inhibited by haem, lead or isonicotinic acid hydrazide and during globin synthesis inhibited by cycloheximide. They showed that iron does not enter the red cell independently of haem synthesis and that there exists a feedback mechanism by means of which haem or haemoglobin prevents surplus accumulation of iron inside the cell.

M.J.G.

Antilymphocyte Serum: Present Status. Shanfield, L., and MacLean, L. D. (1969), *Canad. med. Ass. J.* **100,** 925.

The historical and development aspects of anti-lymphocyte serum are reviewed. Mode of action is discussed and also techniques of assay for the immunosuppressive potency of antilymphocyte serum. The review concludes with short sections on the preparation of, and treatment with, antilymphocyte serum. A concise five-page review of an important topic in the world of transplantation.

M.J.G.

G-6-PD Bangkok: A New Variant Found in Congenital Non-Spherocytic Haemolytic Disease. Talalak, P. and Beutler, E. (1969), *Blood,* **33,** 772.

A new variant of glucose-6-phosphate dehydrogenase was found in a Thai boy who had typical clinical and haematological findings of congenital non-spherocytic haemolytic anaemia. This mutant has normal electrophoretic mobility, normal affinity for substrate G-6 P and NADP and is very labile to heat. It has an activity of about 5% of normal.

M.J.G.

Screening for Vitamin B_{12} Deficiency. Annotation; (1969), *Lancet,* **ii,** 309.

A concise review of methods for screening for Vitamin B_{12} deficiency. The twenty-four recent references listed in the review provide an up-to-date key to the subject and cover all aspects. It is suggsted that the immunofluorescence method for detecting antibodies in serum to gastric parietal cells is a screening procedure preferable to serum vitamin B_{12} assay; the subject is debated. Screening of psychiatric patients is discussed; also the reduced serum B_{12} levels due to folate deficiency.

M.J.G.

New Unstable Haemoglobin Boris: beta88 Leucine → Arginine. Hollender, A., Lorkin, P. A., Lehmann, H., and Svensson, B. (1969), *Nature,* (Lond.), **222,** 953.

This is a report on a new unstable haemoglobin, Boris, causing a haemolytic anaemia with anaemia, an increased tendency towards methaemoglobin formation, jaundice and red cell inclusion bodies following splenectomy. Electrophoresis and "fingerprinting" of the new haemoglobin are described. The amino acid leucine is substituted for arginine in the haem pocket.

M.J.G.

Factor XIII Deficiency; Treatment with Monthly Plasma Infusions. Greenberg, L. H., Schiffman, S., and Stuart Wong, Y. S. (1969), *J. Amer. med. Ass.,* **209,** 264.

A thirteen year old boy with congenital factor XIII deficiency and a history of bleeding since birth was successfully treated with monthly infusions of a single unit of fresh-frozen plasma. Routine laboratory coagulation screening tests did not diagnose this condition; the factor XIII deficiency was confirmed in the laboratory by demonstrating solubility of his clot in 5 M urea.

M.J.G.

Sickle Cell Anaemia — First International Symposium, (1969) *J. trop. Pediat.,* **15,** 50.

This is an interesting three page report of summaries of thirteen papers given at the first International Symposium on sickle cell anaemia held in Jamaica, January 8-10, 1969. Such well-known names as Professor H. Lehmann and Dr E. Huehns are among those giving the papers. Recent techniques for the study of sickle cells are mentioned, ranging from use of lasers to ultra-centrifugation. The significance of high platelet counts in sickle cell patients was discussed. Clinical dangers even in sickle traits are mentioned. It is to be hoped that the papers summarized in the report will be published fully before long.

M.J.G.

Autoantibodies in Healthy Subjects: Whittingham, Senga, Irwin, Janet, MacKay, I. R., Marsh, S., and Cowling, D. C. (1969), *Aust. Ann. Med.,* **18,** 130.

These authors determined the prevalence in an apparently healthy Australian population of the following autoantibodies:—anti-nuclear antibody, rheumatoid factor, smooth muscle antibody, anti-mitochondrial and anti-microsomal antibody, and antibody to adrenal, thyroid and gastric cells. Table I of this paper is well worth consultation. The percentage of females with one or more autoantibodies was greater than that of males throughout life, and prevalence of autoantibodies increased with age in both sexes. Over the age of 60 years, more than 50% of subjects had one or more autoantibodies. These facts must be taken into account when assessing results of auto-immune serological reactions.

M.J.G.

Haemolytic Anaemia with Positive Coombs' Test: Association with Isoniazid Therapy, Robinson, Margaret G., and Foadi, Minou (1969) *J. Amer. med. Ass.,* **208,** 656.

The second known case of haemolytic anaemia associated with administration of isoniazid having a positive direct Coombs' test is reported as above. After a year of isoniazid therapy, a Negro child suddenly developed severe anaemia with haemoglobinuria. A direct Coombs' test with standard gamma Coombs' serum was positive. The Coombs' test became negative when isoniazid therapy was stopped.

M.J.G.

Survey for Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in Polynesians. Nixon, A. D. and Buchanan, J. G. (1969) *Amer. J. hum. Genet.,* **21,** 305.

One thousand, two hundred and forty-five Polynesians living in Auckland, New Zealand, were screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency using the 2, 6-dichlorophenol-indophenol dye decolorisation test. One part-Maori youth with G6PD deficiency was detected among 540 male and 264 female Maoris and part-Maoris tested. His mother and maternal grandmother were heterozygous for G6PD deficiency; they may, however, have inherited the abnormal G6PD gene from a non-Polynesian ancestor. No G6PD deficiency was detected among 199 Samoans, 158 Cook Islanders, 53 Niue Islanders, 20 Tokelau Islanders, and 11 Tongans. The finding of a very low incidence of G6PD deficiency in Polynesians is consistent with the theory of selection for the G6PD deficiency gene in the presence of endemic malaria. Malaria has never been endemic in any part of Polynesia.

M.J.G.

Cytochemical Aspects of Leukaemia and Lymphoma. Hayhoe, F. G. J. (1969), *Semin Haem.,* **6,** 261.

In this paper, Hayhoe reviews the use of cytochemistry in the diagnosis and classification of leukaemic cells, in the control of treatment and in basic research. The four cytochemical methods mentioned are periodic acid — Schiff (PAS) technique, Sudan black B staining, alkaline phosphatase demonstration and peroxidase demonstration.

The whole issue of this journal is devoted to leukaemia and lymphoma including genetic aspects and clinical epidemiology of leukaemia. The following issue, No. 4, is also entirely on the subject of leukaemia and lymphoma, but the papers deal almost entirely with treatment of cases.

M.J.G.

Discovery of the L. E. Cell and Its Morphology. Hargraves, M. M. (1969), *Mayo Clin. Proc.,* 44, 579.

