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Magnesium	$\pm$ 0.3 mg	$\pm$ 0.25	$\pm$ 0.5 mg	$\pm$ 0.25
Iron	$\pm$ 8.0 mcg	$\pm$ 0.25	$\pm$ 8.0 mcg	$\pm$ 0.4
TIBC	$\pm$ 25.0 mcg			
Bilirubin	$\pm$ 0.1 mg		$\pm$ 0.3 mg	
Uric Acid	$\pm$ 0.3 mg		$\pm$ 0.5 mg	
PBI	$\pm$ 0.3 mcg		$\pm$ 0.3 mcg	
T3	$\pm$ 2		$\pm$ 2	
Cholesterol	$\pm$ 10.0 mg		$\pm$ 10.0 mg	
Enzymes	$\pm$ 10%		$\pm$ 10%	

**ADDITIONAL VALUES\***

Constituents	per 100 ml	Normal Adult Range per 100 ml	Method
<b>THYROID PROFILE:</b>			
Butanol Extractable Iodine	3.5 mcg	3.2-6.2 mcg	DADE
Thyroxine (T <sub>4</sub> -isotope f = 78.1)	7.3 mcg	4-11 mcg	Murphy-Pattee
Thyroxine iodine (T <sub>4</sub> - column)	3.6 mcg	3.2-5.4 mcg	DADE
Thyroxine Binding Globulin	13.8 mcg	14-21 mcg	Beirwaltes-Robbins
Protein Bound Iodine	5.7 mcg		AutoAnalyzer (N-56)
<b>LIPID FRACTIONATION:</b>			
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
<b>ENZYMES:</b>			
Phosphatase, Alkaline	1.2 mM units/liter	0.8-2.9 mM units/liter	Bessey-Lowry-Brock
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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.
<b>MISCELLANEOUS:</b>			
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
<b>THYMOL TURBIDITY:</b>			
Coleman, Jr.	4.2 units	0-5.5 units	} Buffer - Tris, pH 7.55 Units - Shank-Hoagland
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A/G RATIO	1.88		
GLUCOSE — Folin-Wu	99 mg		
Nelson-Somogyi	89 mg		
AutoAnalyzer (N-2a)	94 mg		
NON-PROTEIN NITROGEN — Koch-McMeekin	24.2 mg		
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Kawerau (Monoxime)	13.7 mg		
AutoAnalyzer (N-1a)	15.3 mg		
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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 — Flske-SubbaRow	3.6 mμ	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
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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)	15.4 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-Giovannelli	340 mcg		
BILIRUBIN — Malloy-Evelyn	0.5 mg		
Jendrassik-Grot-AutoAnalyzer (N-12b-P)	0.4 mg		
Malloy-Evelyn-AutoAnalyzer (N-12)	0.6 mg		
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DADE TETRA-FORM <sup>TM</sup>	104 units/ml		
LDs — Wroblewski-Gregory	105 units/ml		
LDH ISOENZYMES — DADE ISO-FORM <sup>TM</sup>	LDs (Heart) 17.8%; LD1 (Liver) 12.6% LD2 35.3%; LD3 21.9%; LD4 12.4%		
HBD — Rosalki	215 units/ml		
LIPASE — Cherry-Crandell	0.7 units/ml		
Tietz-Borden-Stepleton	0.4 units/ml		
AMYLAASE — 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	Beckman, Microzone††	Gelman (Cellulose Acetate)	
gm per 100 ml	Percent of Total Protein	HR Buffer 0.05 Ionic Conc'n.	
		Percent of Total Protein	
Total Protein	6.90		
Albumin	4.49	65.0	64.0
Total Globulin	2.41	35.0	36.0
Alpha <sub>1</sub> Globulin	0.23	3.3	4.0
Alpha <sub>2</sub> Globulin	0.52	7.5	6.0
Beta Globulin	0.71	10.3	9.5
Gamma Globulin	0.95	13.8	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.



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duce fluorescin. Cultures which produced fluorescin, were oxidase positive and oxidative, but did not grow at 44°C, produce pyocyanin, liquefy gelatin or reduce nitrate to nitrogen were identified as *Ps. fluorescens*. These 7 tests were considered sufficient to differentiate clearly between *Ps. aeruginosa* and *Ps. fluorescens*. There was little value from urea, malonate or glucose tests due to variable reactions. Growth on 1% tetrazolium agar was not confined to *Ps. aeruginosa* (50% of *Ps. fluorescens* tested also grew on this medium), and utilisation of citrate and hydrolysis of arginine are common to most species of *Pseudomonas*, including *Ps. fluorescens*.

### PYOCINE TYPING

Table III shows the distribution of the most common pyocine types in the towns of New Zealand. Table IV deals with environmental strains, and Table V shows the relationship between pyocine types and source of infection. There is a particularly high percentage of untypable strains in Auckland, of which nearly half were from respiratory infections. A large number of the cultures from Hamilton were from faeces and the majority of these were type 3. It seems likely that the predominance of these types is endemic, and not related to the source of infection. The high percentage of type 1 cultures in Dunedin is due to a number of duplicate specimens. There is a higher percentage of type 10 in Wellington than in other towns (except Tauranga where the numbers are probably too small to be significant) and this is also seen in the environmental cultures, where the majority of type 10 strains were from Wellington. This seems to be the only case where a type not related to any source of infection is more common in one area. Environmental cultures show a somewhat similar distribution to those from human sources, except that the percentage of untypable strains is much lower and the percentage of type 1 is higher.

The pyocine types among isolates from hospital in-patients were compared with those from out-patients and the distribution was fairly similar except for a higher percentage of miscellaneous types amongst out-patients. The relationship between pyocine type, age and source of infection was studied but very little correlation was apparent. The majority of respiratory, wound and urinary infections occurred in the over-50 age group, which is to be expected. It is interesting to note that there were very few cultures from babies and very few burns cultures. Of the cultures from babies, type 1 predominates with very few untypables or miscellaneous cultures. There appears to be an increase of untypable and miscellaneous types in the higher age groups.

An attempt was made to correlate the variable bio-chemical reactions with pyocine types, but in most cases the results do not appear to be significant. About 18% of all cultures produced a blue fluorescence. A fairly high percentage of type 10 cultures

Town	No. of Cultures	Type 1	Type 3	Type 5	Type 10	Untypable	Misc.
Auckland	82	26 32%	2 2%	2 2%	1 1%	45 55%	6 7%
Hamilton	128	16 13%	59 46%	7 5%	5 4%	28 22%	13 10%
Tauranga	16	4 25%	1 6%	3 19%	3 19%	2 13%	3 19%
Wellington	138	48 36%	5 4%	1 1%	20 14%	23 17%	41 30%
Dunedin	64	34 53%	4 6%	---	1 2%	18 28%	7 11%
Others	47	12 26%	1 9%	1 2%	2 4%	16 34%	12 26%
Total	475	140 29%	75 16%	14 3%	32 7%	132 28%	82 17%

Table III: Pyocine type related to town

	No. of Cultures	Type 1	Type 3	Type 5	Type 10	Untypable	Misc.
Plugholes and overflows	81	35 43%	17 21%	1 1%	14 17%	5 6%	9 11%
Mops and buckets	25	11 44%	1 4%	---	8 32%	2 8%	3 12%
"Sterile equipment"	9	2 22%	---	---	7 78%	---	---
Total	115	48 42%	18 16%	1 1%	29 25%	7 6%	12 10%

Table IV: Pyocine types in hospital environment



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## *Pseudomonas aeruginosa*: Isolation, Identification and Typing.

SUSAN HARDING, B.Sc., A.N.Z.I.M.L.T.  
National Health Institute, Wellington.

*Received for publication December 1969.*

Reports of *Pseudomonas* infection have become much more frequent in the last few years. Features which make *Pseudomonas* hard to eradicate are its resistance to most antibiotics, its ability to survive in moist conditions and thus in many types of equipment<sup>10,13</sup> and its relative insensitivity to many disinfectants<sup>3,8</sup>. With the ability of *Pseudomonas* to remain viable in so many situations the source of infection can in many cases only be traced by the typing of strains isolated<sup>1,5,14</sup>.

The aim of this survey was firstly to establish suitable methods for isolation of *Pseudomonas aeruginosa* and differentiation from other species, and then to evaluate pyocine typing as a method for further identification of strains of *Ps. aeruginosa* for epidemiological work. Finally it was intended to determine the predominant types in this country and also to determine whether predominant types are related to specific districts or to types of infection, and whether there is any relationship between variable biochemical reactions such as pigment production and pyocine types.

### Materials and Methods

475 cultures were collected, over a period of 6 months from hospitals and private laboratories throughout New Zealand. (See Table I.)

A smaller number of cultures (115) was collected from the environment, but only from Wellington, Christchurch, Masterton and Hamilton. These cultures were from basin plug-holes and overflows, cleaners' mops and buckets, and "sterile equipment" which included silicone ointment, hand creams, clean blankets, and a humidifier from a baby incubator. Environmental cultures were collected by swabs incubated in tryptose phosphate broth and then plated on 0.03% cetrinide agar<sup>2</sup>, or by impression plates of cetrinide agar.

The following tests were carried out on all cultures:—oxidase, oxidation or fermentation of glucose (Hugh and Leifson), production of pyocyanin or pyorubrin (modified Sierra medium<sup>2</sup>), production of fluorescin (cetrinide agar), utilisation of citrate (Kosers), arginine hydrolysis, liquefaction of gelatin at 37°C, growth in peptone water at 44°C, reduction of nitrate<sup>4</sup>, production of acid in glucose and mannite (1% sugars in peptone water with Andrades indicator), the hydrolysis of urea, utilisation of malonate,

and growth on nutrient agar containing 1% triphenyltetrazolium chloride<sup>15</sup>. Results were read after 1 day, 2 days, and 7 days.

All cultures identified as *Ps. aeruginosa* were pyocine typed by the method of Gillies and Govan<sup>6</sup>. Briefly, this consists of streaking the culture to be tested across a blood agar plate and incubating overnight at 30°C. The culture is then removed with a glass slide, and residual organisms killed with chloroform vapour. The 8 indicator strains are streaked across at right angles to the original inoculum and the plate incubated overnight at 37°C. The pyocine type of the test strain is determined from the pattern of inhibition of the indicator strains. (Figure 1.)

## Results and Discussion

### BIOCHEMICAL TESTS

Table II shows the results of some of the biochemical tests. Cultures were considered to be *Ps. aeruginosa* if the following tests were positive: oxidase, oxidation of glucose, production of fluorescin, and growth at 44°C. All other cultures were discarded. All cultures of *Ps. aeruginosa*, were citrate, arginine and tetrazolium positive within 24 hours, and mannite negative after 7 days. 87% produced pyocyanin or pyorubrin, and 25% utilised malonate. Other results were variable and not always in agreement with other authors.

**Gelatin:** Liquefaction of gelatin occurred within 2 days in 81% of all cultures.

**Nitrate Reduction:** Reduction of nitrate to nitrogen is a characteristic of *Ps. aeruginosa* and one or two other species of *Pseudomonas*<sup>2,9</sup>. Most other species do not reduce nitrate or reduce it to nitrite only.

**Production of Blue Fluorescence:** Certain strains of *Ps. aeruginosa* produce a blue fluorescence rather than the yellowish green fluorescence of most cultures.

**Urea:** Lysenko<sup>9</sup> and Cowan and Steel<sup>4</sup> state that *Ps. aeruginosa* hydrolyses urea, but production of alkali from urea depends on the medium used<sup>4</sup>. Using a commercial urea agar base (without agar) only 6% of cultures were positive within 2 days although more than 50% were positive at 7 days.

**Glucose:** Acid is produced from glucose<sup>1,9</sup> but this also depends on the medium used<sup>4</sup>. In the case of *Ps. aeruginosa* the production of pigment may mask the colour change of the indicator.

### DIFFERENTIATION OF *PS. AERUGINOSA* AND *PS. FLUORESCENS*

For the preliminary isolation and identification of *Pseudomonas* 0.03% cetrinide agar was found to be a satisfactory medium, as most other organisms are inhibited and *Ps. aeruginosa* and *Ps. fluorescens* are the only common strains of *Pseudomonas* to pro-

Town	No. of Cultures	Ears	Respiratory	Wounds	Urnies	Faeces	Misc.
Auckland	82	7	27	23	23	—	—
Hamilton	128	26	4	9	30	58	1
Tauranga	16	7	—	2	6	—	1
Wellington	138	7	17	14	70	20	10
Dunedin	64	—	21	20	12	3	8
Other towns	47	5	10	9	12	7	4
Total	475	52	79	79	153	88	24
			17%	17%	32%	19%	5%

Table I: Source of specimens  
 Note: Miscellaneous specimens include 7 burns, 8 skins, 4 umbilical, 3 C.S.F.'s, and 2 blood cultures.

Biochemical Tests	Human strains (475)	Environmental strains (115)
Gelatin liquefied	92%	97%
Nitrate reduced to nitrogen	99%	99%
Production of pycyanin or pyorubin	87%	87%
Production of blue fluorescence	18%	17%
Hydrolysis of urea	54%	61%
Utilisation of malonate	21%	37%
Acid in glucose	14%	19%

Table II: Results of biochemical tests

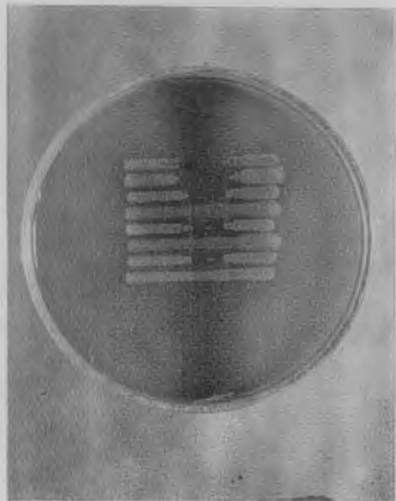


Figure 1: Pyocine type 3

Specimen	No. of Cultures	Type 1	Type 3	Type 5	Type 10	Untypable	Misc.
Ears	52	51%	6%	10%	4%	33%	16%
Respiratory	79	24%	1%	—	4%	54%	17%
Burns	7	43%	—	14%	—	29%	14%
Wounds	79	42%	9%	—	14%	16%	19%
Urines	153	33%	3%	5%	10%	32%	17%
Faeces	88	13%	66%	1%	—	7%	13%
Skin	12	33%	17%	8%	—	17%	25%

Table V: Pycocine type related to source

(42%) show this blue fluorescence and at the same time show an absence of pyocyanin. Of the 56 type 3 faecal cultures from Hamilton, 50 produced blue fluorescence and also pyocyanin. The only other type 3 culture producing blue fluorescence was from an umbilical infection in Hamilton.

32% of the cultures were type 1 and 24% were untypable, which suggests that it could be of value to develop new indicator strains in this country. This would reduce the number of untypables and might also divide the typable strains into smaller groups. Alternatively, introduction of another method such as phage or serological typing in conjunction with pyocine typing would enable the source of infections to be traced more satisfactorily in epidemiological work<sup>1, 2</sup>.

