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The Value of a Background Correction in the Automated Bilirubin Determination

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Received for publication, September 1968.

Of the numerous bilirubin methodologies that have been described, the method of Jendrassik and Grof [4,3,6,7] has been found to adapt best to automation. This is fortuitous because this procedure is generally stated to be the preferred method for the determination of total and direct serum bilirubin. [2] Comparative studies between the manual Malloy-Evelyn procedure [3,5] and the automated method performed both on the SMA-12 (R) and on a single channel analyzer gave poor correlation. The discrepancy was shown to be due to variable background optical density intrinsic in the serum specimen. A method by which this intrinsic error can be circumvented is presented.

Materials and Methods

Total and direct serum bilirubin was estimated by the automated method of Gambino [2] (See Figure 1) and the manual method of Malloy and Evelyn [3,5]. In addition to the routine automated analysis, serum optical density was determined by substituting sulphanilic acid for the Diazo reagent and repeating the total and direct assays. The observed total and direct bilirubin was corrected for the serum optical density.

Reagents were prepared as follows:

1. Caffeine mixture: Caffeine 20 g.; sodium benzoate 30 g.; sodium acetate 50 g.; distilled water Q.S. 400 ml.
2. Diazo I: Sulphanilic acid 5.0 g.; concentrated HCl 15.0 ml; distilled water Q.S. 1,000 ml.
3. Diazo II: Sodium nitrite 500 mg; distilled water Q.S. 100 ml.
4. Diazo reagent: 10 ml. of I plus 0.25 ml. of II. Use within 30 minutes.
5. Tartrate buffer: Sodium hydroxide 100 g.; sodium potassium tartrate 350 g.; distilled water Q.S. 1,000 ml.
6. Ascorbic acid: 1 g. in 25 ml. distilled water.

Approximately 100 normal and patient specimens were tested in this manner. Statistical correlation was computed.

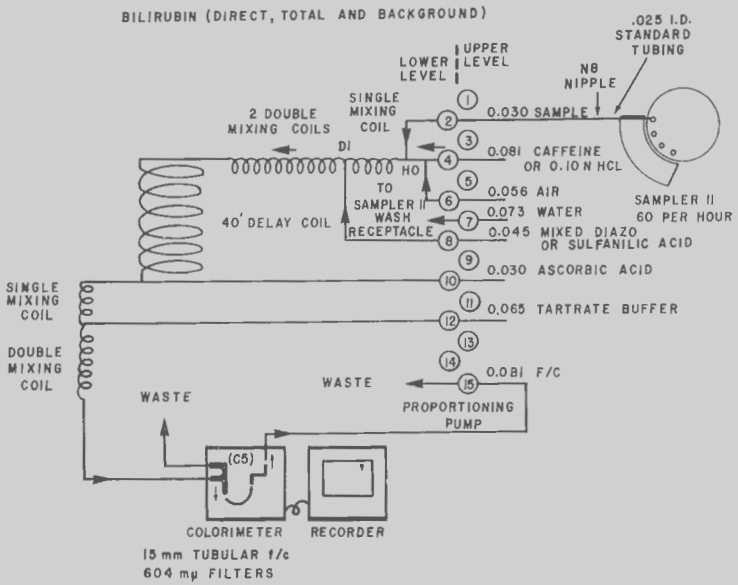

Figure 1

Schematically shown is the flow diagram of the system used in this study. The bilirubin blank is obtained using O.1 N HCl rather than caffeine, and sulphanilic acid rather than Diazo reagent. Direct bilirubin is assayed using 0.1 N HCl and Diazo reagent, and total bilirubin using caffeine and Diazo reagent.

Results

Approximately 100 normal and patient sera were analysed for intrinsic background. All visibly clear specimens gave background levels equivalent to 0.1 mg/100 ml. (±0.02) of bilirubin. Those specimens showing visible turbidity, lipaemia, haemolysis or icterus invariably gave increased background absorbance, occasionally as high as 0.7 mg/100ml bilirubin equivalent. (See Figure 2). The blank optical density reading is essentially the same whether the total or direct systems are used for blank determination; hence, only one baseline cycle is required and it can be used to correct both total and direct bilirubin estimations.

The *t* test fails to show significance between the Malloy-Evelyn procedure and the unblanked Jendrassik-Grof technique when both are compared, using commercial controls and patient sera. Incorporation of the blank in the Jendrassik-Grof technique

Figure 2
Representative tracing of 8 randomly selected specimens are shown. Bilirubin equivalent values are shown in Table I for each sample.

effects t test relevance to within 0.01 in the comparative analysis of total bilirubin. Although the direct reading bilirubin by the two techniques shows straight line correlation, the t test is not satisfied since the Jendrassik-Grof technique gives direct bilirubin values that are approximately 15% higher than those observed with the Malloy-Evelyn procedure. The excellent precision (3% coefficient of variation) of the single channel automated procedure is unaffected by the incorporation of a blank; however, the accuracy of the procedure is apparently enhanced.

TABLE I

SPECIMEN NUMBER	OBSERVED BILIRUBIN, mg/100ml	BACKGROUND (Bilirubin Equivalent) mg/100ml	CORRECTED BILIRUBIN mg/100ml
DIRECT BILIRUBIN			
1	0.30	0.15	0.15
2	0.30	0.20	0.10
3	0.45	0.25	0.20
4	0.45	0.35	0.10
5	0.35	0.15	0.20
6	0.30	0.10	0.20
7	0.30	0.10	0.20
8	0.45	0.20	0.25
TOTAL BILIRUBIN			
1	0.55	0.15	0.40
2	0.45	0.20	0.25
3	0.90	0.25	0.65
4	0.65	0.35	0.30
5	0.60	0.15	0.45
6	0.40	0.10	0.30
7	0.70	0.10	0.60
8	0.60	0.20	0.40

Indicated are the observed and corrected bilirubin values of the eight specimens shown in Fig. 2. The mean correction of the direct bilirubin study was —52%, and the mean correction of the total bilirubin study was —31%.

Discussion

Several reports comment that the background contributed by serum absorbance in the automated Jendrassik-Grof procedure is negligible and may be ignored.[2,9] While clear specimens show consistent background, pigmented or turbid specimens will introduce variable significant errors. In these instances, errors of over 50% of observed readings may occur. The magnitude of this error has recently been cited by Simmons.[8]

The bilirubin estimation with the background correction is probably best accomplished on a single channel system. The Sequential Multiple Analyser (SMA-12) (R) does not provide a serum blank correction and determines only total bilirubin. In addition, a serum control containing 1.0 to 1.5 mg/100 ml.

bilirubin is employed to standardise the bilirubin scale. Since this control serum is turbid and confers a background equivalent of 0.4 to 0.6 mg/100ml bilirubin, a significant subtractive error results in specimens containing bilirubin above the calibrated value and additive errors are seen below this value. If a stable high bilirubin control serum was available, these errors would be partially ameliorated.

Relatively stable primary bilirubin standards provide excellent standardisation in the single channel system. The same curve may be used for both total and direct bilirubin estimation. These considerations and the ease with which a serum blank may be established using a single channel analyser are strong justifications for using a single channel system rather than an SMA-12/30 (R) for estimating bilirubin. The new SMA-12/60 does incorporate a serum blank correction: however, modules for performing direct bilirubin determinations are not yet available.

Although the single channel procedure was originally standardised using a base line in the 98 to 99% T range, it should be pointed out that greater stability may be obtained with lower base line settings. Comparable sensitivity of reading is obtainable in the critical high normal bilirubin range, whether a low or a high base line is set. In this zone clinical reproducibility attains ± 0.05 mg.

Summary

It was observed that serum contributes a variable absorbance to the automated single channel and sequential bilirubin procedure. Clear, non-pigmented specimens contribute a relatively constant 0.1mg./100 ml. bilirubin equivalent background, while turbid, lipaemic, and pigmented specimens may contribute considerably more background. This error may be readily eliminated in a single channel system by performing a blank determination on all serum specimens and correcting the observed result for the bilirubin equivalent of the serum absorbance.

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Review of a Bacteruria Screening Test

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Pathology Laboratory, Cook Hospital, Gisborne.

Received for publication, August, 1968.

Several recent reports have compared the accuracy of the triphenyl tetrazolium chloride (T.T.C.) Bacteruria screen test to standard plate counting methods, Simmons and Williams (1962)[3], with a modified nitrate test Sleigh (1965)[4] or with a combination of the above methods, Kincaid-Smith *et al.* (1964)[2]. The results obtained by these and other authors show a marked lack of agreement.

The present survey was undertaken to determine whether the T.T.C. test, as originally described by Simmons and Williams (1962)[3], was an acceptable screening test for the presence of significant bacteruria, in comparison with the existing method of plate counting all urines with a calibrated loop technique. The object was to effect an economy in media costs if the method proved acceptable.