Dr Hargraves gave this paper at a Symposium in 1968 to commemorate the 20th anniversary of the discovery of the lupus erythematosus cell by Hargraves, Richmond and Morton. Apart from the interesting account of the discovery, the descriptions and details of L.E. cells given are essential reading for all who ever test and search for L.E. cells. Morphology of cells at stages during the test is particularly interesting.

The rest of this September 1969 issue consists of eight other papers on aspects of systemic lupus erythematosus, including methods of detecting anti-nuclear antibodies.

M.J.G.

Quantitative Enzyme Cytochemistry of Leukaemic Cells. Stuart, J., Bitensky, Lucille and Chayen, J. (1969), *J. clin. Path.,* **22,** 563.

A new technique for the quantitative estimation of glycolytic and respiratory enzyme activity in intact and unfixed bone marrow and peripheral blood cells is described. The method gives a two- to three-fold

increase in demonstrable enzyme activity per cell compared with existing techniques using fixed marrow film preparations, and is particularly applicable to the study of relatively fragile cells such as the leukaemic lymphoblast.

M.J.G.

Effect of Incubation Time on the *L. casei* Bioassay of Folic Acid in Serum. Streeter, A. M. and O'Neill, B. J. (1969), *Blood,* **34,** 216.

This report of five pages discusses investigations into a possible source of error leading to non-reproducible results in the bioassay of serum folic acid using *L. casei.* Standard tubes are usually prepared from dilutions of pteroylglutamic acid, but serum contains folic acid in a different form. The authors show that the growth rate of *L. casei* differs in these two environments unless sufficient incubation time is allowed until the available folate is exhausted.

M. J. G.

Effect of Antimicrobial Agents on the Euglena Method of Serum Vitamin B_{12} Assay. Lie, J. T., Ungar, Berta and Cowling, D. C. (1969), *J. clin. Path.,* **22,** 554.

Antimicrobial agents in the serum may affect the results of the Euglena method of serum vitamin B_{12} assay. Sulphonamides suppress the growth of Euglena in concentrations attainable in the serum during treatment; streptomycin, chlortetracycline, erythromycin, kanamycin, and nitrofurantoin bleach Euglena but only when present in concentrations far exceeding the normal peak therapeutic blood levels. False low results of serum vitamin B_{12} assay due to inhibitory and/or bleaching substances in the serum can be readily detected by microscopy of the assay cultures and Euglena cell counts.

M.J.G.

An Evaluation of the Coulter Model S. Barnard, D. F., Carter, A. B., Crosland-Taylor, P. J. and Stewart, J. W. (1969), *J. clin. Path.,* **22,** suppl. (Coll. Path.) **3,** 26.

This paper is another from an issue on automation and it reviews all aspects of the reliability of Coulter Model S fully automatic haematology tests: cell counts, haemoglobin, P.C.V., M.C.V., M.C.H.C., and M.C.H.

M.J.G.

Activated Partial Thromboplastin Time Test in Heparin Therapy. Simmons, A. and Cross, D. E. (1969), *Missouri Med.,* **66,** 430.

The authors studied 101 patients receiving herapin therapy, comparing the conventional Lee-White with an activated partial thromboplastin time (APTT) test. It was concluded that the APTT is a most sensitive and technically more reproducible test than the Lee-White for this evaluation; its use is recommended.

J.H.

HISTOPATHOLOGY

Staining of Immature Collagen by Resorcin-fuchsin in Infant Kidneys. Joiner, D. W., Puchtler Holde, Sweat Faye, (1968). *J. roy. mic. Soc.,* 88, 461-471.

Human kidney tissues obtained at postmortem were fixed in Zenker-formol, 10% formalin and Carnoy's fixative. Paraffin sections were stained by several methods and examined microscopically by ordinary light, polarised light and ultraviolet light. Following fixation in Zenkerformol and 10% formalin binding of resorcin-fuchsin by collagen from infants was abolished, Carnoy's fluid did not show any blocking effect.

Staining properties of resorcin-fuchsin appeared to depend on the

quality of the dye, crystalline resorcin-fuchsin with a strong metallic sheen and no tendency to clump yielded optimum results.

Resorcin-fuchsin stained young collagen moderately to intensely, whereas mature collagen was unstained.

D.T.

A Quantitative Investigation into the Effect of Fixation, Temperature and Acid Strength upon the Feulgen Reaction. Murgatroyd, L. B., (1968), *J. roy. mic. Soc.* **88,** 133-139.

Small pieces of mouse pancreas were fixed in Carnoy's solution, Helly's fluid, 10% neutral formalin and Heidenhain's Susa. Sections were hydrolysed in:—5N HCl at 56°C. for times varying from 5 seconds to 30 minutes, 1N HCl at 56°C. for 1 to 45 minutes and 5N HCl at 20°C. for 1 minute to 4 hours. Following hydrolysis all the sections were stained in Schiff's reagent for 45 minutes. The staining intensity of the nuclei was measured spectrophotometrically on a Barr and Stroud scanning microspectrophotometer at a wavelength of 570 mμ. Graphs are given plotting relative absorbance against hydrolysis time. For quantitative work Helly fixation followed by dydrolysis in either 5N HCl at 20°C. for 2½ hours or 1N HCl at 56°C. for 30 minutes is advocated.

D.T.

MICROBIOLOGY

Interpretation of Disc Sensitivity Tests on Organisms of Intermediate Sensitivity. Waterworth, Pamela W. (1969), *J. med. Lab. Tech.,* **26,** 100-110.

In this article the author discusses interpretation of sensitivity in instances where a control organism is used, and is most emphatic about the use of control organisms in antibiotic sensitivity testing. This paper gives some extremely useful information on interpreting sensitivity in acquired resistance and natural resistance as well as the problem of the resistant Pseudomonas. The laboratory control of chemotherapy has become increasingly complex, and it is really necessary to have sufficient knowledge of chemotherapy to be able to select the drugs appropriate both for the organism involved and the site of the infection, and to be able to interpret correctly the results obtained.

B.M.C.

Quality Control in Bacteriology. Smith, J. P. and Sandlin, Carol. (1969). *Amer. J. med. Technol.* **35,** 531.

This paper describes a comprehensive programme for controlling media and reagent quality. the authors also discuss methods of assessing the overall efficiency of the bacteriology laboratory by the use of "unknown specimens." The difficulties of arranging suitable "unknown," specimens are discussed. The advantages of introducing a Quality Control System in stages and integrating quality control tasks into the daily routine of the laboratory are stressed.

D.G.B.

Occurrence of *Neisseria gonorrhoea* in Routine Genital Cultures. Ellner, P. D. (1969) *Amer. J. clin. Path.,* **52,** 174.

The importance of examining all genital specimens for gonococci whether clinically suspected or not is stressed in this paper. In one year the author's laboratory found that 57% of isolates of *Neisseria gonorrhoea* were from routine genital cultures.