### Summary

590 strains of *Ps. aeruginosa* have been tested for various biochemical reactions and a suitable method of isolation and identification established. The same strains have been pyocine typed and a relatively high percentage of type 1 and untypable strains found throughout the country, but very little correlation between pyocine type and any other factors. It is suggested that new indicator strains could be of value, or introduction of phage or serological typing so that strains could be divided into smaller groups.

### Acknowledgments

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## A Comparison of Diagnostic Tests for Thyroid Disorders

SHIRLEY A. DURNEY,

Radioisotope Unit, Auckland Hospital.

*Abridged from a thesis submitted in partial fulfilment of the examination for Qualified Technical Officer, September 1969.*

### Introduction

The thyroid gland concentrates ingested iodine and combines it with tyrosine for the synthesis of thyroid hormones. These hormones are stored and released according to need. Malfunction of the gland leads to overactive or underactive thyroid states.

There are many tests used in the study of thyroid gland activity. Each test has its own limitations in diagnostic accuracy and it is proposed in this investigation to compare the usefulness and reliability of five of the tests used in the Thyroid Clinic at Auckland Hospital.

The tests under study are:

(a) four hour uptake; (b) forty-eight hour uptake; (c) protein bound iodine estimation (PBI); (d) triiodothyronine resin uptake ( $T_3$  Resin); (e) calculated free thyroxine index.

### Procedure

#### *Clinical material*

Over the past 10 months tests have been carried out on 333 patients attending this clinic. The diagnosis of these patients was made by clinical opinion and only those patients with a definite clinical diagnosis were included. Of the 333 patients selected for detailed study 233 were clinically confirmed as euthyroid, 59 as having thyrotoxicosis and 41 as having hypothyroidism.

### Methods

#### (a) & (b) Determination of Four Hour and Forty-eight Hour Uptakes

If a small amount of radioactive iodine is administered orally to the patient it is absorbed into the blood stream and mixes with the inorganic iodine in the plasma. The thyroid gland is continually removing inorganic iodine from the plasma, to produce thyroid hormones. The rate of uptake of radioiodine into the thyroid is therefore a measure of its metabolic rate.

The uptake of radioactive iodine has been a popular test in the diagnosis of thyroid disorders since 1946.

#### *Method:*

A drink of 15 ml. of a 0.02  $\mu\text{g}$ . per ml. solution of potassium iodide is dispensed and 15 microcuries of radioactive iodine  $I^{131}$  is added. The drink is then measured by placing it in a neck phantom and measuring the amount of radioactivity in the drink on a scintillation counter. The neck phantom is a large perspex block which allows the same amount of absorption and backscatter of the isotope rays as the tissues in the neck. (The phantom is constructed according to the directions of the International Atomic

Energy Commission in order that thyroid counting equipment may be standardised throughout the world.)

The drink is administered to the patient, who returns after four hours and the scintillation counter is held at a fixed distance over the thyroid gland. A measurement is also recorded over the thigh to compensate for the extrathyroidal activity. The net thyroid count is calculated as a percentage of the initial dose administered.

*Calculation:*

$$\text{4 Hour Uptake: } \frac{\text{Neck Count} - \text{Thigh Count}}{\text{Drink Count} - \text{Room Background}} \times 100\%$$

These neck and thigh counts are recorded again at an interval of 48 hours after the administered dose and allowances made for the loss of count due to radioactive decay.

(c) **Determination of Protein Bound Iodine**

The thyroxine level in the blood determines the patient's metabolic rate. As each molecule of thyroxine has four iodine atoms the protein bound iodine estimation is a reflection of the circulating thyroxine. (In fact the thyroxine level in micrograms per 100 ml. = PBI 127 micrograms per 100 ml.  $\times$  1.53).

This test was carried out by the Central Laboratory at Auckland Hospital by an automated technique<sup>4</sup> based on the method of Henry<sup>3</sup>.

(d) **Determination of T<sub>3</sub> Resin Uptake Test**

A method for testing thyroid function was developed by Hamolsky *et al* (1957)<sup>1</sup> using the *in vitro* uptake of triiodothyronine — I<sup>131</sup> by red cells in whole blood. This test was found to be unsatisfactory as it was affected by haematocrit levels,<sup>6</sup> pH of the blood<sup>2</sup> and damage to red cells during the test. Mitchell (1958)<sup>5</sup> reported the use of an ion exchange resin instead of the red blood cells and further modifications of this method have been reported.

This is a test for measuring the unoccupied thyroxine binding sites in blood. As the level of thyroxine increases the number of unbound sites will decrease, and conversely. The thyroxine binding sites have a greater affinity for thyroxine than triiodothyronine. Therefore if radioactive T<sub>3</sub> is incubated with serum followed by addition of resin, the available binding sites in serum compete with sites on the resin for the radioactive T<sub>3</sub>. A high level of thyroxine in serum will give a decrease in the number of free binding sites and a greater amount of T<sub>3</sub> will bind to resin. The radioactive count on the resin is therefore a reflection of the thyroid hormones.

Our method of T<sub>3</sub> Resin uptake is a modification of the method used by Sterling and Tabachnick (1961)<sup>7</sup>.

*Reagents:* Amberlite IRA 400 resin \* converted from chloride form to formate form.

Triiodothyronine I<sup>125</sup> \*\*

\* Obtained from BDH Laboratories Chemicals Division, Poole, England.

\*\* Obtained from Radiochemical Centre, Amersham, England.

**Method:**

A 10 ml. working solution of  $T_3 - I^{125}$  is made up fresh each day with a concentration of 0.01 micrograms  $T_3 - I^{125}$  per ml. of distilled water. Two ml. of serum is pipetted into 25 ml. Erlenmeyer flasks, an accurate 0.4 ml. of working solution is then added and the tops of the flasks closed with parafilm. Two extra flasks are prepared for the standard serum and 100% value. All the flasks are shaken for 45 minutes at a low speed in a shaking incubator at 37-38°C.

Counting tubes each containing 500 mg. of resin are placed in an ice bath. Two tubes are required for each Erlenmeyer flask. When the flasks have completed 45 minutes of shaking they are placed in ice also, for at least 30 minutes. Keeping everything as cold as possible, 1 ml. of serum —  $T_3$  is added very accurately to the resin tube and capped. Each serum is done in duplicate. The two resin tubes from one of the standard flasks are kept back as these are for the 100% value.

The rest of the samples are shaken in the refrigerator for 90 minutes. After shaking, the tubes are put in a rack in the ice bath, some of the iced water is used for washing and approximately 5 ml. of this is added to each tube. It is mixed well with the resin and after settling the supernatant is sucked off, ensuring that no resin is sucked away. This washing process is repeated twice more keeping the resin tubes and water chilled throughout.

All the tubes are counted, including the two 100% standard tubes.

**Calculation:**

$$\frac{\text{Count in resin - background}}{\text{Count in 100\% standard - background}} \times 100 = \frac{\text{percentage resin}}{\text{uptake}}$$

To express the result as a comparison with a standard serum  
 $\% \text{ resin uptake} \times \frac{\% \text{ uptake in standard serum DAY X}}{\% \text{ uptake in standard serum on day of test}}$

**(e) Determination of a Free Thyroxine Index**

It is known that thyroxine is transported to the tissues from the thyroid gland, bound in a reversible association with plasma proteins.



These are thyroxine binding proteins (TBP). A very small portion of thyroxine remains unbound or free, and it is the level of this free thyroxine in the blood which determines the metabolic rate of the body.

The level of free thyroxine is very small and methods for detecting and measuring it are still being developed. An indication of the free thyroxine concentration can be obtained from the combination of the PBI and  $T_3$  resin estimations. This can be calcu-

lated from the following equation, which can be validated mathematically:—

$$T_4 \propto \frac{\text{PBI} \times 100}{100 - T_3}$$

## Results

The normal ranges for each test were calculated from the number of euthyroid patients lying within 95% of the total number of euthyroid patients in each group. The normal ranges are given in Table I.

Table I: Normal ranges

Test	
4 hour uptake	5 - 28%
48 hour uptake	15 - 53%
PBI 127	4 - 8 micrograms per 100 ml.
T <sub>3</sub> Resin uptake	28 - 44%
Free thyroxine index	7 - 14%

## Discussion

### Radioiodine Uptakes

The 4 hour uptake results show a definite overlap between the hypothyroid and the euthyroid group. Two of the patients in the euthyroid group who have results in the thyrotoxic range were found to be iodine deficient. In the thyrotoxic group one patient was receiving treatment with carbimazole (a thyroid blocking agent) and therefore gave a low result. Women on oral contraceptives gave results which did not differ significantly from the others of each group.

The forty-eight hour uptake gave better separation of the three groups than the 4 hour uptake. Of the euthyroid patients giving low uptakes one was found to have a thyroid carcinoma and two had received radio opaque dyes for X-ray examinations within the past few months. The two patients who were iodine deficient gave also high 48 hour uptakes. In the thyrotoxic group three of the results were abnormally low. One was the patient who was on carbimazole and the other two low results were unexplainable.

Radioiodine uptakes are very quickly performed and the results are immediately to hand. The patient suffers no discomfort but the test does involve more than one visit to the clinic. Another disadvantage is that the patient receives a dose of radiation to the thyroid.

The size of the patient's iodine pool also affects the uptake of the radioiodine. In patients with a very small iodine pool (*e.g.* iodine deficiency) the results are elevated and in patients with an increased iodine pool the results are lowered. This last effect causes considerable difficulty in interpreting uptake results as many common pharmaceutical preparations contain iodine.

### *Serum PBI 127*

The PBI results show very good separation between the hypothyroid and euthyroid ranges with no euthyroid results lying in the hypothyroid range. One pregnant hypothyroid patient gave a result in the normal range. Patients who are pregnant or on oral contraceptives give abnormally high results due to the increase in number of plasma binding sites. This in turn causes higher bound thyroxine levels. This can lead to misinterpretation of high PBI results.

A falsely high value occurs in patients who have had a high intake of iodine such as seafoods or cough mixture. This is due to contamination caused by the iodine being bound to organic sites. The test measures all organic iodine not only that put out by the thyroid gland. These results can be extremely high as seen in the top 5 patients in the euthyroid range.

### *T<sub>3</sub> Resin Test*

The T<sub>3</sub> resin test is a fairly simple test for thyroid function and gives good reproducibility as long as the test conditions are strictly maintained. It was found that 4°C was the easiest temperature to maintain and also gave better separation of the ranges. Difficulty has been experienced in maintaining a supply of resin with identical properties and much work is being done to correct this difficulty.

In the pregnant patient, or patient on oral contraceptives, the number of protein binding sites is increased, leading to a high PBI result. However the reverse effect is observed in T<sub>3</sub> resin test because the increased binding sites bind more of the radioactive T<sub>3</sub> leaving less to be taken up by the resin. As can be expected the results show that the majority of patients on oral contraceptives (91%) lie below the normal range. Two of the hypothyroid patients whose results lie well within the euthyroid range were found to be later

- 1) rendered hypothyroid after radioiodine therapy
- 2) rendered hypothyroid after implant for acromegaly

The overlap of euthyroid results in the thyrotoxic range and thyrotoxic results in the euthyroid range was unexplainable but could be due to unreported treatment or technical error.

### *Free thyroxine index*

The results of the calculated free thyroxine index using the PBI and T<sub>3</sub> resin estimations gave an extremely clear picture of the ranges of the three thyroid states.

Excluding the iodine contamination results the figures in Table II indicate that only 2.5% of the results do not fall in the expected ranges. The iodine contaminated PBI estimations gave extremely high index results but this is to be expected. The pregnant patients and those on oral contraceptives gave results in the expected ranges; the effect of elevated PBI 127 and lowered T<sub>3</sub> resin uptake combining together to give a result more indicative of the true level of free thyroxine than either test taken on its own.

Test	No. in series	% Hypothyroid patients in euthyroid range	% Euthyroid patients in hypothyroid range	% Euthyroid patients in thyrotoxic range	% Toxic patients in euthyroid range or below
4 hr uptake	Hypothyroid 10	40%		5.5%	7.9%
	Euthyroid 107				
	Thyrotoxic 38				
48 hr uptake	Hypothyroid 8	0%		7.0%	9%
	Euthyroid 71				
	Thyrotoxic 33				
PBI 127	Hypothyroid 41	4.9%		0%	1.75%
	Euthyroid 227				
	Thyrotoxic 57				
T <sub>3</sub> Resin uptake	Hypothyroid 39	20.5%		10.8%	12.0%
	Euthyroid 232				
	Thyrotoxic 57				
Free thyroxine index	Hypothyroid 39	2.6%		1.8%	0%
	Euthyroid 225				
	Thyrotoxic 55				

Table II



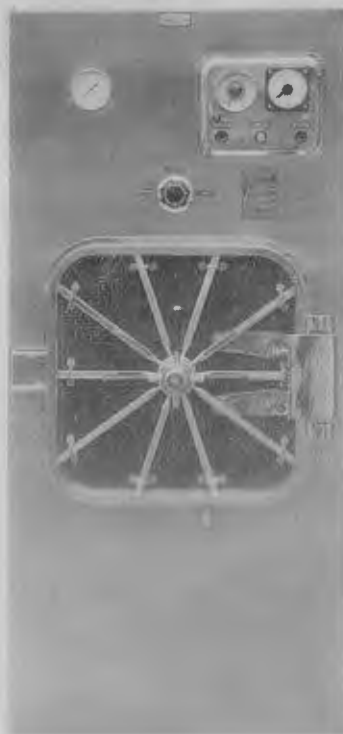
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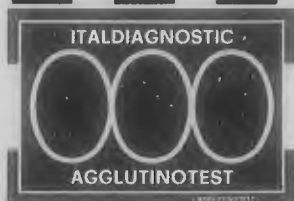
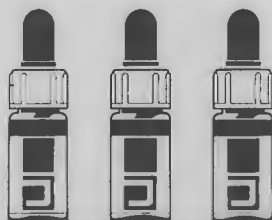
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## Conclusion

### *4 Hour Uptake*

This test is useful for separating thyrotoxic from euthyroid patients providing they are not iodine deficient. It is not satisfactory for separating hypothyroid from euthyroid conditions.

### *48 Hour Uptake*

This test is extremely useful for separating hypothyroid from euthyroid patients, providing they have not received any compounds containing iodine or X-ray examinations using radio-opaque dyes. It is also useful for separating thyrotoxic from euthyroid patients, providing they are not iodine deficient.

### *Protein Bound Iodine*

This test is extremely useful for separating hypothyroid from euthyroid patients. A hypothyroid result will be masked if the patient is pregnant or on oral contraceptives. It is not as satisfactory for separating thyrotoxic from euthyroid patients as high results can be obtained if the patient:—

- (a) has been on compounds containing iodine
- (b) has had X-ray examinations using radio opaque dye.
- (c) is pregnant.
- (d) is on oral contraceptives.

### *T<sub>3</sub> Resin Uptakes*

There is considerable overlap between the three ranges. The results indicate that this test should not be used in isolation. It should be noted that euthyroid patients on oral contraceptives invariably have a hypothyroid result. However this is the only test which gives reliable results in the presence of iodine contamination.

### *Free Thyroxine Index*

This test is the best for separating the three ranges. The only situation where the incorrect result is obtained is if the patient has had iodine containing compounds or radio opaque dyes for X-ray examination.