Materials and Methods:

A total of 453 urines were examined They were obtained from both males and females, either as in-patients, out-patients or private patients. The majority (75%) were in-patients. Specimens were either clean, mid-stream or catheter specimens. If received before 1 o'clock in the afternoon the specimens were cultured and the T.T.C. test carried out on them on that day; if they arrived at the laboratory after this time, culture was carried out on arrival and the T.T.C. test carried out on the following day, the specimen being stored at 4°C until tested.

Triphenyl tetrazolium reagent (T.T.C. reagent) was prepared by dissolving 750 mg. of T.T.C. in 100 ml. of a saturated solution of disodium hydrogen phosphate (Na_2HPO_4). A working solution was prepared by taking 4 ml. of this solution and diluting it to 100 ml. with saturated Na_2HPO_4. Stock and working solutions were sterilised by filtration through an Oxoid membrane filter and stored at 4°C in the dark. The stock solution was found to be stable up to 3 months. Fresh working solution was prepared weekly.

The test was carried out by measuring out 2 ml. of well-mixed urine into a clean test tube and adding 0.5 ml. of the T.T.C. working reagent, using sterile pipettes. The tube was then incubated for 4 hours at 37°C. After this time the deposit and supernatant were examined with the naked eye. A positive result was shown by a red precipitate. It was found important to report any tinge of red or pink as positive. Counts in excess of 10^6 often showed a general reddening of the supernatant also.

The method of quantitative counting used as a routine in this laboratory is as follows:—

A loop of platinum wire, 1.45 mm. inside diameter calibrated to deliver 0.001 ml. fluid, is used. These are obtained from commercial sources and the calibration checked by the method described by Urquhart and Gould (1965)[6]. Samples are plated on to a blood agar and MacConkey agar plate. Colonies are counted after 18 hours incubation and colony count multiplied by 1,000 represents the number of organisms per ml.

Results:

The correlation of bacterial count and T.T.C. test results are shown in Table I. In addition, 5 urines gave a positive reaction with no growth on culture. No reason was found for this.

TABLE I: Correlation of T.T.C. and Bacterial Count in 453 specimens of urine.

Organisms per ml	Total	T.T.C. Positive	T.T.C. Negative
100,000 or more	147	135 (92%)	12 (8%)
10,000 — 100,000	56	13 (23%)	43 (77%)
Less than 10,000	49	4 (8%)	45 (92%)

The above results show that 92% of significant bacterurias were detected by the T.T.C. test. This is fairly close to the results obtained by Simmons and Williams (1962)[3]. The percentage of positive results obtained with urines having a bacterial count of less than 100,000/ml. (16.2%), however, is considerably higher than that quoted by Simmons and Williams.

An analysis of the 12 urines failing to reduce T.T.C. when the bacterial count was above 10^5 revealed that 8 were Gram-negative organisms and 4 Gram-positive. The 8 Gram-negative organisms were all pure cultures; *6Esch. coli, 1Ps. aeruginosa* and *1Proteus mirabilis.* This does not confirm the findings of Simmons and Williams (1962)[3], who found 100% correlation with Gram-negative bacilli; but is in agreement with the findings of Steer and Jackson (1963)[5]. The proportion of positive T.T.C. tests in relation to organisms isolated is shown in Table II.

TABLE II

Bacterial Species	Total Isolated	Positive T.T.C. Test	%
Esch. coli	79	73	92
Proteus species	22	21	96
Pseudomonas aeruginosa	20	19	95
Klebsiella aerogenes	3	3	100
Strep. faecalis	1	1	100
Staph. albus	11	7	63
Mixed organisms	11	11	100

Two hundred and sixteen specimens (47.5%) were tested on the day of receipt and (52.5%) on the day following receipt (after overnight refrigeration). The number giving a false negative report was less in the specimens tested after overnight refrigeration. Nine (5.7%) false negatives were observed in the 216 specimens tested on the same day, compared to 3 (1.9%) of the 237 specimens examined on the day after receipt. The possibility of bacterial growth due to inadequate refrigeration was considered, but repeat plate counts on these specimens failed to show any significant increase in bacterial numbers. No other investigations were carried out to elucidate this point, although the possibility of inactivation by aging of inhibitory or antibiotic substances in the urine seems a possibility worthy of further investigation.

Discussion

Important points in the selection of screening tests are reliability for a wide range of specimens, ease of performance, ease of reading and if possible a substantial cost reduction on existing methods.

As the above results show, the test appears to satisfy the first requirement.

Although the test itself is simple to perform, the solutions are difficult to prepare and store. Other authors have found storage at room temperature in the dark satisfactory, but it was found that one of the batches of T.T.C. used in this study tended to deteriorate after 3-4 days at room temperature when diluted to working strength. The solution remained satisfactory for up to 14 days if refrigerated at 4°C and warmed to room temperature just prior to use. Another batch of T.T.C. from the same manufacturer did not deteriorate. A possible explanation is the high room temperature and humidity existing on the East Coast of the North Island in Summer, when this investigation was conducted, compared with that of Britain and the Northeastern U.S.A. where most previous surveys have been reported.

Experience is required when reading the tests. and the finding of Chard and Coles (1963)[1] that the precipitate is easy to see was not confirmed in this series. The use of a concave mirror and the recording of the faintest trace of pink was found to be essential.

The test effected a marked cost reduction per urine examination. The cost of blood agar and MacConkey agar plates prepared in this laboratory is 4 cents and 3.2 cents respectively. This is for materials only; labour and preparation have not been costed. The cost of each T.T.C. test is 0.76 cents, (again costing materials only).

It is therefore apparent that very substantial reduction of media costs is possible with this method. As an example: if this series had been carried out only by the T.T.C. method, material costs would have been $14.75, (this figure includes the cost of quantitative counting of the T.T.C. positives). The cost of counting all 453 urines by the semi-quantitative method in use was $32.62.

Provided care is taken in the preparation and storage of reagents and the interpretation of tests, this appears to be a most useful and economic bacteruria screening procedure.

Summary:

Comparisons between Simmons and Williams (1962)[3] T.T.C. bacteruria screening procedure and a plate counting method for the detection of bacteruria are described.

The T.T.C. test would seem to be a valuable screening test. 92% of urines showing significant bacteruria in the series were detected by this technique. Certain difficulties which were experienced are described and possible causes discussed. A comparison of the comparative material costs of the two methods is given.

Acknowledgment:

The author is indebted to Mr G. Shone for his able technical assistance.

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The Simultaneous Estimation of Alkaline Phosphatase and Glutamic Oxaloacetic Transaminase by Autoanalyzer

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Clinical Laboratory Services, Dunedin Hospital

Received for publication, February, 1969.

This paper described a simultaneous automated method for determining serum alkaline phosphatase and glutamic oxaloacetic the transaminase, and the thymolphthalein monophosphate method modification of the Fast Ponceau L technique of Morgenstern[4] for the transaminase, and the thymolphtalein monophosphate method first suggested by Coleman in the Technicon Symposium, 1965 [2]. Both methods are mounted on the same manifold but are entirely separate and there is no interaction.

Apparatus:

Standard autoanalyzer modules were used. The Sampler II was provided with a specially cut cam: sampling rate 40 per hour with a ratio of 1/1.

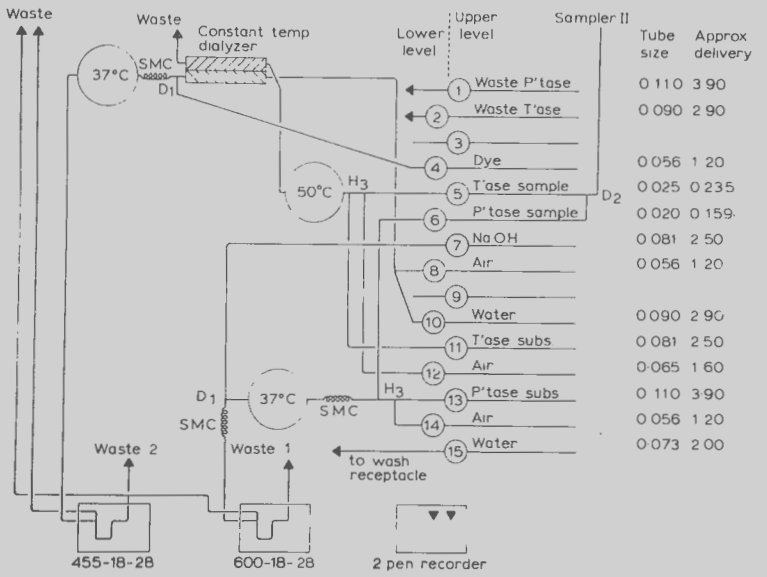

Figure 1.—AutoAnalyzer flow diagram for the simultaneous determination of transaminase and alkaline phosphatase.