D.G.B.

Formaldehyde: A Photothermal Activated Toxic Substance Produced in Middlebrook 7H10 Medium. Miliner, R. A., Stottmeier, K. D., Kubica, G. P. (1969). *Amer. rev. Resp. Dis.,* **99,** 603.

The authors describe the investigations leading to their finding that formaldehyde, in sufficient quantities markedly to inhibit Mycobacterial

growth, is produced from glucose by thermal decomposition and from glycerine, photoactively, in Middlebrook 7H10 medium. A simple modification in the method of preparation of the medium to overcome this problem is suggested.

D.G.B.

Staining of Urinary Leucocytes as an Aid to the Diagnosis of Inflammation of the Urinary Tract. Harris, D. M. (1969), *J. clin. Path.*, **22**, 492-495.

Five hundred specimens of urine were examined for pyuria and bacteriuria, leucocytes being stained by the Sternheimer-Malbin method. The presence of leucocytes in urine was usually related to the bacterial count, pyuria being commonest in urines showing significant bacteriuria. Leucocytes with blue staining nuclei by the Sternheimer-Malbin technique were considered to be indicative of active inflammation, but the incidence of such cells appears to be a reflection of the total leucocyte count of the specimen rather than of its viable bacterial count. The technique appears to have a limited use, restricted to the interpretation of cases in which the results of culture and conventional leucocyte counts are ambiguous.

D.G.B.

Modified Microbiological Assay for Rapid Estimation of Antibiotic Concentrations in Human Sera. Story, S. Ann (1969), *Appl. Microbiol.*, **18**, 31-34.

Because treatment with various antibiotics can cause serious toxic reactions, depending on renal impairment, dosage and mode of excretion, it is often advantageous to know the serum concentration before subsequent dosage. Although assay procedures are available, many small laboratories may not possess the necessary reagents or equipment to perform them. Generally these procedures require 16 to 24 hours to obtain results, which can be too long. This article describes the disc-plate assay method which permits approximation of antibiotic concentration in serum after 5 to 6 hours of incubation using a preincubation of the plates for rapidity of results.

B.M.C.

Timely Topics in Microbiology: Enterics 1967-1968. Brawson, Dorothy (1969), *Amer. J. med. Tech.*, **35**, 176.

This article lists useful and new developments in enteric organisms, disease and techniques, in items taken from the literature of October, 1967-October, 1968. Because new variations of organisms are continually arising, and pigeon-holing them becomes increasingly difficult, the items in this article should present interesting facts for most microbiologists and keep us in touch.

B.M.C.

Fluorescent and Serologic Technics in the Identification of Enteropathogenic *Escherichia coli*. Swierczewski, J. A., Liber, M. and Hill, S. (1969), *Amer. J. clin. Path.*, **52**, 495.

The authors discuss various techniques for the rapid isolation and identification of enteropathogenic *E. coli*. The fluorescent antibody technique was found to be a useful screening technique with a high degree of sensitivity but lacking specificity. A combined technique is recommended.

D.G.B.

Reliability of Biologic Autoclave Sterilization Indicators. Laskaris, T. and Chaney, A. L. (1969), *Amer. J. clin. Path.*, **52**, 495.

This paper discusses the heat resistance and stability of heat resistance after storage of commonly used commercial spore strips for autoclave efficiency testing. The authors present evidence of a considerable loss of heat resistance occurring within the listed expiry period of many strips even when these had been correctly stored. The practice of using *Bacillus subtilis* var *globigii* as an indicator of autoclave efficiency is condemned.

D.G.B.

A Modified test for Brucella Agglutinins. Singh, J. N. (1969), *J. clin. Path.*, **22**, 527.

0.1% protamine sulphate in 0.85% saline is suggested as a diluent for carrying out Brucella agglutination tests. The author claims that this preparation is an adequate substitute for the Coombs test to detect non-agglutinating Brucella antibodies.

D.G.B.

A Quality Control Program for Clinical Microbiology. Russell, R. L., Yoshimori, R. S., Rhodes, T. F., Reynolds, J. W., Jennings, E. R. (1969). *Amer. J. clin. Path.*, **52**, 489.

This paper describes a comprehensive system of quality control suitable for a large microbiology department. The system described is very well thought out and controls all aspects of microbiology, including mechanical equipment and technical procedures. The system has been in operation for one year and is claimed to have effected major improvements in microbiology.

D.G.B.

Effect of Medium Composition on the Apparent Sensitivity of *Pseudomonas aeruginosa* to Gentamycin. Garrod, L. P., and Waterworth, P. M. (1969), *J. clin. Path.* **22**, 534.

This paper discusses variation in the size of inhibition zones of *Ps. aeruginosa* to Gentamycin on various media and variation of the M.I.C. when the antibiotic was incorporated in the medium. These variations were found to depend upon the agar used to solidify the media as well as the general composition of such media. The variation was traced to the magnesium content of the media, a higher magnesium level being associatd with an increased M.I.C. and vice-versa. The authors note that *Esch. coli* does not show this variation with the magnesium content of the medium and point out the desirability of testing the sensitivity of *Ps. aeruginosa* strains in comparison with another strain of the same organism of known sensitivity.

D.G.B.

Cryptococcosis — A Report of 3 Cases with Special Emphasis on Management and a Review of the Local Literature. Supramaniam, J. M. J., Choy, N. K., Tambyah, J. and Woon, G. C. (1969), *Singapore med. J.*, **10**, 155.

Demonstration of the organism in the cerebrospinal fluid is the only absolute diagnostic measure and in the investigation of a problem of meningitis where the causative organism is not obvious, an India ink preparation should be routine. This procedure has proved rewarding, although, occasionally, repeated examinations have to be made.

Recently other workers have found that an agglutination test using latex particles coated with cryptococcal antigen is useful for screening, diagnosis and prognosis. They indicate that occasionally false-negative results may be obtained with an India ink prepartion when the serology is positive.

J.H.

Comparative Study of the Efficacy of Seven Paper-reagent Strips and Conventional Biochemical Tests in Identifying Gram-negative Organisms. Matsen, J. M. and Sherris, J. C. (1969), *Appl. Microbiol.*, **18**, 452.

In this study of 291 strains, excellent correlation was obtained with the oxidase, phenylalanine deaminase, and Voges-Proskauer tests. Indole tests made on liquid medium cultures also gave complete correlation, but some false-negative results with *Proteus* strains were obtained when growth from solid medium was tested by the strip method. Paper strip urease tests were positive within 2 hours with all *Klebsiella* and some *Serratia, Herellea* and *Citrobacter* strains as well as with *Proteus* strains. Approximately 15% of citrate strip test results differed from those of the conventional tests, and reproducibility was poor. The lysine decarboxylase strip test showed

a number of discrepancies and posed problems of interpretation and readability.