The conclusions in this study are based on results of 333 patients. It was not possible to perform all the tests on every patient; however, the number of euthyroid patients in the series should be sufficient to define the normal ranges. Conclusions drawn regarding the separation of the thyrotoxic from the euthyroid ranges for all the tests should be statistically valid considering the number of patients studied. A sufficient number of hypothyroid patients were included in the study to make valid assumptions regarding PBI, T<sub>3</sub> Resin uptake and free thyroxine levels in hypothyroidism. However, the limited number of hypothyroid patients who had radioiodine uptakes was not sufficient to draw valid conclusions in this situation.

To determine the best tests for diagnosing hypothyroid from euthyroid, and thyrotoxic from euthyroid a reliability index was calculated.

$$\text{Reliability index for determining hypothyroid from euthyroid} = \frac{\text{No. of hypo in euthyroid range} + \text{No. of euthyroid in hypo range}}{\text{Total number of patients in euthyroid and hypothyroid range}} \times 100$$

Similarly for Thyrotoxic group

Tests	Determining Hypothyroid from Euthyroid	Determining thyrotoxic from Euthyroid
4 hour uptake	8.6%	4.1%
48 hour uptake	6.3%	5.8%
PBI 127	0.8% (0.4%) <sup>1</sup>	8.1% (1.9%) <sup>1</sup>
T <sub>3</sub> Resin	12.2% (5.2%) <sup>2</sup>	4.5% (3.2%) <sup>2</sup>
Free thyroxine index	1.9% (1.9%) <sup>3</sup>	2.5% (0.7%) <sup>3</sup>

Table III: Reliability index.

The figures in brackets represent the results when:—1. the iodine contaminated results, pregnant patients and patients on oral contraceptives are excluded. 2. pregnant patients and those on oral contraceptives are excluded. 3. the iodine contaminated results are excluded.

From the reliability index of the five tests under study it can be seen that the best test for diagnosing hypothyroidism is the PBI followed by the free thyroxine index, 48 hour uptake, four hour uptake and T<sub>3</sub> Resin. The best test for diagnosing thyrotoxicosis is the free thyroxine index followed by four hour uptake, T<sub>3</sub> Resin, 48 hour uptake and PBI 127.

The best overall diagnostic test for thyroid disorder is the free thyroxine index followed by PBI, radioiodine uptakes and T<sub>3</sub> Resin.

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## ***Mycobacterium bovis*:** **Methods of Isolation and Identification.**

GILLIAN R. BOTT

Pathology Department, Green Lane Hospital,  
Auckland, 3.

*Received for publication, February, 1970.*

### **Introduction**

During the period from June 1964 to December 1969 no less than 5.9% of patients from whom *Mycobacteria* have been isolated at Green Lane Laboratory have proved to be infected with *Mycobacterium bovis*. This unexpectedly high incidence of bovine infections after an apparent absence of several years has led to an investigation of methods for isolating and identifying the organism.

### **Materials**

#### *Lowenstein-Jensen Medium*

This is prepared as in *Mackie and McCartney's Handbook of Bacteriology* Ed. Cruickshank (1962), 10th Edition (E. and S. Livingstone, London), with the addition of 3 ml. tomato juice per 100 ml. of medium, added before inspissation.

#### *Pyruvic Acid Medium*<sup>1</sup>

Potassium dihydrogen phosphate	11.4 g.
Disodium hydrogen phosphate anhydrous	6.0 g.
Magnesium sulphate, hydrated	6.0 g.

Dissolve in 600 ml. distilled water, and sterilise at 15 lb. for 15 minutes.

Break fresh, cleansed eggs aseptically to produce one litre, shake with sterile glass beads, filter through gauze into the salt solution.

Add 10 ml. 2% autoclaved malachite green, and 100,000 units sodium benzyl penicillin dissolved in sterile water.

Add 3.3 ml. 90% pyruvic acid—first adjusting the pH to 8.5 with 2/N NaOH, and sterilising at 10 lb. for 15 minutes.

Heat the medium at 85°C for one hour to solidify.

### **Methods**

When growth of *M. bovis* occurs on Lowenstein-Jensen medium it is observed to be characteristically dysgonic. The colonies are minute, flat, pale cream or white in colour, and may require ten weeks' incubation before becoming visible. Specimens containing many acid-fast bacilli when examined by direct smear may be culturally negative. Sub-cultures may require up to six weeks' incubation and difficulty is often experienced in obtaining sufficient growth on sensitivity media. This partial or complete inhibition of growth is due to the presence of glycerol in the medium. Omission of glycerol results in better growths of *M. bovis*, although the individual colonies are still small. However, growth of *Mycobacterium*

*tuberculosis* is not enhanced as it is on media containing glycerol and colonies are not characteristically eugonic.

When pyruvic acid is substituted for glycerol, growth of *M. bovis* is shown to be greatly enhanced, the colonies being both larger and rougher, and appearing more quickly. The inclusion of a pyruvic acid medium for primary culture in addition to Lowenstein-Jensen medium has greatly increased the number of *M. bovis* isolations in this laboratory. Growth of *M. tuberculosis* is not affected with the exception that some strains are enhanced.

Table I compares the total isolations of *M. bovis* on the two media over one year. Specimens were treated with an equal quantity of 4% sodium hydroxide for thirty minutes including centrifugation. The neutralised deposits were cultured on one slope each of Lowenstein-Jensen medium and pyruvic acid medium, and sloped at 37°C for eight weeks. All slopes were examined weekly under a moveable 60 watt lamp.

Table I

Positive isolations of <i>M. bovis</i> Total	Positive pyruvic acid medium only	Positive Lowenstein Jensen medium only	Positive both media	Growth equal both media	Growth significantly heavier on P.A. medium
42	28	0	14	5	9

### Identification

As a routine procedure new isolations of Mycobacteria were examined by Ziehl-Neelsen stain, tested for growth rate at 37°C, niacin production, photochromogenicity, and sensitivity to streptomycin, INAH and PAS on Lowenstein-Jensen without glycerol.

Niacin production was detected by the method of Runyon, Selin and Harris<sup>3</sup>, after heavy growth had been obtained on one of the egg media incubated with loosened lid. The niacin was extracted from the culture by layering distilled water over the growth for thirty minutes. Equal quantities of 4% aniline in alcohol and 10% cyanogen bromide were added to the extract, and an intense yellow colour developed if niacin had been produced. Later it was found that Tarshis Niacin agar\* with glycerol omitted was preferable as no interference in colour occurred from malachite green. This medium was also suitable for use with niacin test strips.

#### *Photochromogenicity test.*

Young, actively growing colonies were exposed to a 60 watt lamp at twelve inches distance for one hour. Photochromogens develop a bright yellow pigment during the next 24 hours' incubation. The lids of the cultures were loosened during exposure to light. A culture incubated in the dark was used for comparison.

Using these initial criteria, *M. bovis* was suspected by its dys-

\*Difco Laboratories, Detroit, Michigan, U.S.A.



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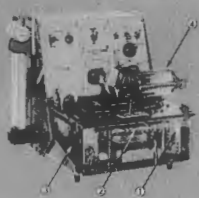
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Table II

	Enhanced by Pyruvic acid	Niacin produced	Nitrate reduced	Growth at 22°C	Growth on TCH medium	Growth on TBI medium	Photo-chromogenicity
<i>M. bovis</i>	+	—	—	—	—	—	—
<i>M. tuberculosis</i>	—	+	+	—	+	—	—
<i>M. kansasii</i>	—	—	+	(slight) +	+	—	+
Other "Atypical" Mycobacteria	—	a	V	+	+	+b	—c

V = variable

a. *Mycobacterium bovis* may produce niacin.b. *Scotochromogens* variable.c. *Mycobacterium balnei* also positive.

Table III

	Enhanced by Pyruvic acid	Niacin produced	Nitrate reduced	Growth at 22°C a	Growth on TCH medium	Growth on TBI medium	Photo-chromogenicity
Number strains tested	77	74	71	45	25	64	77
Positive result	76	0	0	0	0	0	0
Negative result	1 <sup>b</sup>	74	71	45	25	64	77

a. Several strains produced slight growth at 27°C. This test was eventually abandoned.

b. This strain gave typical results in all other tests.

gonic appearance, slow growth (usually greater than 12 days), negative niacin and photochromogenicity tests. Most of our strains from untreated patients were sensitive to the anti-tuberculosis drugs using the resistance ratio method. (It is proposed to present these findings at a later date.) Any culture suspected to be *M. bovis* was then sub-cultured for growth at 22°C, tested for nitrate reductase<sup>5</sup>, and ability to grow on Lowenstein-Jensen media containing (i) 10 µg/ml. thiosemicarbazone (TBI)<sup>2</sup>, and (ii) 10 µg/ml. thiophen-2-carboxylic acid hydrazide (TCH)<sup>4</sup>.<sup>\*</sup> Parallel cultures were set up on Lowenstein-Jensen medium with glycerol, and pyruvic acid medium to observe pyruvic acid enhancement.

*Rabbit inoculations* were initially performed on each isolate, but results varied considerably. Because of the extremely hazardous nature of the test its inclusion was not felt to be justified.

Table II shows the characteristics used to distinguish *M. bovis* from other *Mycobacteria*.

### Results

The results of these tests performed on 77 cultures of *M. bovis* tested in this laboratory are summarised in Table III.

All of the above tests are now performed on suspected *M. bovis* cultures, but ability to grow at 22°C, and on TCH medium has been tested on the latest isolates only. In addition 21 strains were inoculated subcutaneously into rabbits, 20 of these giving at least one positive result out of two or three inoculations.

### Discussion

Seventy of the *M. bovis* cultures were isolated from patients living in the Auckland area. Of these, the majority were adult males, only three being children, and six females. Most were suffering from pulmonary tuberculosis and positive isolations were mainly from specimens of pulmonary sources.

Of fifty patients whose occupations were investigated, thirty-five were found to have had close contact with animals, either as freezing workers or on farms. Three others were known to live in close proximity to members of the first group. In another family, the mother and two children were infected, although the original source of infection could not be traced.

### Summary

It is apparent that tuberculosis caused by *Mycobacterium bovis* is a highly infectious illness, and occurs with sufficient frequency to warrant use of suitable culture techniques for its isolation. The pyruvic acid medium described is strongly recommended for this purpose. A scheme for the identification of *Mycobacterium bovis* is presented.

<sup>\*</sup> Available from Aldrich Chemical Co., Inc., 2371 N 30th St., Milwaukee, Wisc., U.S.A.

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<i>Aerobacter aerogenes</i>	721	82.5
Coliform bacteria	286	91.6
<i>Klebsiella-Aerobacter</i>	263	86.3
<i>Paracolobactrum</i> (all)	250	74.4
<i>Escherichia</i> (all others)	124	86.3
Gram-negative rods	103	64.1

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

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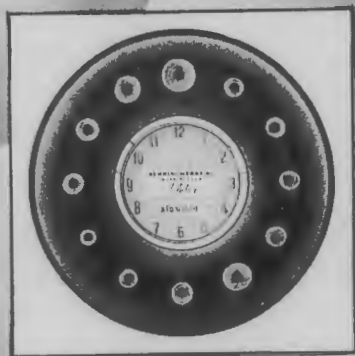
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## Errata

*N.Z. J. med. Lab. Technol.* Volume 24, No. 1 (March 1970).

In the article "The Indirect Fluorescent Antibody Test for the Detection of Toxoplasma Antibodies" by W. A. Beggs:—  
line 3 on p. 3 should read:

"... covered by centrifuging for 20 minutes at approximately 1000 x g."

and line 34 on p. 7 should read:

"... lin before washing, to prevent loss of an important cytotoxin-like"

In the technical communication "Collection of Capillary Blood Samples" by R. D. Allan, the first line of the last paragraph on p.27 should read:

"These tubes hold 400 microlitres . . ."

## A Method for the Estimation of Triiodothyronine ( $T_3$ ) Resin Uptake, Using $T_3I^{125}$

I. H. SYMONDS, B.Sc., A.N.Z.I.M.L.T.,

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16 The Terrace,  
Wellington.

Received for publication November 1969.

### Summary

A method for the estimation of Triiodothyronine ( $T_3$ ) resin uptake, as a test of thyroid function is described.

The use of the method in the diagnosis of thyroid dysfunction is discussed.

### Introduction

For many years the best laboratory estimation of thyroid function, and that most commonly used today, has been the protein bound iodine (PBI)<sup>1</sup>.

A large number of papers have been written enumerating the disadvantages of the PBI as a diagnostic test. Most reflect its susceptibility to organic and inorganic iodine contamination. With the ready availability of radio-isotopes of iodine, especially  $I^{125}$  preparations, indirect measurement of protein-binding sites of thyroid hormones has become possible<sup>3,4</sup>.

These tests are in their turn affected by changes in protein<sup>6,8</sup> but when combined with the PBI the disadvantage of each type of test can separately be largely overcome. Thyroid function can thus be quickly and accurately determined.

### Reagents and Materials:

#### (a) Resin

The resin found to be the most satisfactory was Amberlite I.R.A. 400 (Cl) form, converted to the formate form in the following manner. 500 g. of resin was washed five times in one litre of formate buffer, in a large plastic container on a magnetic stirrer. Care must be taken to ensure that the stirring rate is as gentle as possible, to avoid physical breakdown of the resin. The total washing time should be spread over three hours. The resin is then washed with distilled water three times (10 minutes each wash), and placed on blotting paper to dry at room temperature. When dry the resin should present a light yellow appearance, with a particle size similar to the original (Cl-) form. The dry resin is stored at 5°-10°C and will keep, in a tightly sealed bottle, for at least two months.

#### (b) Formate Buffer

Make up 5 litres of 1M sodium formate and adjust the pH to  $5.0 \pm 0.02$  with 1M formic acid.

(c) *Triiodothyronine I<sup>125</sup> (T<sub>3</sub>): Stock Solution*

A bottle of Amersham Liothyronine-L I<sup>125</sup> Code No. 1M 32, specific activity 0.2mc, is diluted upon arrival to 10 ml. by the addition of 9 ml. of 0.25% bovine albumin. The solution is usable for at least 60 days.

*Working Solution:*

This must be made fresh each day, and contains 0.01  $\mu\text{g}$  T<sub>3</sub> per ml.

To calculate the volume of stock solution required to add to 10 ml. of saline take:

$$\frac{1}{\text{Total T}_3 \text{ Concentration in } \mu\text{g in Amersham Bottle ml.}}$$

e.g.  $\frac{1}{10} = 0.1 \text{ ml.}$

(d) *Pooled Sera*

A pool of normal patient sera was obtained and deep frozen in 10 ml. ampoules.

(e) a device for counting the low level emission of I<sup>125</sup> is required, and for this purpose a Phillips well-type scintillation counter was used.

(f) All counting tubes and pipettes used were cleaned in commercial detergent and rinsed in deionised water.

**Method**

The method is basically that of Sterling<sup>6</sup> together with modifications of White<sup>9</sup>.

1. 500 mg. of prepared resin is added to 7.5 cm. x 3 cm. tubes capped and stored in the deep freeze (resin tubes).

2. Into a set of 7.5 cm. x 3 cm. plain tubes, pipette 1 ml. of test serum. For each run of tests also set up four tubes of pooled serum.