Method:

Transaminase

(I) *Substrate*

33.5g. dibasic potassium phosphate K_2HPO_4
1.0g. monobasic potassium phosphate KH_2PO_4

Dissolve in 800ml of distilled water.

7.05g. l aspartic acid
1.0g. α ketoglutarate
1.0mg. tetra-sodium EDTA
1.0ml. chloroform

Dissolve and dilute to 1 litre. Adjust pH to 7.4. The substrate is stored in 200ml. amounts in plastic bottles at 4°C. (The solution should be discarded if mould or cloudiness develops.)

(II) *Stock Citrate buffer solutions*

A. 42.02g. citric acid per litre of water
B. 58.82 trisodium citrate per litre of water

The growth of moulds in these solutions is inhibited by the addition of 20 mg. of phenyl mercuric acetate per litre.

(III) *Working Citrate Buffer solution*

Mix 535ml. of A
and 465 ml. of B
Adjust the pH to 4.5

(IV) *Dye Solution*

0.4g. of fast ponceau L (Syn. azoene fast red) is dissolved in 100 ml. of working citrate buffer solution. The solution must be prepared daily. 1 drop of Triton X - 405 (Rohn & Hass Philadelphia) is added just before use.

(V) *Dialysis recipient solution*

Distilled water containing 1ml. of Triton X - 405 per litre.

Procedure:

The substrate and dye are kept whilst in use in a container of ice to ensure a stable baseline. The baseline is adjusted to 90% while all reagents are being aspirated. This takes about 20 minutes to appear and stabilise. Any saving achieved by withholding the dye is negligible.

Calibration is achieved by using control sera dilutions. Serial dilutions in saline are satisfactory but to achieve concurrent alkaline phosphatase calibration varying ratios of Versatol ensyme controls, elevated and normal, are used. The ratios suggested by the manufacturers were employed. Results were quoted in Reitman Frankel units as these were the clinically familiar ones in use here. After 80 to 100 specimens have been analysed the dye line is washed out with ethyl alcohol containing 5ml. of saturated sodium hydroxide per 100ml. This removes the precipitated dye. The caustic solution must be thoroughly washed out with water.

Alkaline Phosphatase

(I) *Buffered substrate.*

2.8g. 2-amino-2 methyl 1:3 - propandiol

0.5g. thymolphthalein monophosphate

The magnesium salt was used. (Distillation Products N.Y., U.S.A.)

0.5g. *trisodium citrate.*

Dissolve in distilled water and adjust pH to 10.1 at 20-23°C. Make up to 1 litre and filter, Store in plastic bottles at −20°C.

(II) *2N Sodium hydroxide*

The substrate is also kept in melting ice. The baseline, which appears in 10-12 minutes, is adjusted to 95% transaminase to keep it separate from the transaminase tracings.

Standardisation is achieved with the same control sera dilution used for the transaminase and in this case results are quoted in Bessey Lowry International Units which again were clinically familiar here. Values for the standards are increased by a "blank value" of approximately 3 units (see discussion).

Results

Coefficient of variation:—

The error of the combined method was estimated by running the same serum every seventh specimen and also by running the same serum every day for ten days.

AUTOMATED	MANUAL
26	25
76	86
41	40
52	50
55	52
38	32
38	38
24	24
34	34
33	33
40	37
50	50
44	55
38	32
40	37
42	50
44	50
46	51
26	28
22	20
39	40
24	33
30	35
40	47
46	47
70	66

Table I.—Comparison of results values given are in Bessey-Lowry International Units.

Variation within the run:—

Transaminase 12% at a level of 35 units.
Phosphatase 7% at a level of 50 units

Day to Day Variation:—

Transaminase 8% at a level of 80 units.
Phosphatase 7% at a level of 50 units

Steady State:—

This was established by continuous aspiration and then aspiration of the same serum at the sampling rate.

Transaminase 89%
Phosphatase 94%

This indicates that a good approximation to the steady state is achieved although the wash period is relatively long.

Comparison with Manual Method (Table I)

This aspect was investigated with respect to a phosphatase estimation only, namely Bessey Lowry[1]. It can be seen that the discrepancies are not clinically significant.

Normal Values:—

A small series of apparently healthy blood donors serum was analysed.

Normal range	=	Mean ± 2SD
Transaminase		15 - 34 units
Phosphatase		19 - 44 units

Commercial Control Sera:—

The results obtained with a selection of these sera can be seen in Table II. The manufacturers were asked to comment on the result for Enzatrol. They advised that similar results were obtained with this substrate in their laboratory.

Control Serum	Transaminase		Alk. Phosphatase	
	Found	Claimed	Found	Claimed
Hyland normal	27	30	28	30
Hyland abnormal	78	80	100	123
diluted 50%	37	40	60	61
Hyland Multi-enzyme Standards				
IV	Not applicable		51	49
V			75	82
VI			110	130
Enzatrol	100	85	33	88
Metrix abnormal	125	115	105	115

Table II.—Control sera results.

Discussion:

Transaminase:—

Dye solution:—

This must be prepared every day. Attempts to keep large batches in amounts suitable for a day's use, at −20°C. were unsuccessful. The dye tends to precipitate as flakes on thawing. The addition of polyvinylpyrrolidone increased the reagent colour and only marginally improved the keeping qualities of the dye solution.

Dialysis:—

The Technicon Method[5] dialyses into citrate buffer, whereas Morgenstern[4] used water. No advantage was found from the Technicon modification.

Phosphatase:—

Buffer Selection:—

Three buffers commonly used for the investigation of alkaline phosphatase were prepared to cover the pH range required. The same serum was added to all tubes and analysed for phosphatase by the following manual procedure.

4ml. of substrate (automated formula)
0.2ml. of serum
Incubate for 10 minutes at 37°C.
Add 3.0ml. of 2N sodium hydroxide.

The optical density was read at approximately 600mμ. The results obtained with the three buffers at various pH's are given in Table III. Allowance was made when preparing the buffered substrate at room temperature for the increase in hydrogen ion concentration when the temperature was raised to 37°C.

	BUFFERS		
pH at 37°C.	Carbonate	Glycine	Propandiol
9.0	32	18	10
9.2	34	21	22
9.4	36	24	37
9.5	36*	26	42
9.6	36	28*	47
9.7	34	27	50
9.8	29	26	56*
9.9	28	22	46
10.0	26	20	41
10.1	23	17	37
10.2	18	15	34
10.3	18	12	30

Table III.—*pH optima for three buffers using thymolphthalein monophosphate. Figures represent equivalent activities.

Linearity:—

The reaction of alkaline phosphatase on the substrate is linear over a time period of at least 30 minutes and to a concentration of 130 Bessey Lowry International Units. These results were obtained using a recording spectrophotometer.

Blanks:—

A constant lack of correlation with a normal Bessey Lowry method was found. This was corrected by adding the blank value of the control sera for calibration to the stated values. Blank values were determined by aspirating water in place of substrate. The blank value was usually 3 to 4 units. Only excessively turbid sera required blanks (Serachol gave a blank value of 30 B.L.I.U.) Bilirubin does not normally interfere (paediatric Versatol 20mg. bilirubin has a blank of 3 B.L.I.U.).

Conclusion:

A combined transaminase-alkaline phosphatase method for the Autoanalyzer is presented. It uses a minimum number of reagents, which are inexpensive and readily available. Blanks are seldom required and results compare favourably with present methods.

Acknowledgement:

The author wishes to thank Mr R. D. Allan for his valuable assistance, especially in the preparation of this paper.

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Books Received

Chromatographic and Electrophoretic Techniques: Volume 1, **Chromatography.** Third Edition. Edited by I. Smith, B.Sc., Ph.D., F.R.I.C., M.I.Biol., 1,080 pages with illustrations, 10 in colour. William Heinemann, London, 1969. U.K. Price 130s 0d.

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

To be reviewed in the November issue.

Artist's conception of lymphocyte characteristic of mononucleosis. It shows vacuolated cytoplasm, and eccentric nucleus with coarse chromatin granules.