J.H.

Studies in Urinary Tract Infections. III. Biochemical Characteristics of Coagulase-negative Staphylococci Associated with Urinary Tract Infections. Mortensen, N. (1969), *Acta med. scand.*, **186**, 47.

Forty-six strains of coagulase-negative staphylococci from 13 patients with established urinary tract infections were examined by biochemical tests. It was concluded that contrary to prevailing opinion a negative result of the coagulase test does not exclude association with infection.

J.H.

Bacterial Meningitis Among Newborn Infants. Chevrie, J. J. and Aicardi, J. (1969), *Clin. Pediat.*, **8**, 562.

Purulent meningitis in the first 4 weeks of life remains a serious problem. The authors have treated 36 cases in their neonatal intensive care unit, a referral centre for severely ill babies from other hospitals.

Cerebrospinal fluid examination is the only reliable means of diagnosis. Neither gross appearance of the fluid (it may be turbid, yellow, or even grossly bloody) nor number of cells (4 of the patients had fewer than 50 per cu. mm.) are completely reliable diagnostically. Bacteriological study is the only sure means of diagnosis; it must be performed on every specimen. Delay in diagnosis and starting treatment is a vitally important factor affecting the outcome.

Gram-negative organisms were most often implicated with *E. coli*, *Proteus* and *Ps. aeruginosa* the leading offenders in order of occurrence. Nonhaemolytic streptococci were most prevalent among the Gram-positive cases; *L. monocytogenes* was also common.

Peripheral blood WBC counts may be a clue to prognosis. 7-9 patients with a count of less than 10,000 died.

J.H.

SEROLOGY

Studies on Sera from Men with Sperm Antibodies. Fjallbrant, B. (1969), *Acta obstet. gynec. scand.*, **48**, 131.

Since sperm antibodies in man are considered to be autoantibodies, and since several autoimmune diseases are associated with hyper-or dysgammaglobulinaemia, an intent of this investigation was to determine the level of immunoglobulins in sera with sperm agglutinins as detected by Kibrick's macroscopic direct sperm agglutination test. The levels of IgG and IgM were found to be higher in such sera, which suggests that sperm antibodies are associated with a generally increased production of antibodies.

J.H.

UNCLASSIFIED

The Computer in the Laboratory. Whitehead, T. P. (1969), *Practitioner*, **203**, 294.

In fact, fifty-two pages of this issue of *Practitioner* are devoted to six concise papers on aspects of computers in medicine. Professor Whitehead is well placed to review the subject of digital computers in laboratories. As a professor of clinical chemistry in Birmingham he has practical experience with computers. In this paper he reviews aspects of computer use: data acquisition systems, patient identification, quality control, laboratory management, error detection, reporting systems and interpretation of laboratory results.

M.J.G.

Book Reviews

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.)

This remarkable volume could be subtitled " the anatomy and physiology of the fungi " and as such commands the serious attention of all professional mycologists. It is divided into four sections.

I *Structure and Growth:*—six chapters dealing with such topics as fine structure of fungal cells, apical growth, the fungal colony and spore liberation, dispersal and germination.

II *Function:*—six chapters describing fungal nutrition and metabolism, transport processes, reactions to environmental factors and interaction with other organisms.

III *Recombination:*—four chapters on nuclear division, sexual and parasexual cycles.

IV *Speciation and Evolution:*—two chapters on such topics as hybridization and polyploidy.

An appendix of ten pages lists the classification of the fungi referred to in the text, there are thirty pages of references and an index of the subjects discussed.

The electron micrographs are few in number but of very high quality. The text includes numerous tables and line drawings by Professor Burnett's wife. These drawings are an outstanding feature of the volume and are quite remarkable for their clarity and accuracy.

This book is recommended to any medical technologist who has an interest in mycology and desires to extend his knowledge beyond the bread and butter problems of growth and identification to an understanding of the factors that govern the problems.

It is surprising that in the section on transport processes no reference is made to the liberation of arsine and its alkyl derivatives from substrates containing traces of arsenate by *Scopulariopsis* and *Paecilomyces.* Although the appendix quotes Ainsworth (1961) *Ainsworth and Bisby's Dictionary of the Fungi* 5th Edition as the authority for nomenclature it is disturbing to find *Pullularia (= Aureobasidium) pullulans* referred to in the text and in the appendix as *Pullularia pullans.* A further sense of insecurity is engendered by small typographical errors. On p.191 there is a reference to " soil-inhibiting *Rhodotorula* " which should surely be " soil-inhabiting."

On p.247 *Phycomyces blakesleeanus* is printed as *Phycomyces blakeslecanus* and *Armillariella (= Armillaria) mellea* is mis-spelled *Armillarialla mellea* on p.341.

Nomenclature of the fungi is sufficiently confusing without such errors and it is to be regretted that they should have occurred in a work of this stature.

F.M.R.-M.

An Introduction to Industrial Mycology. 6th Edition. George Smith. Edward Arnold, London (1969). Price $11.00. 390 pages, 173 illustrations.

The latest edition of this important text book has been eagerly awaited and was completed by the author shortly before his death. First published in 1938. Smith's *Introduction* has always been appreciated as the starting point and reference book on moulds for those beginning a study of these organisms. The identification of such may be a stimulating exercise for a medical technologist, but it is increasingly recognised that some of these frequent contaminants may at times be implicated in human

infection. With the aid of easily followed keys and the author's renowned photomicrographs there is no better book available to assist in these identifications.

The first three chapters deal with morphology, classification and nomenclature. Seven are devoted to the various genera of yeasts and moulds and the remaining seven cover general mycological techniques with an appendix on microscopy.

Chapter IX on *Aspergillus* shows important changes and additions and also provides the striking cover photograph of hulle cells of *A. ustus*. Changes in nomenclature are discussed and the medical mycologist is particularly directed to the ruling regarding *Sporothrix* and *Sporotrichum*.

Any review of this book would be incomplete without reference to the scholarly selection of quotations that head each chapter. Two of these are particularly worthy of note: *Chapter I*—"We may rest assured that as green plants and animals disappear one by one from the face of the globe, some of the fungi will always be present to dispose of the last remains." B. O. Dodge, 1940. *Chapter XIII*—"The cultivation of microfungi is still almost wholly an empirical art." H. A. Dade, 1960.

Smith's *Introduction to Industrial Mycology* is a recommended text book for every medical laboratory where specimens are examined for fungi.

F.M.R.M.

A Laboratory Manual of Microtechnique and Histochemistry. A. T. Sumner, M.A., D. Phil. (Oxon.) and B. E. H. Sumner, M.A., D.Phil. (Oxon.). 93 pages. Blackwell, Oxford. (1969). Price in U.K. 20s 0d.