3. Add 0.2 ml. of working T<sub>3</sub>I<sup>125</sup> solution, mix well and place in a 37°C bath for 1 hour.

4. During the serum incubation period an ice bath is prepared, into which are placed the resin tubes.

5. At the completion of the 1 hour incubation time, 1 ml. of the serum-T<sub>3</sub> mixture is pipetted into a resin tube and shaken.

6. The serum-resin mixture is left at 0°C for 75 minutes.

7. During this 75 minutes period a count is taken of each tube to obtain a 100% value.

8. On completion of the ice bath equalibration the resin in each tube is washed in ice cold distilled water. Allow the resin to settle and remove the supersatant. Repeat this step twice.

9. The resin tubes are now counted to give a resin uptake value.

10. *Calculation*

$$\frac{\text{Counts in Resin} - \text{Background}}{100\% \text{ Counts} - \text{Background}} \times 100 = \% \text{ Resin Uptake}$$

## Results

A normal range was calculated by using 500 patient results and this gave a figure of 25% — 38%.

The value of the pool serum after multiple estimation averaged 31%  $\pm$  2%.

Accuracy of the method was 2 SD =  $\pm$  2%, for the duplicate estimations, and on the single tube method described 2 SD =  $\pm$  4%.

In calculating the final result the average pool value is determined and if the figure obtained is outside the allowable limits, *i.e.* 29% — 33%, a correction is applied to all test values in the series.

## Discussion

The proteins involved in the binding of  $T_3$  are those in the inter-alpha 1, alpha 2 globulin region. Upon electrophoresis increases of these thyro-binding globulins are reflected by increased alpha 1 and alpha 2 values. Care must therefore be taken in the interpretation of  $T_3$  uptake results.

Together with the protein bound iodine, a factor known as the free thyroxine (FTI), may be calculated. Full discussions on the validity and use of the FTI have been published<sup>2,5</sup>. The calculation used is:

$$\text{FTI} = \frac{\text{PBI} \times 100}{100 - T_3 \text{ Uptake}}$$

Normal Range = 4.6 — 12.9

The index is not of great value in cases of hyperthyroidism or hypothyroidism, where both the PBI and  $T_3$  will reflect the true thyroid status. Its use is better seen in cases of protein disturbance, *e.g.* pregnancy, where the protein bound iodine is increased and the  $T_3$  resin uptake is decreased.

*Example:* PBI = 9.2 microg/100 ml.  
 $T_3$  Resin Uptake = 24%

$$\text{FTI} = \frac{9.2 \times 100}{100 - 24} = 12.1$$

In this instance the protein bound iodine alone would support a diagnosis of hyperthyroidism, and the  $T_3$  resin uptake that of hypothyroidism, but in fact neither assessment would be correct as shown by the index, which is normal.

The index, however, suffers from being too sensitive to changes in PBI and not sufficiently sensitive to resin uptake variations. There is need for agreement on the factor necessary to compensate for this.

The  $T_3$  resin uptake alone is not a sufficiently sensitive test of thyroid function, but combined with the PBI it is of great

value in the laboratory assessment of thyroid status.

### Acknowledgments:

The author wishes to thank Dr F. B. Desmond for his helpful suggestions in the presentation of this paper, and to Mr D. Cottrell for his technical assistance.

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## South Island Seminar

TIMARU — 2 MAY, 1970

Once again members from the South Island enjoyed the hospitality of the South Canterbury Hospital Board when approximately 50 technologists attended the Annual South Island Seminar.

The opening address was given by Dr M. G. Smedley, who talked about heat stable alkaline phosphatase as an indication of placental function.

The Chairman, Mr J. Case, then guided the meeting through the day. The following papers were presented:—

*A Case of Acquired Factor XIII Deficiency* — B. Rae.

*Neonatal and Premature Coagulation Factor Levels* — Miss S. Hellyer.

*Daily Urines for the Evaluation of Hormone Levels* — W. Bumstead.

*Pyrimethamine-induced Megaloblastic Anaemia* — Miss L. Peterson.

*A Case of Listeriosis in a Newborn* — D. A. Robertson.

*Control Procedures* — R. D. Allan.

Messrs J. Case, B. W. Main and J. D. R. Morgan discussed Institute affairs covering Fellowships, Registration, etc. The paucity of papers allowed ample time for discussion, which was felt to be an advantage. Following the meeting the majority of members retired to the Hibernian Hotel for an excellent dinner.

Our thanks go to the organiser, Mr B. N. Smith, and the South Canterbury Hospital Board for a most enjoyable day. D.T.

## Technical Communications

### Anomalies in Spectrophotometric Methods for the Iodometric estimation of Serum Amylase

Sir,

Owing to dissatisfaction with the current method for the estimation of serum amylase it was decided to investigate the possibility of using the method described by Bartholomew *et al.*<sup>1</sup>. Some anomalies were discovered during these investigations and it was decided also to survey the method of King and Wootton which shares the same principles and is in common use. A summary of findings follows:—

#### 1. The Effect of Temperature:

The absorbance of the starch-iodine complex is dependent on temperature; the lower the temperature the greater the absorbance. This will have little effect on the final result if both test and control are treated in exactly the same manner, but if the control is made from reagents from the refrigerator and the test read at 37°C large errors may be expected.

#### 2. The Effect of Albumin:

Wilding<sup>3</sup> states that "Albumin has a greater affinity for iodine than has starch." This is so, and at normal serum levels the albumin present in the reaction mixture will combine with some of the iodine which would otherwise couple with starch. In the method of King and Wootton the mixture does not contain an excess of iodine; some of this iodine will combine with the albumin, thus reducing the absorbance of the starch-iodine complex and suggesting starch digestion. A concentration of 4g./100 ml. will give a reduction in absorbance which would be equivalent to an amylase activity in the middle of the normal range. This phenomenon can easily be corrected for by running a serum reagent blank. It makes no difference to the measured change in absorbance if the albumin is added to the substrate before or after the addition of iodine provided that sufficient iodine remains to inhibit amylase activity.

#### 3. The Effect of Iodine on Amylase Activity:

0.4ml. of N/100 iodine (Wootton) is insufficient to inhibit amylase activity; 3 ml., as in the method of Bartholomew, *et al.*<sup>2</sup> is sufficient. The absorbance of an amylase-starch-iodine complex decreases quite rapidly when only 0.4ml. of N/100 iodine is added, due to continued starch digestion. A reagent blank of starch plus iodine shows a slight but relatively insignificant change. The excess iodine (Bartholomew *et al.*) has an additional advantage because the absorbance is then unaffected by albumin in concentrations of up to 30g./100ml. There appears to be sufficient iodine present to saturate both the albumin and the starch.

#### 4. Inhibition by Tris. Buffer:

Bartholomew *et al*<sup>2</sup> used Tris-(hydroxy-methyl)-Methylamine (0.05M) as a buffer, but when this was compared with a phosphate buffer (0.066M) results using Tris were approximately half those obtained using phosphate. Maleate and phosphate produced the same activities so it would appear that Tris has an inhibitory effect. This is also suggested by Armador *et al*<sup>1</sup>.

#### 5. Discussion:

By adjusting each variable so that optimum conditions prevail the remaining problem is one of sensitivity. If the amount of enzyme (serum) added is chosen to give reasonable sensitivity the maximum result obtainable is only approximately the upper limit of normal. A wider range can be obtained by increasing the concentration of starch in the substrate, but only with an unacceptable loss of sensitivity.

This problem is a basic one; that of trying to measure a small change in concentration of a substrate which must necessarily be present in relatively large amounts. At present there appears to be no satisfactory way of overcoming this with the type of method described. Methods using an assay of the products of digestion of the starch are free from this limitation.

ALISON M. BUCHANAN,  
Pathology Department,  
Green Lane Hospital,  
Auckland.

(Received January 1970.)

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## Automated Estimation of Pepsin Using the Technicon Autoanalyser

Sir,

The following is a short account of an automated method for the estimation of the enzyme pepsin in gastric juices from augmented histamine test meals, as given to the 25th Annual Conference in August 1969.

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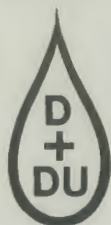
1. Dilution of the gastric juice with 0.01N HCl to bring the final colour within readable range and to obtain the optimum pH of 2.1 for the enzyme reaction.
2. Incubation with haemoglobin substrate at 37°C.



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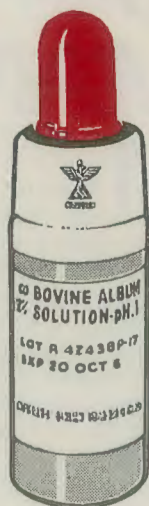
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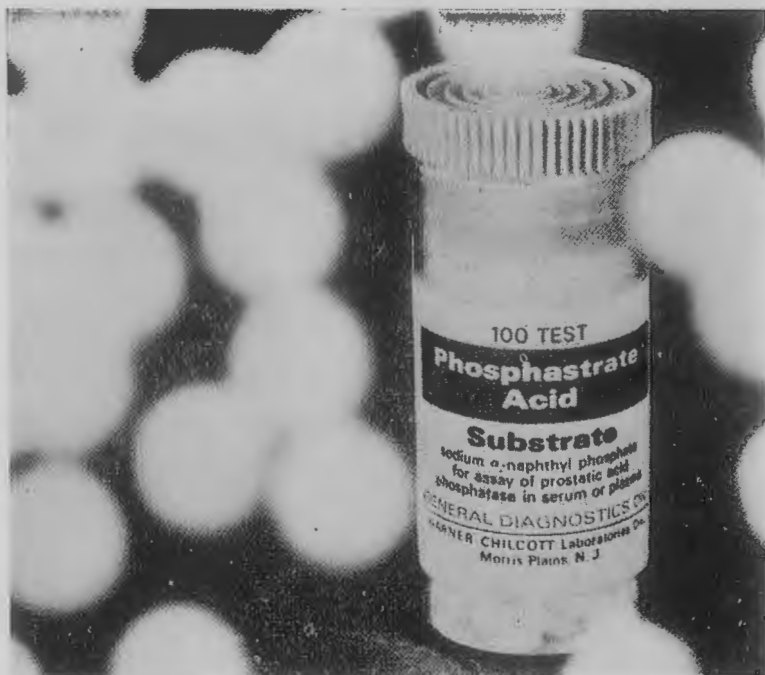


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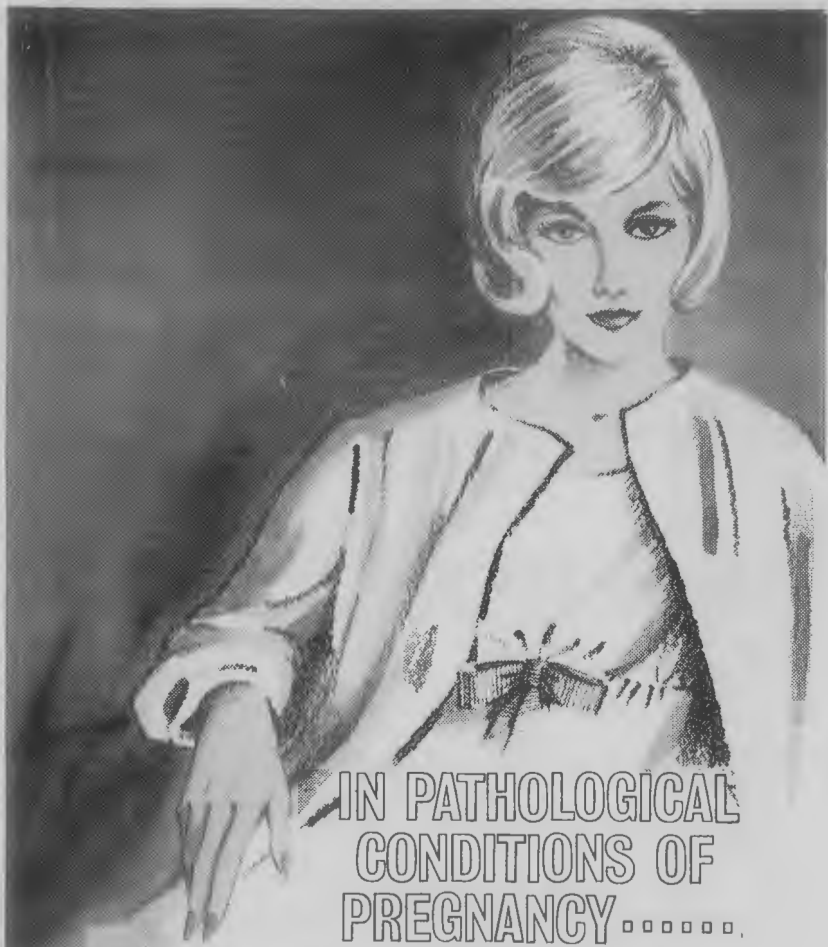
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**Human Haemoglobin Variants and their Identification** (Laboratory Aids Series). L. H. B. Wallett, F.I.M.L.T., F.I.S.T. and J. B. Robinson, A.I.M.L.T. 56 pages. Butterworth, London (1968). N.Z. price \$2.00.

The stated objective of this booklet is to present an account of laboratory methods used in the identification of human haemoglobin variants.

The first 17 pages are concerned with a very brief outline of the structure of the haemoglobin molecule, and various aspects of the haemoglobinopathies. The remaining pages are devoted to techniques used for the detection of abnormal haemoglobins. The methods covered are Heinz body demonstration, alkali denaturation, electrophoresis, chromatography, heat denaturation, solubility and staining for haemoglobin F.

There is considerable variation in the way in which the methods are presented. The bare essentials of some methods are given while others, such as electrophoresis are given in detail followed by a number of useful comments on factors which may effect the results. The reasons for giving three methods for alkali denaturation are not clear. No comment is made on the advantages or disadvantages of any of the methods quoted.

Apart from the section of electrophoresis this booklet has little to recommend it. A more useful contribution could have been made if the authors had deleted "the theoretical basis of molecular biology" and devoted the booklet completely to a discussion of laboratory methods.

A.D.N.

**The Human Reproductive System.** M. Krieger. 72 pages with 40 pictures in colour. Oak Tree Press, London. (1969). U.K. price: 35s 0d.

Sex education remains a topic of controversy unfortunately, for with present-day adolescent education and preparation there can be little doubt that frank and full enlightenment of adolescents is a step towards equipping them to handle new and emotionally important experiences.

At last education material is becoming available to facilitate this education which, in the past, has been inaccurate and has tended to create further emotional confusion.

This remarkable book, in 72 well-illustrated pages, covers succinctly most relevant aspects of sex education in such a way as to convey modern medical concepts, while at the same time maintaining the intrigue and amazement of human functions.

The initial pages cover human anatomy and physiology fully, discussing as well the physiology of the sexual act in the male and female. Coloured diagrams and models are used to advantage. Hormones and their roles are dealt with, giving full explanations of menstruation and pregnancy, and the interrelationships among pituitary, gonads and uterus.

Chapters on fertilisation and development present considerable detailed embryology in a readable manner, and the later chapters discuss in frank detail, and with coloured photographs, the birth process, stages of labour, the puerperium and the physiology of breast feeding. Finally, the last chapter gives a basis of genetics and its application to heredity.

This book contains a very readable account of human reproduction, suitable for a wide age range of receptive intelligence and leaving few questions unanswered. Although not directly applicable to the field of laboratory technology many chapters, especially those on endocrinology and genetics, provide medical accuracy and detail sufficient to be of value to many.