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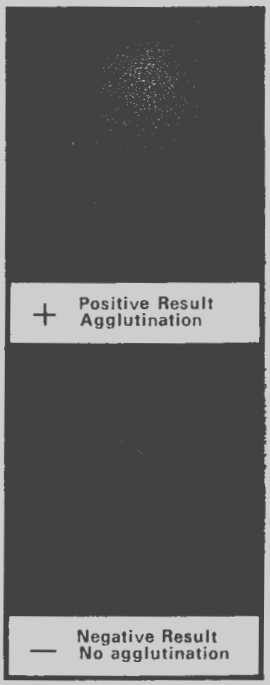

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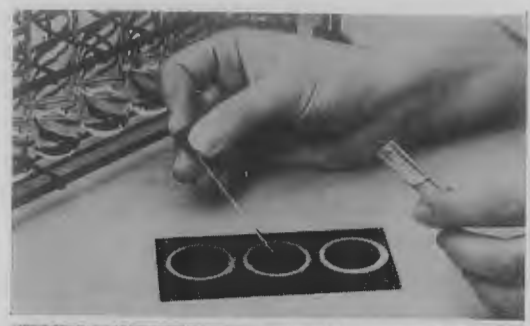

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hen using serum or plasma

ace one drop of diluent into circle on slide. Fill capil- y to mark with serum or plasma and add to diluent. x, add reagent and follow urine procedure

ontrols

own positive or negative *female* urine, serum or plas- may be used as controls.

Microsporum distortum — A Report of a Further Human Isolation

D. C. IRVINE and M. D. McCARTHY, A.N.Z.I.M.L.T.
Drs D. J. Perry and N. W. Fitzgerald, P.O. Box 1164, Dunedin.

Received for publication, November, 1968.

Microsporum distortum is listed in the Medical Research Council's *Memorandum,* Number 23 *(Nomenclature of Fungi Pathogenic to Man and Animals)* as being described by Marples and as the causative agent of *Tinea capitis* and monkey ringworm. Rebell, Taplin and Blank[8] record *M. distortum* as " an uncommon cause of *Microsporum tinea capitis,* so far reported principally from New Zealand and Australia in rural areas, although cases have been described in the U.S. and traced to contact with pet South American monkeys. Infection in dogs and other animals occurs. The natural reservoir of the species is unknown."

Ajello, Georg, Kaplan and Kauffman[1] record the dermatophyte as " known to occur only in N.Z. and U.S." and add, " the fact that the American isolates were recovered from monkeys newly imported from Latin America suggest that this fungus is present also in Central and South America."

World literature lists 27 human isolations of *M. distortum* — three in Australia, one in U.S., and 23 in N.Z. Also recorded are eight isolations from animals — four monkeys and one dog in U.S., and one horse and two dogs in N.Z. The incidence and location of these isolations is as follows:—

1954 Di Menna and Marples[3]: 11 human isolations of *M. distortum* from 10 children and one adult in rural areas of Central Otago. These are the original isolations which led to the naming of this fourth species of the microsporum group and were isolated between 1947 and 1954.

1957 Kaplan *et al.*[6]: Isolated M. *distortum* from four monkeys and one dog in the U.S. These isolations were all made in the period of January to April, 1956, and involved (a) three capuchin monkeys and one spider monkey recently imported from South America, and (b) one Boston terrier bitch and her three puppies. *M. distortum* was isolated from each of these adult animals and clinical lesions were observed on the puppies of the terrier and on 6 humans who had contact with these animals. No laboratory work was done on the human cases.

1959 Brooks *et al.*[2]: One human isolation in the U.S. from a laboratory worker who had a history of handling two monkeys, each of which had shown previous lesions that were clearing after therapy.

1960 Frey, Durie and Becke[4]: Two human isolations from sisters living in the Sydney area.

1962 Marples and Smith[7]: 10 further isolations from the Otago area. Included in this series were two isolations made by B. W. Main and Dr N. W. Fitzgerald from this laboratory in that year. Marples and Smith also listed the only animal isolations in this country, *viz.* of one dog and one horse in Waikouaiti in 1961 and one dog in Ranfurly in 1962.

1968 Frey and Flood[5]: A further human isolation from a child in the Sydney area. Microphotographs in this paper do not reveal the abundance or gross distortion of macroconidia seen as a typical feature of this dermatophyte in microphotographs of prior isolations.

In July, 1968, the first N.Z. isolation of *M. distortum* since 1962 was made in this laboratory, and this paper describes the isolation.

A male European child of one year was referred to this laboratory from Palmerston, a small rural community 36 miles north of Dunedin, with a circular lesion of the scalp. His medical practitioner had referred him to confirm his diagnosis of *Tinea capitis*.

Wood's Light examination showed a circular lesion on the back of the head exhibiting bright green fluorescence typical of a microsporum infection.

Direct examination of the broken hairs in 40% dimethyl sulphoxide and potassium hydroxide showed the presence of numerous small ectothrix spores.

The hair fragments were cultured on Sabouraud's dextrose agar containing cycloheximide and chloramphenicol* and on Sabouraud's dextrose agar with gentamicin, cycloheximide and aureomycin, incubated at 27°C.

After five days there was a recognisable growth, on both slopes, of a finely radiating colony resembling *Microsporum canis* but showing an absence of any yellow pigment. This characteristic lack of pigment resembles *M. canis* var. *album*. Microscopic

* B.B.L. Mycosel Agar.

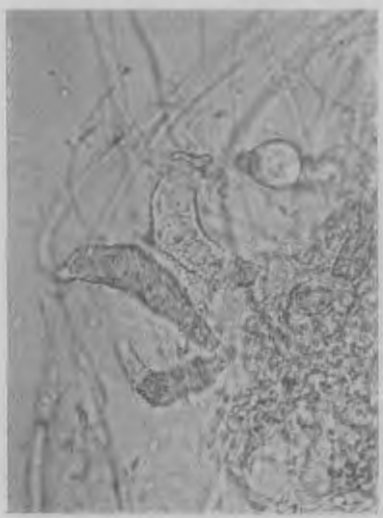

Fig. 1a: Microscopic view of *M. distortum* x 840.

Fig 1b: Microscopic view of *M. canis* x 840.

examination after eight days showed the presence of numerous clavate microconidia and small numbers of distorted macroconidia. A provisional diagnosis of *Microsporum distortum* was made and sterile rice grains were inoculated for confirmation.

After a further eight days incubation of the rice grains, adhesive tape slides[9] revealed the presence of numerous thick-walled, rough-walled, distorted macroconidia characteristic of *Microsporum distortum* (Fig. 1).

The original colonies by this time appeared as large white radiating colonies (Fig. 2), showing a characteristic waxy sheen on the surface.

Fig. 2a: Fifteen day culture of *M. distortum* on Sabouraud dextrose agar.

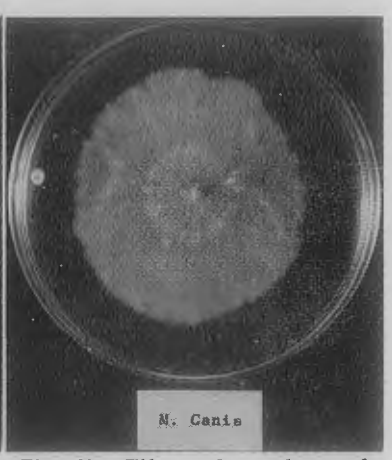

Fig. 2b: Fifteen day culture of *M. canis* on Sabouraud dextrose agar.

Dr J. M. B. Smith, of the Mycology Unit, University of Otago, confirmed the identification and together with Dr N. W. Fitzgerald and one of the authors (D.C.I.) travelled to the farm where the infant lived.

Swabs were taken from noses and faces of the symptomless household cats and farm dogs, soil was collected from the area around the house and kennels and dust was sampled from the household vacuum cleaner. Swabs were also collected from the nose and face of one deceased kitten. The kitten exhibited a lesion above one eye and this, too, was swabbed.

The lesion from the kitten yielded *M. canis,* the soil samples *Trichophyton (Keratinomyces) ajelloi* and *Microsporum cookei* but no evidence of *Microsporum distortum.* The samples from the household vacuum cleaner and from the remaining animals did not reveal the presence of any fungi.

The child returned to this laboratory three weeks following the original isolation and the Wood's Light revealed continued

activity in the original area as well as two smaller and newer lesions lower on the scalp due to auto-infection. All sites gave good growths of *M. distortum*.

The work of Kaplan *et al.*[6] demonstrated that the American infections were most likely due to contact with the monkeys and that the infection could be passed from animal to animal and from animal to human. However, in N.Z., no such correlation exists between animals and humans. Three sets of sisters are included in the N.Z. series, but evidence points to separate infections rather than transfer of infection[7]. Marples and Smith (1962)[7] suggested the possibility that this dermatophyte is an inhabitant of the soil and that animal and human infections are from this source. Further work on Otago soils has not confirmed this and a paper by Smith *et al.*[11] on animals as a source of human ringworm in N.Z. fails to reveal the presence of any further cases of animal infection with *M. distortum*.

Rush-Munro[10] has not recorded any isolations of *M. distortum* in Auckland, nor has he had any referred to him from the North Island. This would indicate that the Otago region is still the only area of infectivity in N.Z.