The authors of this manual intend it as an introduction to practical microtechnique and the histochemistry of animal tissues, primarily for undergraduates and research workers. This is not necessarily a disadvantage to the technologist in histopathology since the approach of academic workers can often suggest additions to the repertoire of everyday techniques.

In a book of less than 100 pages a comprehensive treatment of the subject cannot be expected, but some of the omissions are unfortunate. The section on fixatives is quite reasonable, but processing to paraffin wax is poorly covered with no choice of fluids suggested, and the method for determining the "end-point" of decalcification should not be used in a diagnostic laboratory. Because of the lay-flat characteristic given to the book by wire binding, the section devoted to staining techniques is easy to use on the laboratory bench. However the absence of many of the routine diagnostic stains *e.g.* reticulin. Van Gieson, melanin etc., make the book of very limited value. A saving grace is the sensibly concise theoretical note at the end of each method.

Approximately 40 pages are devoted to histochemical methods and since these include a reasonable coverage of the periodic acid-Schiff reaction and the identification of lipids, this is a most useful and interesting section of the manual. The cryostat microtome is mentioned earlier, but no comment is made about its use in enzyme histochemistry, and many of the methods use paraffin embedded material which is often likely to give poorer results. However, the methods are given in a straightforward manner and should encourage experiment in the routine laboratory, where histochemistry is still viewed with some awe.

The authors refer frequently to the standard textbooks on histological technique, and for the trainee technologist such books would give a much wider — and safer — grounding in the subject. For the technologist venturing into histochemistry this book can give a gentle introduction to much deeper waters.

B.G.-J.

NEW

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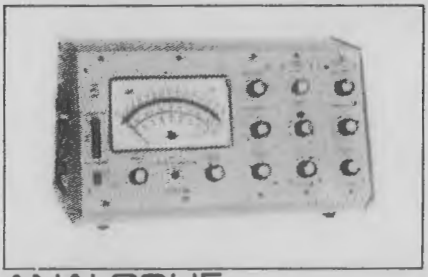

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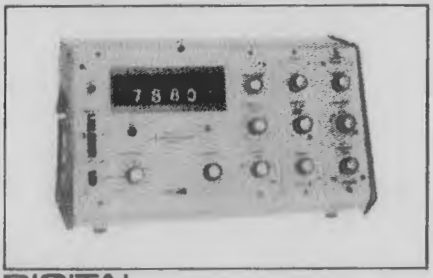

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Both of the Acid-Base Analyzers are fully transistorized, high-precision pH-meters incorporating the latest achievements within the field of modern amplifier technique. They can be used as separate pH-meters or they can be mounted on the Acid-Base Cart, type ABC1. When fully equipped, the Acid-Base Cart enables a complete acid-base analysis to be carried out, either according to the equilibration technique or on the basis of a pH and a Pco_2 measurement.

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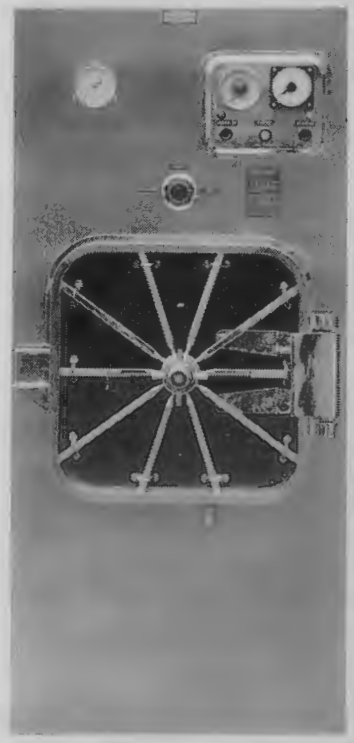

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A Short Textbook of Microbiology. D. C. Turk, D.M., M.R.C.P., M.C. Path., and I. A. Porter, M.D., M.C. Path. 335 pages. English Universities Press, London. (1969). Price in U.K. Library Edition 38s 0d, Paperback 28s 0d.

This is a further book in the University Medical Text series and is written primarily for medical students. The author's objective has been to help students to understand the relationship between man and his microbial parasites and to provide an intelligent approach to the investigation, prevention and treatment of microbial disease.

No detail of laboratory methods has been given nor practical instruction in the use of sterilisers, antiseptics, immunising agents or anti-microbial drugs. Instead, the emphasis has been on bringing the student to understand the use of these. The authors have collected some of their material from such books as Topley and Wilson's *Principles of Bacteriology and Immunity*, Mackie and McCartney's *Handbook of Bacteriology*, and the *American Review of Medical Microbiology* by Jawetz, Metnick and Adelberg. References to other books or sources of material relating to individual chapters are indicated by the suggestions for further reading made at the end of these chapters.

The book is divided into 6 major sections:

I Introduction — historical perspective and approaches to microbiology.

II Biological background — including ecology and physiology.

III Pathogenesis of microbial diseases — transmissions of pathogens, pathogenicity and host defences, outline of immunological mechanisms, immunological response harmful to the host.

IV Micro-organisms of medical importance — Bacteria, Rickettsiae and Bedsoniae, virus, fungi and protozoa.

V Laboratory diagnosis of microbial diseases — collection and examination specimens, diagnostic serology and skin testing.

VI Prevention and treatment of microbial diseases - Principles of prevention, sterilisation, some special problems of hospitals, bacteriology of water, milk and food; immunisation, anti-microbial drugs.

This book can be recommended as a complementary handbook for the trainee microbiologist, although the information presented can be obtained in greater detail from the "classical" text books.

It would benefit those trainees who already have a sound working knowledge of techniques and want to acquire a basic knowledge of the clinical approach to the disease processes.

The concise presentation of facts, useful for revision purposes, may appeal to examination candidates.

M.H.A.

Systemic Mycoses. A Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Ruth Porter. J. and A. Churchill, London (1968). Price $8.65.

This symposium was held at Ibadan University, Nigeria, in 1967; with 23 contributors from 13 countries, including England, U.S.A., France, Germany, Belgium, Mexico and Russia. Four papers are presented on pathology, two on clinical aspects, four on epidemiology and three on antifungal antibiotic therapy. Each paper is followed by a spirited discussion and relevant references are included. Contributors of the calibre of W. St.C. Symmers, L. Ajello, C. W. Emmons, E. Drouhet, F. Mariat, P. K. C. Austwick (now in New Zealand), H. P. R. Seeliger, J. E. McKinnon, R. Vanbreuseghem and A. Gonzalez Ochoa ensure that this volume will command attention. Although New Zealand is fortunate in its comparative freedom from systemic mycoses, the possibility of such may need to be considered and no large hospital laboratory can afford to be without this text. Any medical technologist studying mycology will find it to be of absorbing interest and permanent value.

F.M.R.M.