W.J.R.

**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.

This small book in the Laboratory Aids series is compiled by two medical technologists and covers most aspects of tuberculosis bacteriology. It concisely lists the most commonly-used methods with well-presented technical details and references.

A chapter is devoted to the often-neglected safety precautions, emphasising their importance.

Two concentration techniques are presented: The n-acetyl-l-cysteine method is discussed and formulae are given for a selection of culture media, including sensitivity media.

A useful comprehensive scheme for identification based on tests described in the book identifies *Mycobacterium tuberculosis*, *Mycobacterium bovis* and the more commonly encountered Atypical Mycobacteria. Brief descriptions of each organism are included.

The resistance ratio sensitivity method is covered sufficiently for routine testing, although I would question the use of such a large inoculum as recommended in the method given.

A chapter on methods for assaying the anti-tuberculosis drugs in body fluids will prove a helpful reference for some laboratories.

This book will be an invaluable practical handbook for anyone endeavouring to upgrade tuberculosis methods, as it summarises most of the recent developments, especially in the field of identification. It should be welcomed by trainee technologists as a comprehensive basic coverage of the subject of Mycobacteria.

G.R.B.

**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.

This book is specifically designed for candidates for the Membership of the College of Pathologists. Virology and public health sections have not been published, as the authors assume that those using the book will attend courses on the particular topics and will visit virological and public health laboratories.

This book is essentially a handbook of practical methods in bacteriology which have been used and have worked successfully in the hands of the authors. The text is divided into 35 sections, each comprising a practical assignment, a discussion of the theory of the exercise and an outline of supplemental practical work.

The authors have succeeded in their intention of producing a practical bench manual and some of their comments on techniques are not readily accessible in other standard texts on microbiology. One suggestion they make is that one of the reasons for failure of streptococcal grouping could be the fact that polysaccharide of group B streptococci may be heat sensitive and would be better extracted in 0.07N HCl at 50°C for two hours or longer, and this illustrates the essentially practical bias of this book.

Each section is fairly short, an average of 6-7 pages, and is well tabulated. The suggested identification tests in the sections on identification are commendably simple and kept within reasonable bounds for the average laboratory. The nomenclature is up to date and it is a pleasure to read through the sections and not have the confusion of adherence to some of the older names for the more common bacteria.

With an increased interest in *Brucella abortus* testing in animals in this country it is pleasing to see that the authors give a detailed account of both the standard agglutination and Coombs' techniques for the detection of Brucella antibodies.

The mycology section is the largest: 17 pages. It is illustrated by adequate line drawings and contains fairly tersely written descriptions of the dermatophils. This section would have been better if it had been illustrated with plates, as mycology is not well served by only the written word and line drawings for illustration.

The book is presented with a spiral binding necessary for a bench manual and a very soft cardboard cover. The cover has not lasted well in

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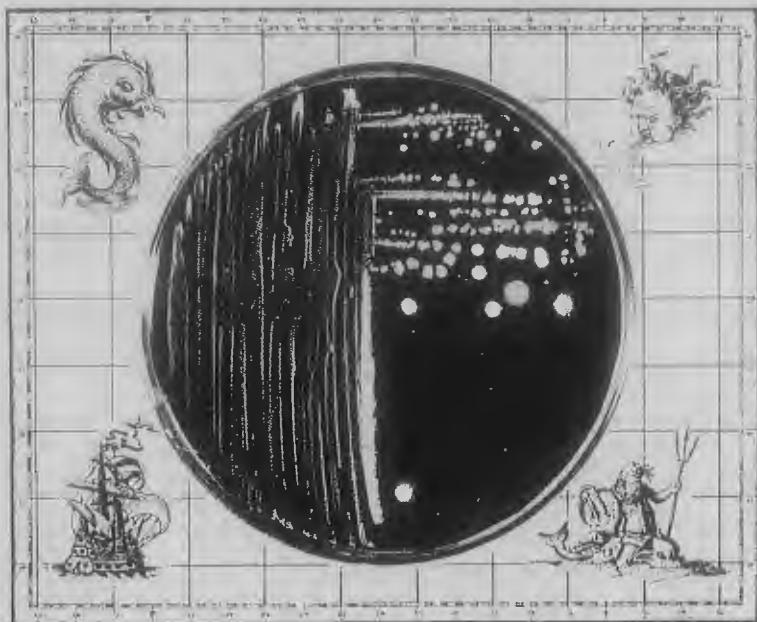


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the period of this review and would disintegrate with more usage. In future editions it could well be replaced by a cover of more substantial material.

This is a book that has been a pleasure to review because of its practical aspects and detail only to methods that have worked for the authors. They do not claim that these methods are the ultimate of identification or methodology for serological techniques, but these are practical methods for any size of laboratory.

This book is of good value for any microbiology laboratory where it would be available to both trainees and pathology registrars as a practical book for their respective examinations.

M.D.McC.

**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.

The book is one of a series published under the editorship of F. J. Baker in the Laboratory Aids Series.

It is intended to assist candidates reading for the I.M.L.T. examinations, and to be useful for all laboratory workers in this field. It contains useful details on the laboratory aspects of efficiency tests and controls of sterilisation procedures, the use of gamma irradiation, and a very useful table on the sterilising properties of various plastics. The book covers all aspects of sterilisation in a superficial manner, and as such may be of value to the student, but of little value to the practical technician to whom it is in part directed. There would appear to be many important omissions, such as details of the handling of beta-propiolactone, the type of peptone used in the Rideal Walker test, failure to mention the standard specifications for the examination of disinfectants issued by the British Standards Institution and many other basic facts fundamental to an appreciation of modern sterilisation procedures.

The book itself: The type is clear and the paper good; however, I doubt if the binding and cover would long survive the average student handling. Lastly — a point of interest — it would appear that the published price in Great Britain has been doubled in transport to New Zealand, surely excessive for so small a tract.

L.R.

## Books Received

**Elementary Principles of Laboratory Instruments.** 2nd Edition. L. W. Lee, B.S., M.T.(ASCP). 215 pages, illustrated. C. V. Mosby Co., St. Louis. 1970. U.S. price \$10.00.

**A Laboratory Guide to Clinical Diagnosis.** 2nd Edition. R. D. Eastham, B.A., M.A., D.C.P., Dip. Path., F.C. Path. 276 pages, paper covers. John Wright and Sons, Bristol. Price in U.K. 20s 0d.

## Selected Abstracts

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

### CHEMICAL PATHOLOGY

**A Modified Technique for Serum Lipase Determination.** Searcy, R. R. Hayashi, Shinichiro and Berk, J. E. (1968), *Clin. Biochem* 1, 311.

Examination of certain serum lipase kinetics led to the adoption of an assay technique that features the use of high levels of substrate, bile salt, and a one hour incubation period to permit precise titration. The modified method was applied to sera from healthy subjects to establish a normal range. Analysis of sera from patients with acute pancreatitis disclosed a close relationship between serum lipase and amylase activity.

A.B.

**Problems in the Analysis of Urinary Mucopolysaccharide Excretion.** Linker, A., Evans, L. and Madsen, J. (1969) *Biochem. Med.* 2, 448.

In a study of polysaccharide excretion in patients with mucopolysaccharidoses large differences in yield, as well as pattern depending on the assay procedure used, were observed. If urines were not dialysed prior to analysis large amounts of heparitin sulphate were lost, if they were dialysed considerable losses of chondroitin sulphate B occurred. Both factors significantly affect any attempt to correlate disease classification with urinary excretion pattern. An improved method is proposed and checks for possible losses suggested.

A.B.

**Analyses of Extravascular Fluids.** Shafer, W.H. (1969) *J. Amer. med. Ass.*, 210, 1087.

The formation of transudates and exudates and the significance of the gross appearance, protein content and cell count are discussed. Causes of serious effusions of the abdominal, pleural and pericardial cavity are given and synovial fluid is discussed in detail. A table is given of the normal characteristics of synovial fluid together with those found in nine types of joint disease.

A.B.

**Myeloma Proteins and Antibodies.** Metzger, H. (1969) *Amer. J. Med.* 47, 837.

Most myeloma and Waldenstrom proteins are normal synthetic products by structural criteria, and "M" proteins result from a massive production of one globulin selected at random from a family of normal ones. In the past few years techniques have been developed to study antibody binding sites and from the observed facts it is provisionally accepted that the observed binding of "M" proteins is similar to that found in induced antibodies. It would appear that "M" components are products of cells already participating in an immune response at the time they undergo neoplastic change.

A.B.

**Spectral Properties of Hemopexin-heme: The Schumm Test.** Rosen, H. and Sears, D. A. (1969) *J. Lab. clin. Med.* 74, 941.

Previously the Schumm's test of human plasma with ammonium sulphide has been considered as specific for methaemalbumin when a strong absorption maximum appears at 558m $\mu$ . However, these authors report that a solution of haemopexin-heme also shows this absorption maximum. They also partially purified haemopexin, the heme-binding p<sub>1</sub>-globulin.

M.J.G.

**Serum Enzyme Activity and Bromsulphalein Retention during use of Oral Contraceptives.** Wilbert, L., Severidt, E., Kallmann, T. and Creutzfeldt, W. (1969) *Germ. med. Mth.*, 14, 451.

The effects of two oral contraceptives, Anovlar and Eugynon, were studied covering 66 women in the reproductive age. With one exception the serum enzymes glutamic pyruvic transaminase, glutamic oxalacetic transaminase and alkaline phosphatase were normal. 27 (41%) had increased leucine aminopeptidase levels and an abnormal bromsulphalein retention of 7-16% was observed in 40% of cases.

A.B.

## HAEMATOLOGY

**Chronic Erythromonocytic Leukemia.** Brown, G. O. (1969) *J. Amer. med. Ass.*, 47, 785.

Three cases are presented which demonstrate an association between refractory sideroblastic anaemia and chronic monocytic leukaemia. The cases are characterised by ineffective erythropoiesis, a hyperplastic erythroid bone marrow showing megaloblastoid changes and numerous ringed sideroblasts and hypochromic microcytic red cells. The leucocytes show an absolute monocytosis in peripheral blood with mature but atypical monocytes; the bone marrow shows a slow but progressive increase in monocytic cells. The course is prolonged and relatively benign.

M.J.G.

**An Evaluation of Aryl Sulphatase Activity in Leukaemic Cells.** Boysen, G. (1969) *Scand. J. Haemat.*, 6, 246.

This paper describes the findings of a histochemical method for demonstrating aryl sulphatase activity in cells from peripheral blood and from bone marrow of 22 cases of leukaemia of different types. The findings in these cases do not confirm the difference in distribution of activity in myeloid and lymphatic cells which other papers have reported and suggested as an aid in the classification of acute leukaemias.

M.J.G.

**The Many Forms of Thalassaemia: Definition and Classification of the Thalassaemia Syndromes.** Necheles, T. F., Allen, D. M. and Gerald, P. S. (1969) *Ann. N.Y. Acad. Sci.*, 165, 5

This survey of the thalassaemia syndromes states there are at least eight varieties and that the criteria for diagnosis have become more complex. The old morphological criteria have been discarded and have been replaced by a series of biochemical criteria. However, the classification of the various thalassaemias is still far from clear-cut.

This paper is just one of the many papers from the "Second Conference of the Problems of Cooley's Anaemia" published in this journal which covers over four hundred pages and includes papers by many names famous in the field of haemoglobin synthesis. The concluding 40-page summary of panel discussion is of great value as a survey of recent thinking on the subject of thalassaemia.

M.J.G.

**Thalassaemia Research and Abnormal Hemoglobins.** Lehmann, H. and Huntsman, R. G. (1969) *Ann. N.Y. Acad. Sci.*, 165, 339.

The variants of human haemoglobins are very concisely surveyed and there is discussion of Ingram and Stretton's theory that a thalassaemia may be caused by a pathological haemoglobin that cannot be distinguished from HbA by present Jay methods. The table of known haemoglobin substitutions and deletions in the alpha and beta chains is of interest together with the novel diagrammatic representations of messenger RNA and ribosome activities.

M.J.G.

**The Separation of Normal Human Leukocytes by Density and Classification by Size.** Zucker, R. M. and Cassen, B. (1969) *Blood*, 34, 591.

Leukocytes of haematologically normal human blood have been studied by separating them in bovine serum albumin (BSA) gradients. The blood



cells of the density fractions have been characterised by electronic volume sizing and microscope differential counts. The electronic volumes of different leucocyte populations have been determined from density gradient experiments. Lymphocytes of various sizes can be separated by differences in density. The small lymphocyte population is the most dense while the large population is the least dense. The density of blood cell populations in BSA in order of increasing density were: Monocytes, lymphocytes, granulocytes and erythrocytes. M.J.G.

**Leukocyte Alkaline Phosphatase.** Ray, P. K. and Pinkerton, P. H. (1969). *Acta Haemat.* 42, 18.

The leukocyte (neutrophil) alkaline phosphatase (NAP) has been determined in 389 males and 227 females, using a histochemical method. Activity of this enzyme is significantly higher in females than in males from the second to the fifth decades; thereafter no significant difference is noted. In both, the NAP score declines with age and, in the elderly (over 70 years), an overlap with patients of similar age with chronic myeloid leukaemia may be seen. The need for caution in interpreting low NAP scores in suspected chronic myeloid leukaemia in the elderly is stressed. M.J.G.

**Haptoglobin in Thalassemia.** Yamak, B. and Ozsoylu, S. (1969). *Acta Haemat.* 42, 176.

The type and presence of haptoglobins were determined in the serum of 35 and 42 patients with thalassaemia major and minor respectively. Haptoglobins could not be shown in the starch gel electrophoresis in 74 and 17% of these groups of patients respectively. No correlation was found between the age, haemoglobin concentrations, transfusion frequency, size of spleen, liver, reticulocyte count, nucleated red blood cells and the presence or absence of Hb A electrophoretically in thalassaemia major cases. It is pointed out that the incidence of ahaptoglobinaemia among thalassaemia trait cases seems to be more frequent. M.J.G.

**Circulating Siderocytes in Human Subjects.** Kurth, Doris, Deiss, A. and Cartwright, G. E. (1969) *Blood*, 34, 754.

A report of a study of the occurrence of iron-containing granules in circulating erythrocytes. Both reticulum and siderotic granules were searched for in 80 normal adults, 7 newborn infants and 486 patients. The siderocytes did not exceed 0.3 per cent. in normal adults. M.J.G.

**Metabolic Disorders of the Erythrocyte.** Editor (1970). *J. Amer. med. Ass.*, 211, 820.

This is a very concise summary of aspects of the physiological and biochemical effects of erythrocyte enzymopathies. It draws attention to the fact that the number of unclassified cases of hereditary non-spherocytic haemolytic anaemias has become greatly reduced, and that red cell carbohydrate metabolism may partially regulate the oxygen affinity of haemoglobin. M.J.G.

**Effects of L-Asparaginase in Acute Myelocytic Leukaemia.** Ohnuma, T., Holland, J. F., Nagel, G. and Arneault, G. St. (1969). *J. Amer. med. Ass.*, 210, 1919.