A case of infection with *M. distortum* is described. Efforts made to trace the source of infection failed to reveal any new information as to the ecology of this fungus.

Acknowledgements:

The authors gratefully acknowledge the valuable assistance of the following people: Dr I. M. Harper for the clinical material and Dr N. W. Fitzgerald and Dr J. M. B. Smith for assistance with the collection and processing of the animal and soil specimens.

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Footnote:

Since the preparation of this manuscript a further isolation has been recorded.

Technical Communication

Semi-Quantitative Bacterial Counts on Urine Specimens

Sir,

Considerable interest has been evident in recent years in the devising of reliable, technically simple, and if possible economical methods of enumerating the bacteria present in clean catch specimens of urine. In view of this the following modification of the blotting paper technique originally suggested by Leigh and Williams (1964)[3] may be of interest. I believe that, in particular, the medium adopted in this laboratory offers significant advantages over the MacConkey's agar used in the original method.

The medium adopted was the modification suggested by Bevis (1968)[1], of Mackey and Sandys (1966)[3] cystine, lactose, electrolyte deficient medium, (CLED medium). This modification incorporates Andrade's indicator in addition to the brom thymol blue indicator used in the original formulation. This is claimed (and was found) to give considerable assistance in detecting lactose fermentation.

Tests of the modified medium in comparison with blood agar and trypticase soy agar were carried out. Organisms tested were those used for preparing calibration curves as shown below, plus *Streptococcus pyogenes* and some diphtheroid species. Tests were carried out by making tenfold broth dilutions of overnight cultures of the organisms and using these dilutions for surface viable counts by the Miles and Misra techniques (1938)[4]. With all the species tested growth was found to be satisfactory. The medium was almost equal to trypticase soy agar in the luxuriance of growth as judged by colonial size. No significant differences in surface viable counts were observed between the three media.

The colonial appearances of organisms on this medium differ considerably from those seen on blood or MacConkey's agar. This caused some difficulty when the modified CLED medium was first adopted, but with increased experience of the medium there has been little difficulty in distinguishing common pathogens.

Calibration curves, to correlate colony count per blotting paper impression with the actual bacterial count, were carried out as described by Leigh and Williams (1964)[3]. The following organisms, all isolates from urine specimens, were used to obtain the calibration curves.

Gram Negative Organisms: *E. coli, Proteus mirabilis, Proteus vulgaris,* Klebsiella species, *Ps. aeroginosa, Acinetobacter anitratus* and *Citrobacter freundii.*

Gram Positive Organisms: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis.*

Some difficulty was experienced in finding a suitable blotting paper. The type suggested by Leigh and Williams (1964)[3], (Postlip Mill 633 fibre free), is not readily obtainable in New Zealand. After trying several brands of blotting paper and three grades of Chromatography paper the 28lb per ream blotting paper supplied through Government Stores to all New Zealand hospitals was found to be the most satisfactory. The manufacturer of this varies from batch to batch, and although this may seem rather unsatisfactory it has been found so far that reproducibility of results between batches of this paper has been excellent and far superior to any of the other blotting papers tried.

In addition to the investigations described above, before adopting the technique as a routine, 400 urines were examined by this method in parallel with the calibrated loop technique used at that time. This comparative trial showed excellent (98.5%) correlation between the two methods. Discrepancies were investigated by repeating both tests and carrying out a Miles and Misra (1938)[4] count on the urine specimen. Six discrepancies were found. Four of these were resolved by the Miles and Misra (1938)[4] count in favour of the blotting paper method. The error of 0.5% is felt to be acceptable in a screening method of this type, particularly as it produces false positive results.

This technique is a very economical screening test for significant bacteriuria. The cost per plate (materials only costed) of the modified CLED agar is 3.97 cents. Eight urines can be examined on one plate; thus, ignoring the cost of blotting paper which is infinitesimal, the cost per urine examination is 0.49 cents. In contrast, the calibrated loop technique previously employed in this laboratory used a blood and a MacConkey agar plate at a total cost of 7.2 cents per urine examined.

D. G. BOLITHO,
Cook Hospital,
Gisborne.
March, 1969.

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It is standardized for physical appearance, pH. moisture content, cake weight, heat stability, sodium and calcium content, Factor V and fibrinogen levels. After reconstitution it is stable, if refrigerated, for 1 working day.

It is convenient and economical. Simplastin-A permits the laboratory to hold samples arriving during off hours so all prothrombin time tests can be run at the same time, by the same technologist, under the same conditions. In the pro-thrombin-proconvertin test the availability of Simplastin-A eliminates the need for special preparation of prothrombin-free plasma.

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**Factor V and fibrinogen are added to Simplastin-A specifically for the prothrombin time testing of patient plasmas over four hours old. For plasmas less than four hours old, Simplastin, the standard thromboplastin-calcium, is recommended.*

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Selected Abstracts

BLOOD BANKING

Serological Investigation of 1,358 Transfusion Reactions in 7,400 Transfusions. Ahrons, S. and Kissmeyer-Nielson, F. (1968), *Dan. med. Bull.*, 15, 259.

In the series tested, most of the reactions were febrile and in half of them leucocyte antibodies were present.

There were 66 haemolytic reactions, 51 of them delayed, and erythrocyte antibodies detected included anti-Fy^a, anti-Jk^a, anti-K, anti-C, anti-E, anti-C^w, anti-c, anti-Lu^a, anti-Le^a, anti-Le^b, and anti-P_1. In three cases with severe haemolysis no erythrocyte antibodies were found.

CHEMICAL PATHOLOGY

Assessment of an Assay System (Urograph) for the Determination of Urea Nitrogen. Logan, J. E., Taada, D. R., Krynski, I. A. and Allen, R. H. (1969), *Can. med. Ass. J.*, **100**, 335.

The precision of Urograph was found to be outside the limits of error considered acceptable for a quantitative method. Generally, Urograph tended to give high values in the normal range and low values in the upper range. Urograph should prove useful as a screening tool if duplicate analyses are carried out carefully. Data from single determinations should be subject to confirmation by a regular quantitative procedure, particularly for readings in the 25 to 40 mg.% range.

J.H.

Some Observations on the Use of Azostix for the Determination of Blood Urea Nitrogen. Eby, P. W. and Logan, J. E. (1969), *Can. med. Ass. J.*, **100**, 125.

It was concluded that, while the product should be able to screen out those patients with levels of 50 mg.% and above, further improvement in the performance of Azostix in the borderline region seems necessary before it can be considered a completely reliable screening test. J.H.

Measuring Ionised Calcium. Arras, M. J. (1969), *Postgrad. Med.*, **45**, 57.

Serum ionised calcium determinations should become invaluable as diagnostic aids with the availability of the improved specific electrode described in this article. The calcium electrode functions in a manner similar to that of the conventional pH electrode. Calcium electrodes also depend on a voltage change, but accomplish this with a liquid ion exchanger (a calcium salt of an organophosphoric acid) which has a high specificity for ionised calcium.

These measurements in a variety of diseases will undoubtedly clarify and amplify the understanding of disorders that occur in calcium homeostasis. J.H.

Creatine Phosphokinase Determination in Myocardial Infarction. Kierkegaard-Hansen, A. and Kierkegaard-Hansen, G. (1969), *Dan. med. Bull.*, **16**, 53.

Eighty-one patients suspected of having coronary occlusion were examined, using simultaneously two methods differing by the addition of 2-mercaptoethanol; it was concluded that methods not involving a reactivation of the sulphydryl groups of the creatine phosphokinase should gradually be abandoned. J.H.

HISTOPATHOLOGY

Acid Orcein-Iron and Acid Orcein-Copper Stains for Elastin. Lillie, R. D., Gutierrez, A., Madden, Dolores and Henderson, Raljean (1968), *Stain Tech.*, **43**, 203-206.

A procedure is described for staining elastin tissue in formalin fixed paraffin section. In essence it is Taenzer's Orcein solution (Orcein 1 gm., 70% alcohol 99 ml., conc. HCl. 1 ml.) followed by 0.02% ferric chloride (or 0.02% copper sulphate) in 70% alcohol for 3 minutes. The usual purple brown elastin colour is changed to black or reddish black. Useful counterstains are picro-methyl blue or flavianic acid, ferric chloride, acid fuchsin mixture. D.T.

Eosinol Counterstain for Routine Paraffin Embedded Tissue. Lee, F. W. (1969), *J. med. Lab. Technol.*, **26**, 36-37.