Books Received

Antibiotics and their Laboratory Control. (Laboratory Aids Series). M. C. Bryant, A.I.M.L.T. 72 pages. Butterworth, London. (1968). N.Z. price $2.00.

Blood Groups. A. E. Davis, S.R.N., R.N.T. and M. R. Gurr, S.R.N., S.C.M., R.N.T. 79 pages. Taylish Universities Press, London (1970).

Exfoliative Cytology in Gynaecological Practice. Second Edition. Erica G. Wachtel, M. D. 245 pages (with many photomicrographs). Butterworth, London. (1969). N.Z. price $9.25.

Handbook of Haematological and Blood Transfusion Technique. Second Edition. J. W. Delaney, F.I.M.L.T. and G. Garraty, F.I.M.L.T. 422 pages. Butterworth, London (1969). N.Z. price $9.75.

Human Haemoglobin Variants and their Identification (Laboratory Aids Series). L. H. B. Wallett, F.I.M.L.T., F.I.S.T. and J. B. Robinson, A.I.M.L.T. 56 pages. Butterworth, London (1968). N.Z. price $2.00.

Mycobacteria: Isolation, Identification and Sensitivity Testing (Laboratory Aids Series). B. Allen, F.I.M.L.T. and F. J. Baker, F.I.M.L.T., F.I.S.T., F.R.M.S. 75 pages. Butterworth, London. (1968). N.Z. price $2.00.

Parasitology: Indentification of Helminths (Laboratory Aids Series). R. A. Lambert, F.I.M.L.T. 64 pages. Butterworth, London. (1969). N.Z. price $2.00.

Practical Bacteriology. Nuala Crowley, M.R.C.S., L.R.C.P., F.C. Path., Jean M. Bradley, M.B., M.C. Path., D.C.H. 288 pages. Butterworth, London. (1969). N.Z. price $7.75.

Sterilisation: Methods and Control (Laboratory Aids Series). M. R. Breach, F.I.M.L.T. 72 pages. Butterworth, London. (1968). N.Z. price $2.00.

What's New

THE PYE MODEL R GAS CHROMATOGRAPH

A new gas-chromatograph system which can be built up to individual requirements from a choice of modules is announced by *Pye Unicam Ltd.*, York Street, Cambridge, England. The chromatograph, known as the Pye Model R, is designed to fill the gap between routine analytical chromatographs and the very costly instruments designed for basic research. It can incorporate any or all of the following main features:

1. a large oven, suitable for running two columns simultaneously and extensible in stages to accommodate exceptionally long columns. The side panels can easily be removed for fitting electric or gas leads as required;

2. interchangeable detectors of four different types — flame-ionisation, thermionic, electron-capture, and katharometer. Up to four detectors of up to three different types can be used simultaneously;

3. gas-flow controls, injection-point heaters and temperature read-out;

4. programmable control of column temperature, including automatic cooling if desired. A complete run can be carried out unsupervised, and the instrument then rapidly re-sets itself to the starting temperature ready for injection of the next sample;

5. programmable control of column gas-pressure — preferable to temperature programming in some preparative work;

6. a pyrolyser unit for solid and liquid samples, using the "Curie point" technique to produce accurately reproducible results.

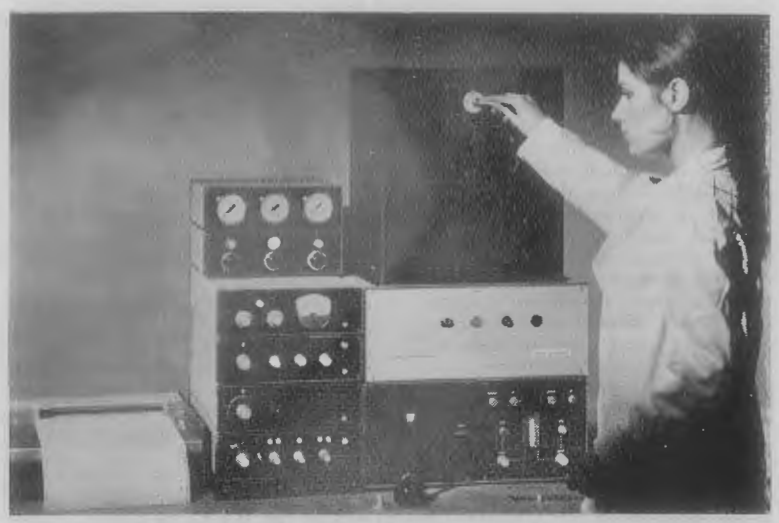


Fig. 1 The Pye Model R gas chromatograph with typical selection of modules.

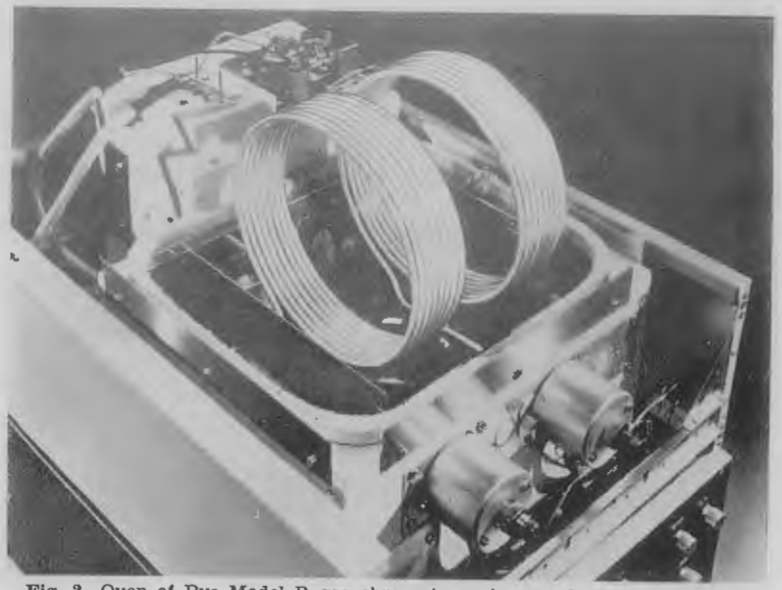
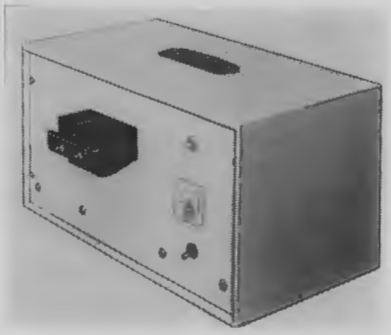


Fig. 2 Oven of Pye Model R gas chromatograph opened to show injection-point heaters (in front), columns (centre) and detector units (behind).