This is an interesting report of a case of acute myelocytic leukaemia under treatment with l-asparaginase, an enzyme with anti-leukaemic effects in man. Both haematological and biochemical laboratory test results are fully tabulated during 20 weeks after injection and during a period of toxicity in the patient. M.J.G.

**New and Sensitive Reaction for Automatic Determination of Inorganic Phosphate and its Application to Serum.** Belle, H. van (1970), *Analyt. Biochem.*, 33, 132.

In 1966, Itaya and Ui proposed a new approach to the determination of phosphate in that the phosphomolybdate was complexed with a basic dye, Malachite Green. The resulting spectral shift was found to be a very sensitive measure of the phosphate present and the method was

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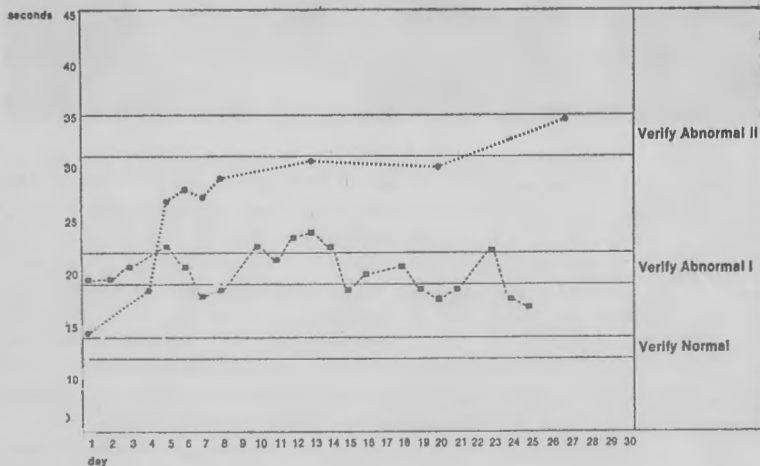
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claimed to be 30 times more sensitive than the Fiske-Subbarow method. Based on this idea, but using Methyl Green OO, a new, specific method is presented. Other advantages are the very short reaction time (2½ min.) under relatively mild conditions (1.25 N HCl), its extreme simplicity, uptake of much less sample, and negligible hydrolysis of labile phosphates. The reproducibility is very good and analyses of control sera reveal excellent accuracy and complete recoveries of added phosphate. J.H.

**Transient Stomatocytosis with Hemolysis: A Previously Unrecognised Complication of Alcoholism.** Douglass, C. C. and Twomey, J. J. (1970), *Ann. intern. Med.*, 72, 159.

Stomatocytes are RBCs whose normal central circle of pallor is replaced by a slit or mouth-like unstained area. The presence of stomatocytes together with haemolysis is a rare clinical entity; approximately 20-30 per cent. of such patients' RBCs in peripheral films are stomatocytes.

Stomatocytes comprised < 4 per cent. of 40 control subjects but of 40 patients with acute alcoholism, 4 had more than 10 per cent. and 7 had 5-10 per cent. J.H.

**Agnogenic Myeloid Metaplasia: Its Current Management.** Silverstein, M. N. (1970), *Minn. Med.*, 53, 257.

Agnogenic myeloid metaplasia is a myeloproliferative disorder closely associated with polycythaemia vera, chronic granulocytic leukaemia, and haemorrhagic thrombocythaemia. At the Mayo Clinic of Rochester, Minnesota, U.S.A., the author's group sees 35 new cases of agnogenic myeloid metaplasia each year; the disease thus approximates the frequency of chronic granulocytic leukaemia in their practice. J.H.

**Hematologic Changes in Septicemia.** Emerson, W. A., Zieve, P. D. and Krevans, J. R. (1970), *Johns Hopkins med. J.*, 126, 69.

A study was made of haematological changes in 61 patients with 69 episodes of septicemia.

Toxic granulation was noted in 26 patients (38%) examined on the day of septicemia and 38 patients (55%) had toxic granulation at some time.

Vacuolisation of the cytoplasm of neutrophils was found in 47 of the blood films (68%) examined on the day of sepsis. Seven patients had vacuolated neutrophils subsequently. Vacuolisation was present for several days before and after positive culture in a number of patients; it was often transient, lasting only 12-18 hours. Large numbers of vacuolated neutrophils were of no prognostic significance.

Dohle bodies were found in 21 patients (31%) on the day of septicemia and infrequently thereafter. Two patients with Dohle bodies had been treated with cytotoxic agents, but neither had received cyclophosphamide, a drug reported to produce these inclusions. Half of the patients with Dohle bodies did not have an initial leucocytosis. J.H.

**Etiology and Laboratory Diagnosis of Infectious Mononucleosis: I. Epstein-Barr Virus (EBV) Mononucleosis.** Escobar, M. R., Allison, M. J. and Dalton, H. P. (1970), *Va med. Mon.*, 97, 138.

Evidence has recently been presented that the mononucleosis-like syndrome may be aetiologically related to two common viruses of the herpes group: the EBV and the cytomegalovirus.

Evaluation of an indirect antibody fluorescent antibody test for EBV is in progress in the authors' institution. It may be useful, at least, in cases where (a) confirmation of a positive heterophil test is necessary, (b) a false negative heterophil test is being considered, or (c) the specific aetiological agent of the infectious mononucleosis syndrome needs to be established. J.H.

## HISTOLOGY

**Electrically Heated Forceps for Histology Laboratories.** Bovis, S.C. and Harrington, E. (1969) *J. Sci. Technol.* 15, 99-100.

This paper describes the construction of an inexpensive and easily constructed pair of electrically heated forceps for use in "blocking out" procedures.

D.G.B.

## IMMUNOHAEMATOLOGY

**Anti-Gn<sup>a</sup>, A New Antibody Reacting with a High-incidence Erythrocytic Antigen.** Fox, J. A. and Taswell, H. F. (1969) *Transfusion, Philad.*, 9, 265.

The new antibody, active by the antiglobulin technique, was found in the serum of a blood donor, along with anti-D. It agglutinated all cells tested except the donor's own and those of three relatives. The letters identifying the antigen have been taken, according to custom, from the surname of the donor, Mrs Gonsowski. No negatives were found in testing 2,600 random bloods. J.C.

**The Stolzfus Blood Group, a New Polymorphism in Man.** Bias, Wilma B.; Light-Orr, Jeannette K.; Krevans, J. R., Humphrey, R. L., Hamill, P. V. V., Cohen, Bernice H.; and McKusich, V. A. (1969) *Amer. J. hum. Genet.*, 21, 552.

An antiglobulin-active antibody defines a new blood group antigen which has been designated Sf<sup>a</sup> (Stolzfus) and which was found to be present in approximately 40% of the United States population.

The antigen was found to be unstable, maintaining a 3+ reaction for up to 4 days storage in ACD at 4°C, diminishing to 2+ for a further 8 days and then showing little or no reactivity thereafter. Red cells stored at -20°C or after freezing in liquid nitrogen were shown to be inactive.

It is assumed that the patient developed the antibody as a result of pregnancy, but the 3 Sf<sup>a</sup> positive children of Mrs Stolzfus were not jaundiced after birth, as would be expected since fractionation studies showed the antibody to belong to the IgA class of antibodies. J.C.

**Histocompatibility Testing.** Limber, G. K. (1970), *Va med. Mon.*, 97, 238.

The histocompatibility antigens are a part of the most complex immunogenetic system known in man. The majority of the important genes for tissue antigens are located near each other in the HL-A sector of the chromosome. Two other important areas are the 9-locus, situated some distance from HL-A, and the 5-locus still further away — if not on another chromosome. Within each of these loci are sub-loci and sub-sub-loci, each controlling the expression of a certain antigen on the cell surface.

White cells bear transplantation antigens in common with tissues, therefore, they are amenable to use as indicators of histocompatibility between donor and recipient pairs. Histocompatibility is usually tested by the cytotoxicity method (some antigens are better detected by leucoagglutination). The cytotoxicity method consists of the incubation of donor or recipient white cells with known antisera in the presence of complement. After incubation, trypan blue, which is taken up by dead cells, is introduced. If more than 35 per cent. of the cells are killed the antigen is present on the cells. In addition, a direct crossmatch should be carried out in which donor cells are incubated with recipient serum. Any significant cell death is cause for donor rejection as it has been associated with hyperacute graft rejection. J.H.

**Clinical Aspects of the Leukocyte Antibody Reaction.** Cannon, D. C. (1970), *Postgrad. Med.*, 47, 51.

Leucocyte antibodies are responsible for most febrile transfusion reactions. These antibodies are present in about 20 per cent. of females who have been pregnant. In persons who receive multiple transfusions, leucocyte antibodies develop with a frequency roughly proportional to the number of transfusions and are present in perhaps 50 per cent. of individuals who have received 25 or more transfusions.

Fortunately, two simple but relatively efficient methods for removing leucocytes from blood are now within the technical ability of virtually all blood banks: (a) high-speed centrifugation of blood units with subsequent removal of red cells from beneath the layers of plasma and buffy coat and (b) filtering fresh heparinised blood through a nylon column.

Since it is not technically feasible to screen all patients for leucocyte antibodies before transfusion, leucocyte antibody reactions cannot be

completely eliminated. The logical and currently attainable goal is to educate physicians and blood bank technologists so that repeated reactions in the same patient can be avoided. J.H.

### MICROBIOLOGY

**A Selective Medium for the Isolation of Haemophilus from Sputum.** Baber, K. G. (1969) *J. Med. Lab. Technol.* 26, 391.

This paper describes the preparation and use of a selective medium for Haemophilus in sputum. The medium described is a heated blood agar containing bacitracin 10 units/ml. This is claimed to suppress most of the other organisms present in sputum allowing a high recovery rate of Haemophilus. D.G.B.

**Isolation of L-forms from Clinical Material.** Brem, A. M. (1969) *Amer. J. med. Technol.* 35, 702.

The author describes two simply prepared media suitable for the isolation of L-forms from clinical material. Sixteen organisms were isolated by this technique which routine procedures failed to detect. The routine procedures are not described. The author emphasises the unestablished relationship between L-forms and disease and also stresses the fact that the technique suggested is not a complete answer to L-form culturing but a method suitable for use in any laboratory. D.G.B.

**Effect of Mixed Cultures on Antibiotic Susceptibility Testing.** Shahidi, A. and Ellner, P. D. (1969). *Appl., Microbiol.*, 18, 766-770.

In this paper the authors have sought some factual basis for the objection to direct sensitivity testing. Their careful studies of the antibiotic susceptibility of mixtures of bacteria encountered in clinical cultures have shown that results obtained by direct sensitivity testing are completely unreliable. They have shown how mixtures of both sensitive and resistant species appeared either as "resistant" or "sensitive" depending on the organisms and the antibiotic. A number of sensitive species gave reactions interpreted as resistant when tested in combination. Since reactions of bacterial mixtures are completely unpredictable, the authors emphasise that antibiotic sensitivity testing should be limited to pure cultures. Although monobacterial infections, (i.e. blood cultures and spinal fluid cultures, can have direct sensitivity testing.

This is a paper which should be read by all laboratory workers.

B.C.

**Subacute Bacterial Endocarditis Due to Haemophilus Aphrophilus.** Spicer, A. J. and Mitchell, E. S. (1970), *J. Ir. med. Ass.*, 63, 57.

In this case report, the patient was hypersensitive to penicillin; bacteriological cure was achieved with cephaloridine and streptomycin. The source of *Haemophilus aphrophilus* and its portal of entry are unknown, but there is some evidence that dogs may act as a reservoir. J.H.

**Polymyxin-Coagulase-Mannitol Agar: I. A Selective Isolation Medium for Coagulase-Positive Staphylococci.** Orth, D. S. and Anderson, A. W. (1970), *App. Microbiol.*, 19, 73.

A selective, differential plating medium was developed for the isolation and identification of coagulase-positive and mannitol fermenting staphylococci. Coagulase produced by growing *Staphylococcus aureus* caused an opaque zone of fibrin to form around each colony. The addition of polymyxin B to the medium selectively retarded the growth of *S. epidermidis* and minimised false-positive reactions caused by citrate-utilising Gram-negative rods. J.H.

**Gram-Negative Pneumonitis: A New Postoperative Menace.** Atik, M. and Hanson, B. (1970), *Am. Surg.*, 36, 198.

This report was published to bring attention to a unique type of Gram-negative pneumonitis, sudden in onset, overwhelming in nature, devastating to respiratory organs, complicated by shock and a bleeding tendency, which follows uncomplicated procedures.

The six patients reported had pneumonitis caused by *Pseudomonas aeruginosa*, *Aerobacter aerogenes*, or both. In four of the patients, similar organisms were traced to respirator apparatus used during anaesthesia, and in one patient, in the intensive care unit.

There is a discussion of the proper use, cleanliness and sterile maintenance of ventilatory machines. J.H.

**Isolation of Anaerobic Bacteria from Clinical Specimens.** Zabransky, R. J. (1970), *Mayo Clinic Proc.*, 45, 256.

During a 6 month period, 482 isolations of obligately anaerobic bacteria, including the *Bacteriodaceae*, anaerobic cocci, *Clostridia*, and miscellaneous Gram-positive bacilli were made from 1,223 specimens. Specimens included miscellaneous body fluids and exudates, tissue, and material from wounds and abscesses. More than one-fourth of these specimens (323) had at least one anaerobic species. This represented 40 per cent. of the specimens that were found to contain any bacteria, aerobic or anaerobic. Seventy-six per cent. of the anaerobic species were isolated from anaerobic culture plates made directly from the specimen. *Bacteriodes fragilis* was the most common isolate, being present in almost one-half of the specimens yielding anaerobes. Anaerobic bacteria were found in mixtures with aerobic flora 65 per cent. of the time but in pure culture 21 per cent. of the time. The significance of anaerobic bacteria in infectious processes can be assessed only after proper cultural techniques are used for their isolation and identification. J.H.

**Incidence of Mucoid *Pseudomonas aeruginosa* from Clinical Sources.** Doggett, R. G. (1969). *Appl. Microbiol.*, 18, 936-937.

Encapsulated *Pseudomonas aeruginosa* are quite rare in nature; however, in 78 patients diagnosed as having cystic fibrosis 70% of their sputums contained mucoid strains of *P. aeruginosa*.

Because of its antibiotic resistance and the fact that an increased incidence of *P. aeruginosa* is usually associated with long-term chemotherapy in chronic illness, one would expect similar frequencies of mucoid strains in other chronic diseases. However, this is not the case but is an apparent mutation resulting from environmental factors within the host.

These mucoid strains are not unusual strains but have been shown to be the commonly encountered strains. B.C.

**Isolation of L-forms from Clinical Material.** Brem, A. N. (1969). *Amer. J. med. Technol.*, 35, 702-705.

Though it is not fully established as to the part L-forms play in the infectious process, enough data is available to necessitate its consideration when routine laboratory procedures fail to explain clinical signs and symptoms.

It has been shown that it is possible to increase the number of positive cultures for blood, urine and spinal fluid in the laboratory, using a culture procedure that enhances the growth of some L-forms.

This paper discusses the use of a brucella broth media with added sucrose and a solid media using Noble agar to induce growth of the organism.

The facts presented indicate that an L-form growth procedure should be available in clinical and hospital diagnostic laboratories for immediate use when requested, or if felt warranted by the senior technologist.