Acid eosin was prepared by dissolving 5 g. of eosin in 10 ml. of distilled water and adding glacial acetic acid and hydrochloric acid. After filtration the dried eosin was dissolved in a mixture of ethanol and acetone and the supernatant was added to 1,500 ml. of carbol-xylene to make a stock solution. The working solution consisted of 150 ml. of stock in 1,000 ml. of xylene. Staining time was 40 seconds followed by a rinse in xylene. before mounting. The stock solution was used for rapid frozen and cryostat sections. D.T.

A Modified One-Step Trichrome Stain for Demonstration of Fine Connective Tissue Fibres. Sweat, Faye; Meloan, Susan N. and Puchtler, Holde (1968), *Stain Tech.*, **43**, 227-231.

Gomori's one-step trichrome method was modified to improve coloration of fine connective tissue fibres. Autopsy material was fixed in a variety of fixatives. Paraffin sections were mordanted for 1 hour at 56°C in Bouin's solution then stained for 1 minute in a trichrome solution consisting of chromotrope 2 R, phosphomolybdic acid and aniline blue WS, adjusted to pH 1.3 with HCl. After a rinse in 1% acetic acid the sections were dehydrated and mounted. The method requires no differentiation and no fading was observed in sections stored for more than 8 years. D.T.

MYCOLOGY

Isolation and Recognition of Dermatophytes on a New Medium (DTM). Taplin, D., Zaias, N., Rebell, G. and Blank, H. (1969), *Archs Derm.*, **99**, 203.

The medium contains a mould inhibitor, two antibacterial antibiotics, and a pH indicator. Growth of a dermatophyte changes the medium from yellow to red. The medium has been designated Dermatophyte Test Medium (DTM). J.H.

SEROLOGY

Antinuclear Factor (ANF) Test—Its Diagnostic Value. Garewal, G. S. and Deodhar, S. D. (1969), *Cleveland Clin. Q.*, **36**, 53.

The ANF test has gained considerable popularity in recent years as an aid to diagnosis of various diseases that may have an underlying basis of autoimmunity. In the present study, human splenic tissue obtained at the time of operation was employed. Imprints were made and a drop of the patient's serum added. After incubation the slides are washed, then overlaid with fluorescent antihuman gamma-globulin and incubated. Positive fluorescence was apparent as an intense apple-green coloration of the nuclei, nuclear membranes, and/or nucleoli. It is suggested that the ANF test is of great value as an aid in the diagnosis of systemic L E and as a screening test for some of the other autoimmune diseases. J.H.

MISCELLANEOUS

Electronic Data Processing in the Clinical Laboratory. Richterich, R. and Ehrengruber, H. (1969), *Minn. Med.*, **52**, 69.

The system installed in the authors' laboratory was designed not only to reduce clerical work, but to facilitate interpretation of results and to eliminate any type of transcribing errors in the wards. Special care was taken to improve the medical significance of the results, by giving age-and-sex-adjusted normal ranges for each test, by marking abnormal results with asterisks, and by printing a daily list of abnormal results for each ward and daily cumulative case record sheets for each patient. J.H.

Versatile Non-cycling Programmer for Automating Laboratory Procedures. Wheatley, V. R. and Selmanowitz, V. J. (1969), *Med. Res. Eng.*, **8**, 34.

This simply-constructed device is essentially a converted strip-chart recorder made to function like a photoelectric Pianola. Application of the device to the procedures of lipid fractionation and analysis is described, though the unit is capable of much wider application. J.H.

Book Reviews

Exfoliative Cytology of the Stomach. D. D. Gibbs, B.A., O.M., M.R.C.P., D.C.H., 147 pages, including illustrations. Butterworth & Co., London (1968). N.Z. price $7.75.

This book covers the history of gastric cytology, methods of collection and staining of specimens, the cells both benign and malignant found in gastric washings, and gastric cytology in relation to the site and character of the cancer (this chapter includes a number of case histories).

In the last chapter he writes about gastric cytodiagnosis in relation to incidence and prognosis of gastric carcinoma and comes to the depressing although probably valid conclusion that early diagnosis does not affect the prognosis anyway.

D. D. Gibbs is a physician, and in his book there are many details which will interest only the pathologist or clinician. However the technologist, also, will enjoy the account of cell types and particularly the large collection of photographs.

The history of cytology is written in considerable detail (perhaps too much) but I found it interesting.

It is a nicely written little book, useful for reference, and pleasant to read for both the pathologist and the technologist. R.H.S.

Practical Haematology. Fourth Edition. J. V. Dacie, M.D., F.R.C.P., F.C. Path., F.R.S. and S. M. Lewis, B.Sc., M.D., D.C.P., M.C.Path. 568 pages, 104 illustrations. J. & A. Churchill, London, 1968. Price in U.K. 50s 0d.

Thicker than its predecessor by more than 130 pages, the latest edition of this popular textbook brings up to date the practical aspects of haematology and continues to provide a handbook that is indispensable in any haematology laboratory.

Obsolete techniques have been dropped in favour of those more commonly in use today, and others have been revised in step with modern advances. The cyanmethaemoglobin method now takes pride of place in the section devoted to the measurement of haemoglobin, for instance, and this is proper recognition for the technique which must be by far the most widely used; though, strangely enough, the micro-method for haematocrit estimation is not similarly recognised.

Among new techniques treated are: platelet counts by electronic methods, certain additional cytochemical staining techniques, a couple of new lysis tests for PNH, the Rose Waaler test, assay techniques for coagulation factors, tests for fibrinolytic activity. estimation of haemoglobin A_2, and electrophoresis of haemoglobins on cellulose acetate, on agar gel and on starch gel. Other new techniques are more briefly mentioned.

The familiar scheme for the investigation of auto-immune haemolytic anaemia has been considerably expanded, and much of the chapter on haemolytic anaemias has been rewritten to include reference to the different types of human immunoglobulins, and the part played by the antibodies of the I-i system is recognised. The final chapter, containing appendices on the preparation of reagents, glassware, cleaning and such like, has been lengthened to include some material that was formerly embodied in the main chapters.

Many of the old familiar illustrations are still present, but others have been dropped, most notably the one depicting the preparation of glass capillary "prickers" which, though still mentioned in the text, are not to be compared with the sterile, disposable lancets now so cheaply available. There are a few more photographs of red cell abnormalities and two new drawings illustrating abnormal autohaemolysis, as well as other useful graphs.

Dacie and Lewis have certainly succeeded in their aim to bring their book up to date, and the price for its purchase represents a bargain by any standards. J.C.

Volunteer Service Abroad

Miss Patricia August, of Greymouth, a laboratory technologist who is at present working on Volunteer Service Abroad in the British Solomon Islands, watches one of her trainees at work in the laboratory of Central Hospital, Honiara.

VSA has other assignments in the Pacific for laboratory technologists. Fares, insurances and clothing, living and rehabilitation allowances are paid. Those interested in a term of service overseas, giving urgently-needed assistance and gaining valuable experience, should write to VSA, P.O. Box 3564, Wellington, for further details.

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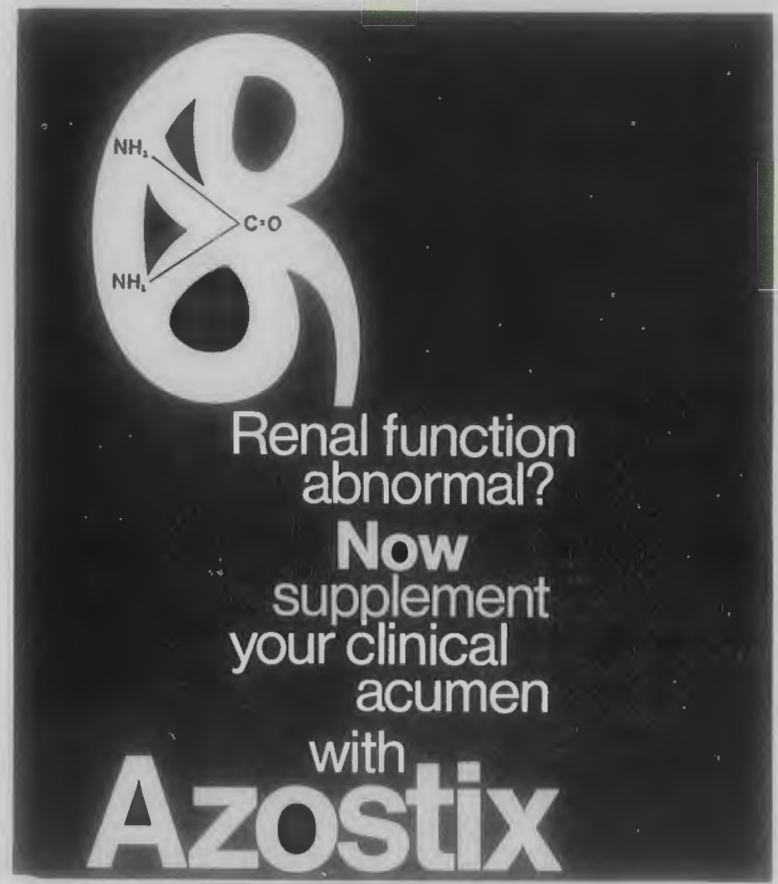