BENCH WELDER FOR SEALING P.V.C. COMPONENTS AND BAGS



Stanelco - Thermatron Ltd., manufacturers of high frequency plastics welding equipment, have, as can be seen in the illustration, produced a compact bench type welder for the welding of small P.V.C. components and the sealing of P.V.C. bags after filling.

Though primarily designed for medical applications including blood transfusions, saline intravenous solutions and plasma bags, it has in fact, a very wide range of uses throughout industry especially where the packing of small components, accessories and piece parts is carried out before despatch or where long storage life can be ensured by the use of P.V.C.

Known as the Type "BP.80" it is operated by means of a foot switch, thus leaving the operator's hands free to hold the product.

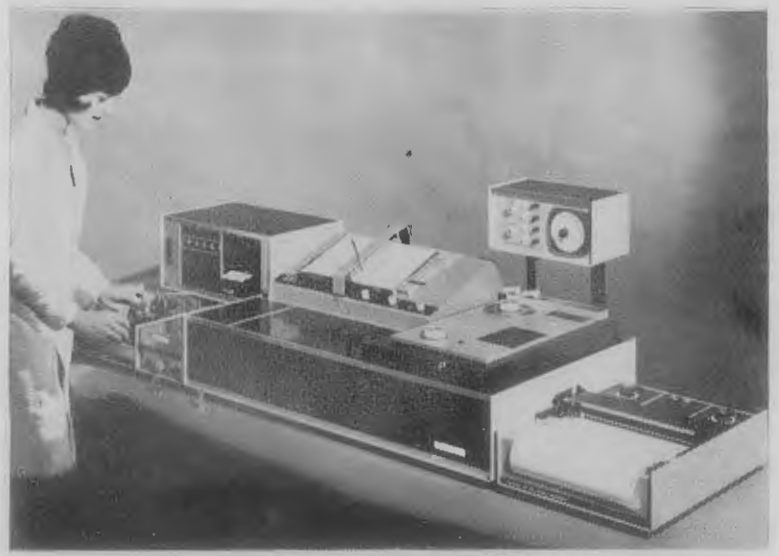
Normal welding times are in the region of one to two seconds and power is automatically switched off at the end of the weld period, pressure being maintained for a pre-set time to enable the P.V.C. to cool.

Further particulars may be obtained from *Stanelco-Thermatron Ltd.*, 4 Elstree Way, Boreham Wood, Herts, England. (Telephone: 01-953, 4031).

THE UNICAM SP1800 ULTRAVIOLET SPECTROPHOTOMETER

The new Unicam SP1800 Ultraviolet Spectrophotometer available from *Pye Unicam Ltd.*, York Street, Cambridge, England, is a low-cost double-beam instrument operating in the ultraviolet and visible wave-length regions. It is designed for use either on its own or as the basic unit for a range of instrumentation systems whose analytical capability and degree of automatic control can be chosen according to the user's requirements. Wavelength ranges of 190-700nm (SP1800A) or 190-850nm (SP1800B) are available, each instrument providing an accuracy of ±0.5nm and reproducibility of 0.5nm over the whole range.

On the basic unit, results are presented on a built-in meter with a scale 5½in (140 mm) long. A switch selects one of four linear absorbance-ranges (0-2A, 0-1A, 0-0.5A or 0-0.2A) or a linear concentration-range. Zero-shift controls with a wide range of adjustment allow high values of absorbance to be displayed on the more sensitive scales.



The new Unicam SP1800 Ultraviolet Spectrophotometer in a configuration which includes (from the right) an SP40 automatic sample-changer, an SP46 digital printer, an SP1805 programme controller, an SP1803 wave-length selector and an AR25 chart recorder.

Wavelength Scanning

The basic SP1800 spectrophotometer, manually operated, permits precise measurements on a variety of samples; but with the addition of accessories, the user can choose a configuration suited to his own requirements as regards degree of automatic control, type of analysis to be performed and presentation of results. Thus scanning-spectrophotometry, for example, can be carried out by combining with the basic unit an automatic wavelength drive unit (SP1804), a wavelength synchronisation accessory (SP1808) and an external recorder (AR25). The wavelength drive unit enables the extent of the scan (i.e. initial and final wavelengths)

to be set, and the user can select any one of five scanning-speeds. Fixed-wavelength operation is also possible. The recorder provides seven chart-speeds, so a wide range of wavelength-scale expansions can be obtained. Synchronisation of the wavelength drive unit with the recorder chart is fully automatic, and repeat scans may be superimposed if desired.

Automatic Programming

Adding an automatic cell-changer (SP830) and a programme controller (SP1805) to the basic SP1800 spectrophotometer substantially increases the versatility of the system. Four sample- and four reference-cells can be accommodated in the cell-changer, and are then interchanged automatically according to a preset programme. Scanning or fixed wavelength measurements can be carried out either at predetermined time intervals or when triggered by external equipment. Up to four reactions can thus be simultaneously followed, each against its own reference-solution, enabling rate—concentration relationships and reaction orders to be calculated. It is also possible to follow the kinetics of organic reactions and to determine isobestic points.

Another important accessory, particularly for work on multi-component systems, is an automatic wavelength selector (SP1803) which enables measurements to be made on a series of samples at up to five pre-selected wavelengths. Results can be presented in sequence on the external recorder or fed to a digital printer (SP46).

Other units which can be used in conjunction with the SP1800 instrument are a spectrofluorimeter accessory (SP860), an accessory for measuring light reflected from solid and powdered samples (SP890), an automatic sample-changer (SP40), a temperature programmer (SP876) and a constant-temperature cell housing (SP870).

Optical Features

In the SP1800, light from a tungsten lamp or a deuterium arc lamp (both are included in the unit) is filtered, monochromated by a constant-bandpass Ebert monochromator incorporating a high-resolution grating (see Fig. 2), and reflected to a fixed beam-splitter. The two emerging beams are directed by a system of mirrors through the sample-cell and reference-cell respectively, and are then received by a photomultiplier detector. Solid-state circuitry compares the two signals, and produces an output voltage proportional to the absorbance of the sample, which is displayed on the meter.

Two features of the optical system are worth special mention: an additional sample-position close to the detector is provided, allowing accurate measurements on turbid or light-scattering samples; and a beam-balance control is included which equalises the two beams of light from the splitter and thus ensures that a linear baseline can be maintained over the whole wavelength range.

THE UNICAM SP8000 ULTRAVIOLET RECORDING SPECTROPHOTOMETER

A new recording spectrophotometer known as the Unicam SP8000 is announced by *Pye Unicam Ltd.*, York Street, Cambridge, England. It is available in two versions, the SP8000A with a wavelength range from 190-770 nm, and the SP8000B with a range from 190-850 nm.