B.C.

**Differentiation of Haemophilus Species Using Satellite Methods.** Klein, Marilyn and Blazevic, Donna (1969), *Amer. J. med. Technol.*, 35, 695-701.

Seventy-six *Haemophilus* species were tested for satellite growth, using staphylococci and enterococci streaks and X and V factor discs on brain heart infusion agar. It was found that 20% of the *H. influenzae* strains produced slight visible satellite growth near the staphylococci. *H. influenzae* and *H. haemolyticus* strains did not grow at all near the enterococci or around the V factor disc, and they grew only between the X and V factor discs. The *H. parainfluenzae* and *H. parahaemolyticus* strains all produced significantly more growth when tested with the three satellite methods.

These methods worked well aerobically or in increased carbon dioxide.

B.C.

**Characterisation of Ornithine Decarboxylase-Positive, Non-motile Strains of the Klebsiella-Enterobacter Group.** Matsen, J. H., and Blazer, Donna. (1969) *Appl. Microbiol.*, 18, 566-569.

A problem which appears to be coming more prevalent is the identification of strains of organisms belonging to the Klebsiella and Enterobacter groups. For obvious clinical and academic reasons accurate reporting is most important. This paper deals with 36 organisms which were non-motile and ornithine positive. Biochemical and serological testing showed that five strains conformed to *Escherichia coli*, three Klebsiella, while the remainder were Enterobacter. This paper emphasises that well-chosen biochemical procedures must be used in identification of Klebsiella-Enterobacter groups.

B.C.

**Nalidixic Acid (Negram) Disc Technique for the Rapid Isolation of Faecal Streptococci from Mixed Flora.** Devaraj., S. K., and Zinnemann, K., (1969) *J. med. Microbiol.*, 2, 366-368.

The importance of isolating faecal streptococci from urinary infections in which a mixed flora predominates is obvious with regard to antibiotic therapy. In many instances gram stains indicate gram-positive cocci and gram-negative bacilli with the culture showing only gram-negative bacilli. Suppression of growth by the more rapidly growing gram-negative bacilli occurs and the streptococci are missed. By using a 30 µg. disc of nalidixic acid on a plate, plated with the mixed flora, inhibition of the gram-negative bacilli is sufficient to isolate the streptococci.

This short paper states some interesting points of use in the routine laboratory.

B.C.

**Identification of the Mima-Herellea Group of Organisms and Selected Members of the Genus Neisseria.** Marrara, R. V., and Rheins, M. S. (1969) *Appl. Microbiol.*, 18, 575-578.

It will no doubt be accepted that difficulties can arise in identification of members of the genus Neisseria and organisms exhibiting similar characteristics, such as *Mima polymorpha*. This paper discusses the problem and sets out a useful scheme which could be adopted for routine use when handling specimens from the genito-urinary tract. From 82,273 specimens handled, 728 were from the vaginal/cervical or urethral sources, and by employing the scheme suggested 12 isolates of the Mima-Herellea group were identified. This indicates the possibility of erroneous identification, and far-reaching social implications.

B.C.

**Techniques for Culturing Mycoplasma species from Clinical Specimens.** Praznik, J. (1969) *Amer. J. clin. Path.*, 52, 784.

This paper describes methods for the routine isolation and identification of Mycoplasma species from clinical specimens.

D.G.B.

**Evaluation of a Reagent Impregnated Strip for Detection of Nitrate Reduction by Bacterial Isolates.** Yu, P., Birk, R. J. and Washington, J. A., II (1969) *Amer. J. clin. Path.* 52, 791.

The authors compared the performance of a prepared paper strip Nitrate reagent (W6565, Warner Chilcott Laboratories) with the broth method of detecting nitrate reduction described by the committee on bacteriological technique of the Society of American Bacteriologists.

The methods showed good correlation with all bacterial species tested except *Staph. aureus*. The authors note the desirability of doubling the incubation time recommended for the strips to reduce the incidence of false negative reactions. It is the authors conclusion that these strips are a useful addition to the range of rapid bacterial identification tests now available.

D.G.B.

**Anaerobic Indicators: A Review.** Drollette, D. D. (1969) *Amer. J. med. Technol.*, 35, 12, 758.

The author lists seven methods of preparing anaerobic indicators.



Methods of preparing the indicators are described but no comparison of their efficacy is made. D.G.B.

**Gram Negative Bacilli in Burns.** Davis, B., Lilly, H. A. and Lowbury, E. J. L. (1969) *J. clin. Path.*, 22, 634-641.

The authors discuss the percentages of various types of Gram negative bacilli other than *Pseudomonas aeruginosa* found in burns and examines the antibiotic sensitivity patterns of these organisms. It is noted that a high proportion of the *Escherichia coli* and *Proteus mirabilis* strains isolated were resistant to ampicillin. D.G.B.

**Sensitivity of Gram Negative Bacilli to Ampicillin after Six Years' Clinical Use.** Slocombe, B. and Sutherland, R. (1969) *J. clin. Path.*, 22, 644-648.

The authors examined over 1,000 isolates of Gram-negative bacilli for resistance to ampicillin. Only 20% of resistant *Escherichia coli* strains and 10% resistant *Proteus mirabilis* strains were encountered. This (1967) survey compared favourably with an earlier survey carried out in 1961 which demonstrated almost identical sensitivity patterns to this antibiotic.

The authors also examined cultures for transfer of resistance to ampicillin. They conclude on the basis of the material available to them that infective or transferable resistance was a feature of ampicillin resistance before the antibiotic came into general clinical use. D.G.B.

**A Simple Quantitative and Qualitative Microbiological Screening Test for Bacteruria.** Mackay-Scollay, E. M. (1969) *J. clin. Path.* 22, 651-655.

The paper describes a semi-quantitative method of enumerating bacteria in clean catch specimens of urine. The method employs a simple non-inhibitory medium which detects lactose fermentation and suppresses the swarming of *Proteus* species. This medium is distributed in a thin layer around the inside of a screw cap plastic vial by rolling. The shelf life of the prepared media is claimed to be indefinite. In use the vial is filled with urine, the urine is then discarded, the vial capped and incubated overnight. A standard area of the surface is then counted using a mask. The author claims that the method is accurate, that the medium gives sufficient differentiation of common pathogens to make these organisms readily identifiable and also describes a technique for carrying out direct antibiotic sensitivity tests without adversely affecting the accuracy of the bacterial count. D.G.B.

**A Comparison of Methods for the Quantitation of Bacteria from Burn Wounds: I. Experimental Evaluation.** Georgiade, N. G., Lucas, M. C., O'Fallon, W. M. and Osterhout, S. O. (1970) *Amer. J. clin. Path.*, 53, 35-39.

**A Comparison of Methods for the Quantitation of Bacteria from Burn Wounds: II Clinical Evaluation.** Georgiade, N. G., Lucas, M. C. and Asterhout, S. O. (1970) *Amer. J. clin. Path.*, 53, 40-42.

In these two papers the authors compare the efficacy of the gauze capillarity method of Brentano with a moist swab technique. The authors conclude that the moist swab technique is as accurate and less time consuming and expensive than the gauze capillarity method. D.G.B.

**An Improved Dip Spoon Method for use in the Screening of Urines for Significant Bacteruria.** Roberts, G. (1970) *J. med. Lab. Technol.*, 27, 109-111.

This paper describes an improvement of the dip spoon method, as described by Mackey and Sandys (1965), of examining clean catch urines for significant bacteruria. The main points of difference between this modification and the original method are:—use of a smaller, transparent spoon; use of MacConkey's agar in place of cystine lactose electrolyte, deficient agar and the incorporation of acti-dione in the medium suppress fungal contamination.

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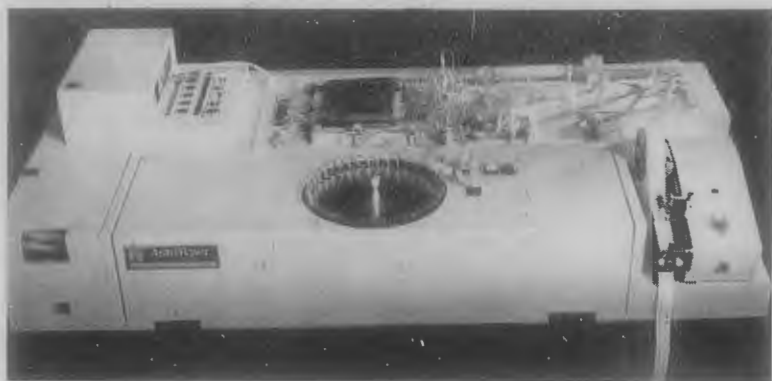
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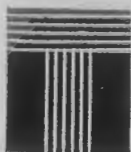


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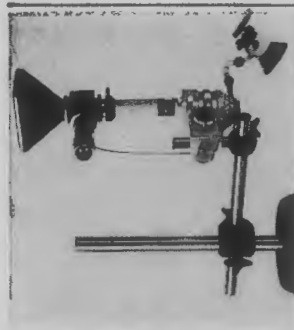
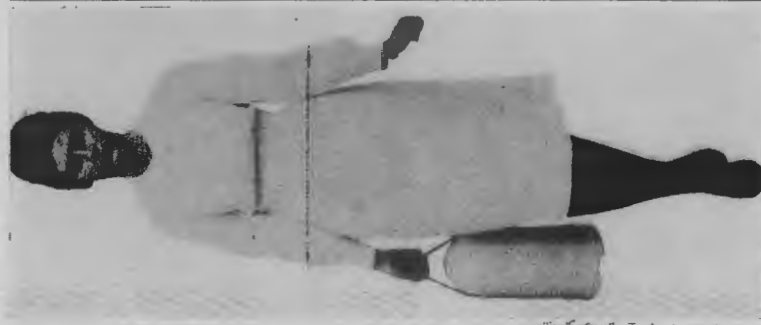
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The author claims that these modifications make the counting of colonies and differentiation of lactose fermenting and dactose non-fermenting colonies easier.

D.G.B.

**Some Observations on the Detection of Staphylococcal Deoxyribonuclease.** Stickler, D. J. (1970) *J. med. Lab. Technol.*, 27, 83-85.

This paper describes a modification of the staphylococcal deoxyribonuclease test (DNase test) which can be read after 4 hours incubation. The author states that 587 strains of *Staph. aureus* were studied for DNase production at 2-4-6 and 24 hours. 88% correlation was obtained between 2 and 24 hours incubation and 100% between 4 and 24 hours.

D.G.B.

**Salmonellosis. Report of an Unusual Case.** Westerlund, N. C. and Bierman, A. H. (1970) *Amer. J. clin. Path.*, 53, 92-94.

The authors describe the isolation of *Salmonella enteritidis* from a shoulder joint aspirate. Stool and blood specimens were negative. The paper discusses possible routes of infection.

D.G.B.

**Observations on the Effect of Membrane Filters on Inhibitory Dyes in Solid Media.** Drake, W. J., Elsie, J. R. and Nichols, G. (1970) *J. med. Lab. Technol.*, 27, 37-42.

The authors studies have demonstrated that cationic dyes, such as crystal violet which are used to selectively inhibit Gram positive organisms in culture media, are ineffective when these media are used to support the growth of organisms on membrane filters.

The growth of a variety of organisms on selective media containing dyes when directly inoculated on to the medium and when grown on membrane filters is compared. The lack of inhibition is constant for all organisms compared.

The dye retaining properties of the filters were tested, using both cationic and anionic dyes. It was found that cationic dyes were absorbed by the filters and that soaking the filters in distilled water did not extract the dyes.

The physical reasons underlying these observations are discussed.

D.G.B.

**A Study of Some of the Methods of Urinary Collection in Children.** Conn, H. K. (1970). *J. clin. Path.*, 23, 81-84.

A comparison of three methods of collecting urine from children for bacteriological examination.

Methods investigated were clean catch, with and without toilet, adhesive plastic bag technique and suprapubic bladder aspiration.

Analysis of results indicate that in the great majority of cases a satisfactory bacteriological diagnosis can be made on specimens passed naturally and collected with care. In only a minority of cases is bladder aspiration indicated to provide conclusive results. The results also indicate that preliminary toilet is of little value with clean catch specimens and that provided the specimen is maintained at low temperature after collection some delay in removing adhesive plastic bags after passage of the specimen will make little difference to the count.

D.G.B.

**Direct Microscopical Examination of Tube Cultures for the Detection of Trichomonads.** Barrow, G. I. Ellis, C. (1970) *J. clin. Path.*, 23, 91.

This technical note describes a method of culturing Trichomonads in sterile tissue culture tubes, thus allowing direct microscopical examination of the culture without the necessity to prepare wet films.

D.G.B.

**Quality Control Experiences with Febrile Agglutinins in Serum.** Carter, P., and Schoen, I. (1970) *Amer. J. clin. Path.*, 53, 364-369.

A commercial, polyvalent positive control antiserum for febrile agglutinins proved stable and reliable for at least 6 months at 4°-6 C. Estimates of precision using 22 replicates over this period indicated that a



two-fold change in titre is in excess of technical variation and indicates significant biological change. A quality control survey covering several laboratories is described. This showed interlaboratory variability to be twice intralaboratory variability for febrile agglutinins.

D.G.B.

**A Study and New Description of *Corynebacterium vaginale* (*Haemophilus vaginalis*).** Dunkelberg, W. E., Skaggs, R. and Kellog, D. S. (1970) *Amer. J. clin. Path.*, 53, 364-369.

A new study of *Haemophilus vaginalis* type strain 594 and four other reference strains. The authors conclude, on the basis of an extensive range of identification tests, that the proposal of Zinnemann and Turner should be adopted and this species be reclassified as *Corynebacterium vaginale*.

D.G.B.

## UNCLASSIFIED

**Types of Error within a Clinical Laboratory.** McSwiney, R. R. and Woodrow, D. A. (1969) *J. med. Lab. Technol.* 26, 340-346.

The authors of this paper discuss the findings of a four-week survey carried out to detect gross errors occurring in their laboratory.

Although the survey covered a clinical chemistry laboratory, the paper is applicable to all branches of medical laboratory work, as it is concerned not with quantitative precision but with clerical, organisational and technical errors which are common to all disciplines.

The survey revealed a surprising number of errors (2.3% of all results). A further survey showed that apparently 2-3% error is the irreducible minimum of error which can be attained using human operators. Examples of errors occurring in other human activities are given. The authors stress the importance of clear, written directions, and for constant skilled supervision in reducing organisational and technical errors.

D.G.B.

**The Computer in the Clinical Biochemistry Laboratory.** Fleck, A. and Reekie, D. (1970), *Internat. J. bio-med. Comput.*, 1, 41.

There are two methods of handling data by computer in the laboratory. Either the computer is directly linked to the analysers (on-line) or the data are first converted into machine-readable form for subsequent batch processing (off-line). It is the authors' opinion that off-line procedures solve many of the problems arising from the ever increasing workloads of biochemistry departments. While on-line processing has been more popular in early research-oriented applications, the advantages have not been as great as expected. In contrast, off-line processing, which is a relatively straightforward computer application, can be successful and moreover, by using data links to increase the workload offers considerable economic advantages.

J.H.

**The Computer in the Hospital Laboratory: Is it Premature?** Summers, H. (1970), *Internat. J. bio-med. Comput.*, 1, 31.