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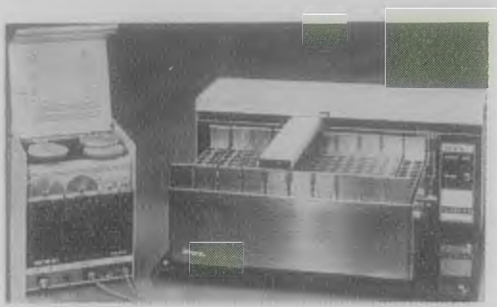
What's New

A NEW CHEMICAL FRACTION COLLECTING SYSTEM



A new system of collecting chemical samples, which is capable of producing accurate and reproducible fractions as small as 0.1 ml, is announced by *Baird & Tatlock (London) Ltd.*, of Chadwell Heath, Essex, England. The system, know as the B.T.L. Fraction Collecting System, eliminates the need to count drops and operates as a closed loop in which the outlet from a peristaltic pump is connected to the inlet of a chromatograph column, whose outlet is connected directly to a dispensing unit. This arrangement permits both the fraction size and the flow rate to be correctly matched and controlled from the inlet side of the assembly, which, in turn, enables small fractions to be reproduced very accurately.

The fraction collecting system comprises two separate units — a pump, known as the 'Chromapump,' and a fraction collector — the 'Chromafrac.' The pump is driven by a constant speed motor and has two variable gear trains. One can be set to give any of 17 fraction sizes between 0.1 ml. and 15 ml. and drives a microswitch which controls the movement of the dispensing head; the other gear train controls the speed of the pump, so that the flow rate can be matched with the fraction size. The 'Chromafrac' fraction collector consists of a rack which can hold up to 200 test tubes in each of which a fraction is collected and a dispensing head which, under the control of the microswitch in the 'Chromapump,' moves over the test tubes and dispenses a single fraction into each tube. A flow analyser can be connected between the chromatograph column and the dispensing head. A suitable analyser and recorder can be supplied as optional extras.

For simpler applications the 'Chromafrac' can be used without the 'Chromapump.' It can be gravity-fed from an eluent reservoir and a timer used to control the dispensing head which then operates as a time-flow fraction cutter.

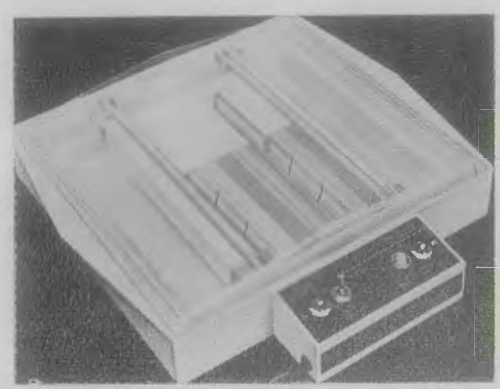
DEMONSTRATION ATTACHMENT 10x

The projected reproduction of microscopic pictures offers advantages not only during lectures, but also during discussions on microscopic specimens by a smaller circle of persons. To this end, *Carl Zeiss-Jena* have developed the Demonstration Attachment 10x.

This attachment may be used with all *Zeiss* transmitted-light microscopes. It has been designed for microscope objectives of the tube length 160 mm. and may be applied to the head of the microscope arm in place of the observation tube.

The Demonstration Attachment is provided with a projection eyepiece 10x, a flap-type shutter and a projection screen with a *Fresnel*-lens of 160 mm. diameter. The frame of the projection screen has four boreholes, into which ordinary stage clips are introduced, with whose aid transparent paper for tracing, foils with graduations or square plates for measuring and counting, as well as other means of evaluation may be fixed on the projection screen, thus considerably extending the range of application of the Demonstration Attachment. In addition, the projection screen can be easily exchanged for a 9 x 12 cm. dark-slide holder or an adapter for the Polaroid cut-film holder 500.

NEW APPARATUS FOR ELECTROPHORESIS



A variety of electrophoretic techniques which use filter paper, cellulose acetate, starch gel, agar gel etc, can be carried out on the new Electrophoresis Apparatus after Kohn, Model U77, announced by *Shandon Scientific Company Ltd.* of London N.W.10. A range of accessories can be supplied which include a comprehensive set of equipment for micro - immunoelectrophoresis and a cooling platen for separating samples subject to degradation by heat — e.g. enzymes.

The U77 can be arranged to provide a bridge gap of 1 cm. to 21 cm. in 1 mm. steps. This enables a wide variety of techniques to be used — e.g. for short-run high voltage electrophoresis (200V over 2 cm.). It is also suitable for thin layer electrophoresis on 20 x 20 cm. plates. The tank is a one-piece plastic moulding. It is easily set up and incorporates a labyrinth connection between buffer and electrode compartments, obviating filter paper or cotton wicks. Arrangements for tensioning and supporting the strips are simple. The whole unit may be totally immersed for cleaning.

Connection to the power supply may be either direct or via a polarity reversal and safety switch unit which is available as an accessory.

UNIVERSAL SEPARATING CHAMBER FOR THE ZONE ELECTROPHORESIS

The universality of the new separating chamber, by *Carl Zeiss-Jena,* exists in the fact that all methods of zone electrophoresis known so far may be carried out with it, as well as paper electrophoresis on membrane foils, in agar gel, in starch gel, or in other layers. In addition to paper electrophoresis, the other methods are carried out in the so-called microformat (about the size of the specimen carrier). This variability is achieved because different inserts (the separating chambers) may be accommodated in one housing. Further inserts are in the course of development.

The separating chambers are equipped with platinum electrodes and with an electrode system especially developed for zone electrophoresis. The electrodes in question are reversible ones, with the aid of which the electrolytic effects and the associated displacements of the pH-values are avoided. Due to these aforementioned electrodes and to the special construction of the chamber, very stable separation conditions have been achieved, although only 100 ml. of buffer solution is required per buffer chamber. The dimensions of this chamber, which is made of PVC material, have been deliberately kept small.

VACUTAINER MULTIPLE SAMPLE NEEDLE

Difficulties associated with the collection of multiple blood samples from one patient by the Vacutainer system are overcome by the latest addition to the system's range of items.

The new product, by the *Becton-Dickinson Company,* is similar to the familiar two-ended Vacutainer needle but features a latex rubber sleeve that occludes the stopper-puncturing end of the needle.

The removal of one blood specimen tube and its replacement with another can thus be accomplished without seepage of blood into the holder.

Further details from: *Smith-Biolab Ltd.*, P.O. Box 36007 (or branches in Wellington and Christchurch).

A NEW RANGE OF DIAGNOSTIC REAGENTS

Now available in New Zealand is a new range of latex serological reagents from *Italdiagnostic* of Italy.

The range consists at present of reagents for the diagnosis of four diseases—toxoplasmosis, echninococcosis, infectious mononucleosis and rheumatoid arthritis.

Each kit contains sufficient reagent for at least 40 tests, together with buffer solution and positive and negative control sera.

Further details from *Smith-Biolab Ltd.*

ELECTROPHORESIS APPARATUS FOR MULTIPLE SEPARATIONS

The MULTI-MICROBAND Electrophoresis Apparatus announced by *Shandon Scientific Company Ltd.* of London N.W.10, is designed for rapid, economical routine examination of large numbers of samples in hospitals, research establishments etc. It will separate 10 or 16 mechanically-applied samples simultaneously in 20 minutes. The medium employed is cellulose acetate membrane and the separations are sharp and precisely located for rapid comparison. The bench space required is only 35 cm. x 24 cm.

The 10 or 16 samples are applied to a 150 x 78 mm. sheet of cellulose acetate by special applicators. These have 10 or 16 square teeth each grooved to retain a reproducible volume when dipped into pools of sample. The sample pools are pipetted into marked positions on a glass or PVC strip. The applicator is then applied to the cellulose acetate, transferring the samples. Sample volumes are 0.4 microlitres for the 10-sample applicator and 0.25 microlitres for the 16-sample applicator. The sample positions are numbered and there is an extra wide gap between the first and second teeth of the applicators to aid identification.