The instrument provides a read-out linear in absorbance units (A), which is recorded against a linear wavelength scale on a built-in flat-bed recorder. Two absorbance ranges are provided, selected by a switch. One has a span of 0.2A and the other of 1.0A, and the provision of a zero back-off control with a range of 1A enables the former to be set anywhere between —0.2A and +1.0A and the latter anywhere between —0.2A and +1.8A. Higher absorbance values can be measured if an attenuator of known value is inserted in the optical system.

Fig. 1 The new Unicam SP8000 ultraviolet spectrophotometer made by Pye Unicam Ltd. — shown here with SP40 automatic sample-changer (left front), SP46 digital printer (left rear), SP8005 programme controller (rear) and an external recorder (right) in addition to the built-in recorder.

The instrument has a photometric accuracy better than ±0.005A in the 0.2A range and ±0.01A in the 1.0A range.

Constant-Wavelength Operation

One chart measuring 11in x 6in (28 x 15 cm) covers the whole of the visible or ultraviolet spectral regions, and the wavelength scale can be expanded or contracted to permit detailed examination of parts of the spectrum. The chart can also be displaced sideways in a series of equal steps so that several short spectra can be placed side by side or superimposed. If the wavelength drive is uncoupled altogether, the recorder plots the variation of absorbance with time at constant wavelength.

Two Sample-Positions

The sample-compartment of the instrument is designed to accommodate cells of up to 40 mm pathlength in standard holders. It will also accept semi-micro and micro cells, flow-through cells, cells of variable pathlength, and special cells of 10-cm pathlength for liquids and gases.

In addition, there is a second sample-position — situated at a focus very close to the detector — which enables accurate measurements to be made on many "difficult" samples such as turbid or light-scattering solutions, single crystals, or paper chromatograms.

Automatic Programming

The addition of accessories converts the Unicam SP8000 spectrophotometer into an automatic analytical system. The main units are:

(a) the SP40 automatic sample-changer. This holds up to 50 samples in 10-ml containers, and transfers them in sequence into a special flow-through cell in the spectrophotometer, using either a piston pump or a vacuum transfer system. This can be used in conjunction with (b).

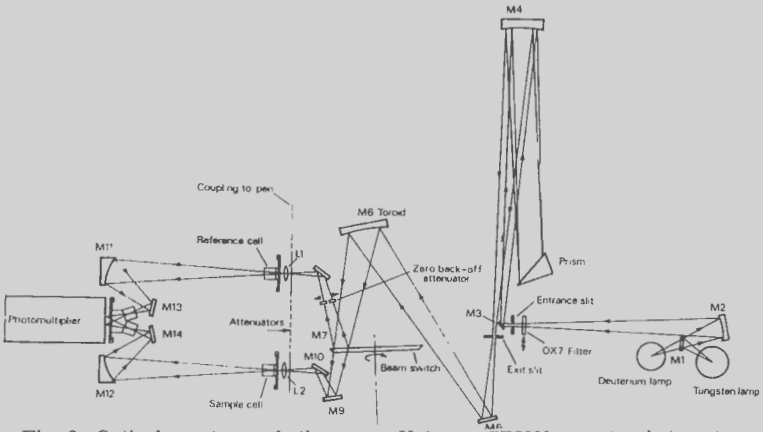


Fig. 2 Optical system of the new Unicam SP8000 spectrophotometer.

(b) the SP8003 automatic wavelength selector. This enables up to 15 wavelengths to be pre-programmed. The absorbance of each sample in turn is measured at the selected wavelengths, and repeat measurements on a single sample at pre-set time intervals are also possible.

(c) the SP8005 programme controller, designed primarily for studies of reaction kinetics. This enables scans or fixed-wavelength measurements on up to four sample- and four reference - cells held in a special cell-changer to be programmed.

(d) an external recorder. Controls on the main instrument enable a read-out direct in concentration units to be obtained, or either of the standard absorbance ranges to be expanded by a factor of 1, 2 or 5 for measurement of small changes.

(e) the SP46 digital printer, which converts the analogue output signal (in absorbance or concentration units) into digital form.

Also available are constant-temparature and temperature-programming control units, and an accessory for measuring light reflected by solid and powdered samples.

Optical System

The SP8000 is a double-beam spectrophotometer using the optical null principle. Radiation from a light-source is passed through a sealed, thermostatted Littrow monochromator incorporating a 30° silica prism of high purity. The resulting beam of monochromatic light is split into two by means of a rotating sector mirror, and switched alternately through the sample cell and a reference cell. The two beams converge on a photomultiplier detector, and if the sample has absorbed radiation at the particular wave-length of the light, an error signal is generated. This is amplified, and powers a servo-driven optical attenuator which is moved into the reference beam until the two beams are equal in intensity and the error signal is eliminated. The attenuator is directly coupled to the recorder pen, which thus records the absorbance of the sample against wave-length.

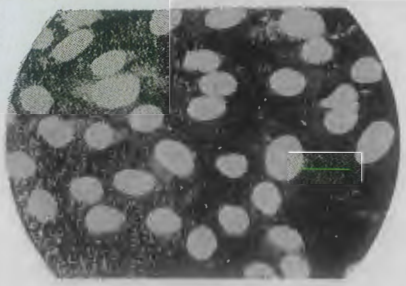
The instrument uses two light-sources, a deuterium arc lamp and a tungsten filament lamp. Change-over between the sources, and control of light filters, slit-width and amplifier gain are all automatic, making operation of the instrument very simple. Solid-state electronics are used extensively.

MICROBIOLOGICAL SCIENCES INC.

n·b·a KIT

NUCLEAR
BINDING
ANTIBODY

Kit Contents — Human Tissue Culture Cells. 10 Cover Slips; Antihuman Globulin (Fluorescein Labelled) 1 ml; PBS 10 X, 50 ml.
Note: Individual items of the kit may be purchased separately.



Positive L.E. Reaction

Negative L.E. Reaction

A routine procedure to assist in the diagnosis of LUPUS ERYTHEMATOSUS by the Fluorescent Antibody Technic using stored human tissue culture cells. The n•b•a KIT provides for the first time a reliable procedure that requires minimum processing time (1 hour) and rapid reading time (15 seconds or less).
n•b•a KIT — 80 TESTS — $60

WHEN RELIABILITY AND TIMING ARE IMPORTANT

The n•b•a kit provides for the first time, to the discreet technologist, the necessary reagents to run a highly reliable Nuclear Binding Antibody test.
The fluorescent NBA test has been accepted by leading Medical Centres to be more sensitive and specific than the classic L.E. reaction. A higher degree of reliability can be expected from this technic over the latex agglutination method.

By using the n·b·a KIT You *DO NOT* have to:

- grind the blood clot and collect the white cells.
- be concerned about the activity of the phagocytes as they do not affect the test.
- count the L.E. Cells and differentiate them from the "TART" cells.
- count the conventional 1000 cells per slide.

ALSO — The Pathologist does not have to study the slide as a histological section.

Contents

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