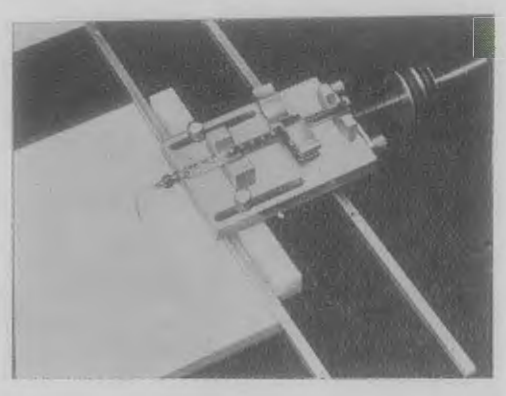
The special power supply may be connected to the apparatus either directly or via a polarity reversal and safety switch unit available as an accessory.

STANDARDS FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY

Now available from *British Drug Houses Ltd.* are ready-made standards for the rapid determination of trace metals by atomic absorption spectrophotometry.

Each standard contains 1 mg. of the metal per ml. and is prepared in N acid to prevent hydrolysis and mould growth.

CHROMATOGRAPHY SAMPLE STREAKER



Shandon Scientific Company Ltd. of London N.W.10 now manufacture a low-priced Chromatography Sample Streaker enabling a thin line, or streak, of sample to be deposited on filter paper, thin layer or preparative layer chromatographic media. The streak is quantitatively uniform throughout its length, very reproducible and it can be accurately positioned on the medium.

The streaker may be used with a hypodermic syringe or (with a special drive unit) an 'AGLA' micrometer syringe. The syringe is fitted with a right-angled Luer-fitting needle to which is attached a short length of sample-transfer tubing. The chromatographic medium is placed on the baseboard against the guide rail. The syringe is then clipped to the carrier which slides across the baseboard against one of the two guide rails, the tip of the sample-transfer tubing being in contact with the medium. A drive wheel, rotating on a threaded shaft on the carrier, bears against the syringe plunger, discharging the sample as the carrier is moved across the baseboard. A special twin-wheel drive unit is used for the 'AGLA' syringe. A guide bar on the carrier may be adjusted to position the streak on the chromatographic medium.

South Island Seminar

The South Island Seminar was held at Timaru Hospital on May 3, 1969, and was attended by approximately fifty members representing a large number of members.

The opening address was given by Dr M. Brookfield, who spoke of the importance of quality control in the diagnostic laboratory.

The programme consisted of short papers, followed by discussion periods. Papers presented were:—

Problems of Enzyme Units G. McLaren
The Augmented Histamine Test in the Diagnosis of Peptic Ulceration F. L. N. Corey
An Instant Romanowsky Stain T. E. Tanner
An Unusual Haematology Case Miss B. Don
Mycosis Fungoides — Sizary's Syndrome J. Rees
What Makes a Clotting Profile? B. Rae
Medical Technology in the Islands Miss M. M. Eales

Mr J. D. R. Morgan also gave a brief resume of Council activities and Mr J. Case detailed the recent proposals made by the Institute for amendments to the Hospital Employment Regulations.

The South Canterbury Hospital Board again provided lunch and morning and afternoon teas, and a number of the delegates rounded off the day with dinner at the Hibernian Hotel.

P.L.L.

Obituary

FLORA SMITH, B.Sc., M.A., M.B.,Ch.B., F.C. Path., M.C.P.A.

The death occurred in Auckland, on 22 May, 1969, of Dr Flora Smith, Senior Morbid Anatomist at the Central Laboratory, Auckland Hospital.

Educated in Wellington, Dr Smith had intended to follow a teaching profession, and to this end completed a M.A. degree at Victoria University. Her entry into the medical world was as a trainee bacteriologist at the Wellington Public Hospital laboratory under Dr J. O. Mercer. It was during this period that she added a B.Sc. degree to her list of academic achievements. After working for some years in the private laboratory of Dr P. P. Lynch she began the medical course, finally graduating M.B., Ch.B. from Otago University. Returning to Wellington Hospital as a house surgeon, she decided to specialise in pathology and found herself back once more in the laboratory. In November 1954, she moved to Auckland to fill the position she held for the remainder of her career.

Dr Smith was interested in all branches of pathology, but it was in morbid anatomy that her interests chiefly lay. She was a prolific worker for whom only the best would do, both from herself and her subordinates. She was intolerant of short cuts or any form of work that was not up to standard. However, for all her enthusiasm, her feet were always firmly on the ground, and she was at all times prepared to risk the displeasure of a clinician for refusing to be caught in the current of over-enthusiasm. Nevertheless, she enjoyed a very good relationship with her colleagues and the many slides referred to her were testimony of the high esteem in which her opinions were held. Her orderly mind always allowed her to see things in their proper perspective. She never attempted to simplify the complicated nor to complicate the simple.

Outside of medicine her interests were many and varied. Sport played a big part in her life. She played tennis and golf regularly and in the summer months enjoyed a daily swim. She was a member of the Ruapehu Alpine Club and had always been interested in skiing, mountaineering and tramping. She had travelled extensively both at home and abroad. Her other interests included such things as music and drama and even metal work and mechanical engineering, courses of which she had taken at evening classes. To her an active mind was a healthy mind and there can be no doubt that she lived up to this axiom.

Dr Smith was a person of many qualities. Space does not permit them to be dwelt on here. However, at a time such as this two of these come to mind. The first is her loyalty to those with whom she was associated, be they friends or colleagues; and the second is the courage with which she faced death. During her illness, which had lasted over a year, she had carried on, neither seeking sympathy nor feeling resentment. She was at her bench to within a few weeks of her death. Dr Smith was indeed a noble woman who will be missed by many.

To her brother, Mr J. Smith of Wellington, we offer our deepest sympathy.

R.J.P.

Directions for Contributors

Adherence to the following instructions is necessary in order to ensure uniformity of presentation, and all contributors are urged to study them before submitting their manuscripts.

Manuscripts should be typewritten on one side only of good quality quarto paper, be double spaced and have a one inch margin all round. They should bear the author's name (male authors give initials and female authors one given name), address and (if this is different) the address of the laboratory where the work was carried out. Carbon copies are not acceptable, and nothing should be underlined unless it is to be printed in italics. The use of italics to denote emphasis should be avoided, if possible.

ILLUSTRATIONS

The *Journal* will bear the cost of a reasonable number of illustrations, but these should be used sparingly. Graphs, line drawings and photographs are all referred to as "Figures" and should be numbered in the order of their appearance in the text, using Arabic numerals. Drawings should be made in Indian ink on stout white paper, somewhat larger than required for reproduction. Legends should be typed on separate pieces of paper, and their approximate position in relation to the text should be noted in the typescript. Elaborate tables should be kept to a minimum, should be typed on separate pieces of paper and numbered in Roman numerals.

NOMENCLATURE

Scientific names of micro-organisms should be in conformity with the style adopted in the latest edition of *Bergey's Manual of Determinative Bacteriology* and should be underlined to indicate that they are to be printed in italics. Abbreviations such as CSF for cerebro-spinal fluid are permissible, but their meaning must be clearly indicated when first introduced. Conventional abbreviations such as ml. for millilitre are acceptable without explanation, but authors should note that the correct abbreviation for gram (or grams) is g. and not gm. or gms.

REFERENCES

Only papers closely related to the author's work should be quoted. Authors should study past issues of the *Journal* for examples of the preferred method of making reference. All references are brought together at the end in alphabetical order and numbered, the appropriate numerals being used in superscript within the text. In the list references should include the surname of the author, followed by initials (or by one given name if the author is a female), the year of publication in brackets, the abbreviated title of the publication (underlined to denote italics), the volume number and the page number. If there are three or more authors, the first author's name may be used in the text followed by the words *et al.*, but the names of the co-authors must be given in the list. The abbreviation of titles of periodicals may be copied from *World List of Scientific Periodicals,* but the derivation is largely common sense. Broadly, all prepositions and conjunctions are omitted, nouns commence with a capital letter, adjectives lower case, and words are foreshortened consistent with understanding. Example: *The American Journal of Clinical Pathology is Amer. J. clin. Path.* References to books should include name(s), year, title (underlined), edition, page number(s), name of publisher and place of publication in that order.

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PROTEUS SET

Urease Tablets
Voges-Proskauer Test Tablets
Indole IPA Tablets
Mannitol Fermentation Tablets
Lactose Fermentation Tablets
Gelatin Strips

PSEUDOMONAS SET

Dextrose Fermentation Tablets
Key Gluconate Tablets
Key Oxidase Test Tablets
Key Nitrite Test Tablets
Key Motility-Nitrate Tablets
Key Gelatin Strips

SALMONELLA-ARIZONA SET

Lactose Fermentation Tablets
Sucrose Fermentation Tablets
Salicin Fermentation Tablets
Adonitol Fermentation Tablets
Salmonella-Arizona Phage
Lysine Decarboxylase Tablets
Ninhydrin Tablets
Brilliant Green Agar Tablets

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