Seven reasons are given for the increase in the amount of biochemical work carried out in hospital laboratories: two of these reasons are considered less admirable. The role of the computer is considered in the context of data processing in hospital laboratories with advantages and limitations being analysed. The author stresses the value of the computer in the clerical side of laboratory work, but doubts its importance in the field of biochemical estimations.

It has been stated that a computer is excellent for those who have a problem to be solved which needs its facilities for storage or calculation but a computer is a menace in the hands of those who have the machine, and then try to think what to do with it.

J.H.

## What's New

### THE UNICAM SP90 SERIES 2 ATOMIC ABSORPTION SPECTROPHOTOMETER

A new version of the Unicam SP90 Atomic Absorption Spectrophotometer, announced by *Pye Unicam Ltd.*, of Cambridge, England, incorporates improvements in sensitivity, in simplicity of operation and in the display of results. The instrument, known as the Unicam SP90 Series 2, now has a built-in meter giving a readout linear in absorbance units, which enables concentrations to be read off directly in most cases. Another important feature is that the burner system is fully enclosed to ensure safety, minimise operator fatigue and improve the stability of the flame. Accessories such as an automatic sample-changer and digital printer can be added to the basic instrument to form a fully automatic analytical system (Fig. 1).

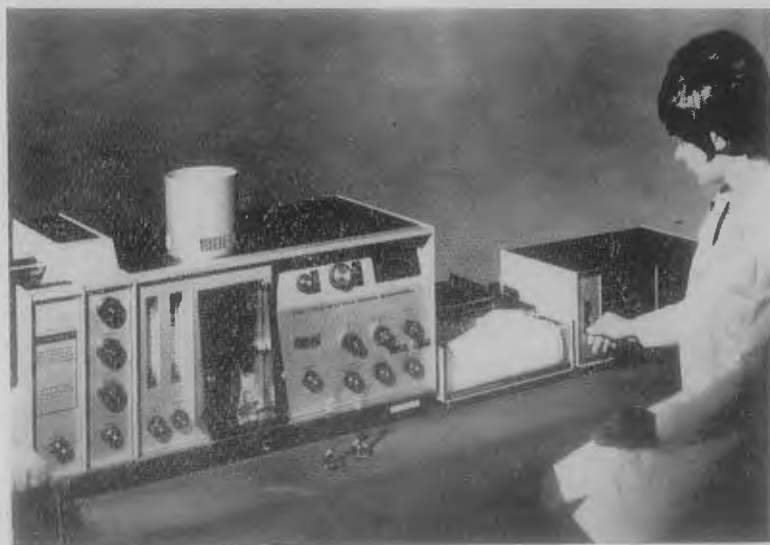


Fig. 1 The Unicam SP90 Series 2 Atomic Absorption Spectrophotometer set up for automatic operation.

Atomic absorption spectrophotometry has established itself as a rapid and highly sensitive method for the quantitative determination of more than 60 chemical elements, present in a wide variety of samples. Typical applications include the examination of blood serum for the presence of calcium, copper, iron, potassium, lithium, sodium and zinc. More than 70 method-sheets describing proven applications of the instrument are available from the manufacturer.

#### *Improved Optics*

In the SP90, the sample is atomised in a flame, and the absorption of light by the flame is measured at a particular wavelength corresponding to a strong spectral line of the element concerned. Suitable light is provided by a hollow-cathode lamp containing the element in question; this ensures that the light is rich in the wavelength being used and, since the spectrum is discontinuous, permits precise isolation of that wavelength by a monochromator. Wavelengths between 190 and 852 nm can be used. The

instrument can also be used for flame emission spectrophotometry.

Improved optical alignment in the new instrument has increased the sensitivity of detection achievable with many elements. The hollow-cathode lamps are held in special holders which permit rapid focusing. A valuable accessory is a turret containing three of these lamp-holders, the appropriate lamp being chosen by rotation of the turret. Each lamp is run continuously at its correct operating current to eliminate warm-up delays.

To minimise interference from the flame background, the power supply to the lamp is modulated and the amplifier for the photomultiplier detector is tuned to the same frequency.

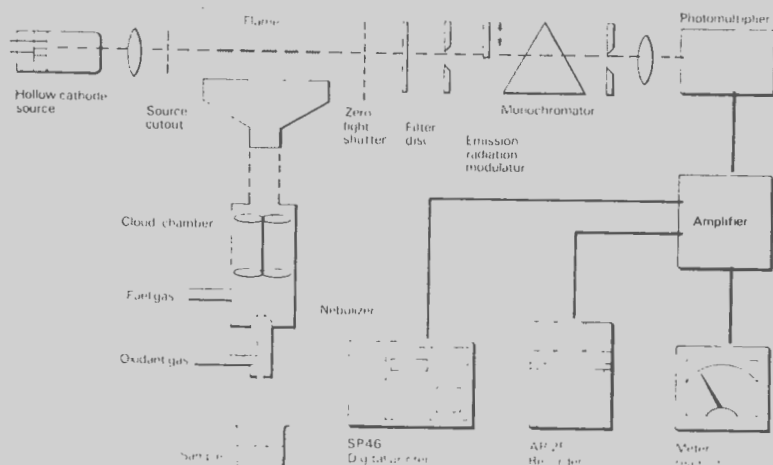


Fig. 2 Schematic diagram for the Unicam SP90 Series 2.

### Linear Display

The output from the amplifier is displayed on the built-in meter. Readout linear in either absorbance or transmittance units can be selected, and a single continuously-variable control permits expansion of any part of the absorbance scale by up to 10 times, and of the transmittance scale by up to 50 times. Output points for a chart recorder and a digital printer are also provided.

### Stable Flame

The design of the mixing and burning system ensures a flame of constant height and luminosity, even with slow-burning flames. The sample is first mixed with an oxidant gas by aspiration, then passes through a nebuliser where it is formed into an aerosol of small uniform droplets. A stainless-steel nebuliser is standard, but a special inert nebuliser is available for corrosive solutions. The mixture next passes into a cloud chamber where fuel gas is added, and finally passes to the burner head.

Various burners for different oxidant/fuel gas mixtures are available. With the standard instrument, two 10-cm laminar-flow burners are supplied for use with air/propane and air/acetylene mixtures, together with a Meker head for use with air/acetylene in flame emission spectrometry. The burner heads can be changed rapidly, and the flow-rates of the oxidant and fuel gases are continuously monitored and easily adjusted. An accessory is an air-compressor supplying up to 10 litres of clean air per minute.

Two other accessories are burners allowing the use of a hydrogen/argon mixture as the fuel or of nitrous oxide as the oxidant. A special gas-flow control unit is supplied with the nitrous-oxide burner to ensure safety, and

## 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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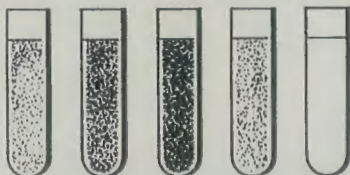
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# RHEUMATEX<sup>T.M.</sup>

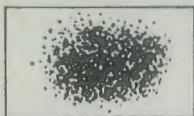
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AGGLUTINATION TEST PERFORMED EITHER IN  
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IN A WELLED TRAY



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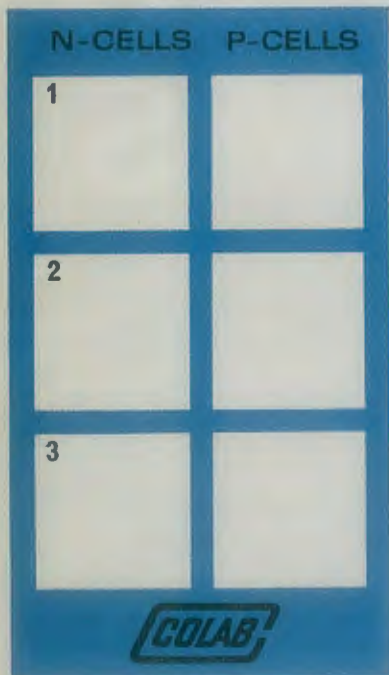
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Sera inactivation unnecessary

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Suitable for 50-60 slide tests



PRODUCTS FROM

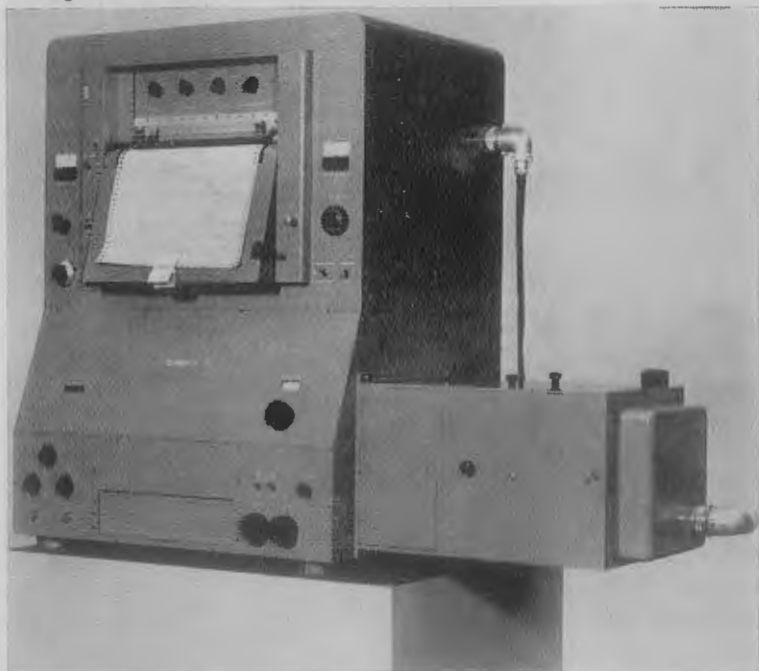


the burner head itself is of a new type with a raised slot to reduce carbon formation.

For concentrated solutions containing up to 12% dissolved solids, a multi-slot burner is used. This accessory will support both air/acetylene and air/propane flames. It is supplied with an auxiliary air-supply which allows higher flow-rates without impairing efficiency.

### 'ORGANIC MS20'—A NEW MASS-SPECTROMETER FROM AEI

AEI Scientific Apparatus Limited, a member of the GEC-Elliott Automation group, announce the introduction of the ORGANIC MS20 — a fast-scanning mass-spectrometer for chemists who need a combined gas-chromatograph/mass-spectrometer capable of making analyses at high sensitivity. The ORGANIC MS20 is stated to give the user an analytical performance and instrumental facilities hitherto associated with instruments costing twice as much.



The ORGANIC MS20 can be fitted with gas chromatographs using packed or capillary columns, and other attachments include an all-glass heated inlet and a probe, which is both heatable and coolable, for direct insertion into samples. The many applications thereby made possible include quantitative analysis, determination of molecular weight and qualitative analysis of organic gases, liquids and volatile solids. The instrument is extremely sensitive, and a resolution better than 400 (using the '10%-valley' definition of resolution) can be obtained with  $5.10^{-8}$  grams-per-second of sample in the source at a scan-speed of 1 second per decade of mass. At a scan-speed of 10 seconds per decade, the resolution is better than 500. Stability and reliability in operation are ensured by using advanced solid-state circuits in the electronic control units.

Further information from J. A. Race, A.E.I. Scientific Apparatus Ltd., Barton Dock Road, Urmston, Manchester M31 2LD, England.

## LEUCONOR-2 ELECTROMECHANICAL COUNTING DEVICE

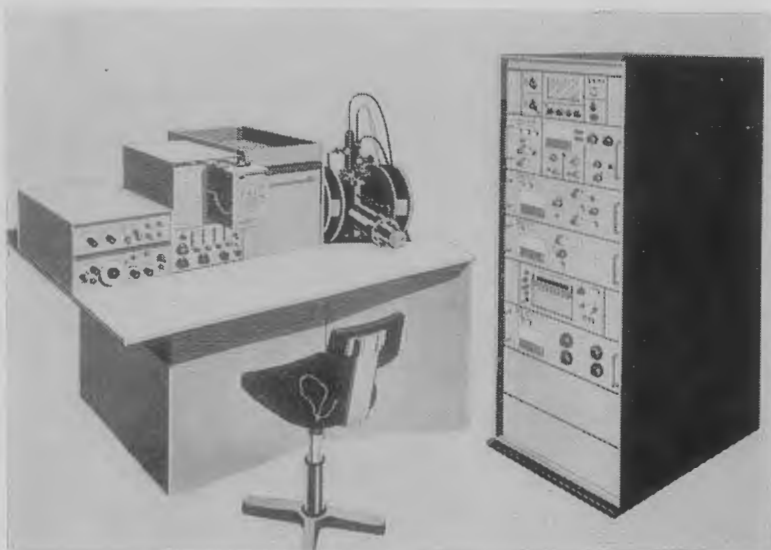
Medical, academic and industrial laboratories engaged in microscopic analysis frequently employ the method of differential counting of components. Such time-consuming, tedious counting often keeps highly trained personnel from doing the qualified work they are paid for. The LEUCONOR-2, an electromechanical counting device, has been developed to facilitate and economize counting procedures, such as in recording various types of flaws and tolerances, in culture counts and spray density scanning. It is of particular advantage in haematological laboratories of clinics and hospitals, where it is applicable for counting white blood cells. Simple operation and small space requirement make the instrument expedient and versatile. Its shape and colour are styled in accordance with latest ergonomic aspects.

The instrument has eight six-digit individual counters arranged in two rows, with two rows of correlated release buttons. There is also a six-digit totalising counter which is provided with an adjustable arresting mechanism and switches the instrument off as soon as a preselected count number is reached (e.g. 100 when counting blood cells). All counters can be reset individually. The release buttons are set in a white Decelith plate bearing symbols to correlate the buttons to the different components to be counted.

The ingeniously designed electrical circuitry and the use of independently working counting modules guarantee absolutely fail-safe operation and ease of maintenance.

Further details from:—VEB Rathenower Optical Works, Carl Zeiss Strasse, Rathenow, German Democratic Republic.

## NEW ALL SOLID STATE RECORDING SPECTROPHOTOMETER FROM CARY





A new, high performance recording spectrophotometer, the Cary 17, was introduced and demonstrated for the first time at the 21st Pittsburgh Conference in Cleveland, Ohio, March 1-6, 1970.

Produced by Cary Instruments, a Varian subsidiary, the solid state instrument covers the wavelength range 186 nm to 2.65 microns. It incorporates as standard equipment several of the accessories Cary users have found most beneficial in the past, plus various new features not previously available. Visually, the 17 is similar to the Cary 14 and makes use of some of this instrument's time-proven design elements. However, it does not replace the 14, which will continue to be manufactured.

*Solid state electronics* lead the list of Model 17 features. Most of the electronic components are mounted on a single swing-out circuit board which is easily accessible from the back of the instrument. Major benefits derived from solid state construction include high reliability and reduced maintenance and parts replacement.

*Coupled scan and chart drive through digital techniques.* The introduction of solid state digital circuits permits electronic synchronisation of the scanning mechanism and the chart drive in both forward and reverse modes. The selected chart scale expansion remains constant even when the scanning speed, which automatically governs chart speed, is increased or decreased. It is simple, fast, and convenient to change scan and chart presentation parameters. Separate stepping motors drive the wavelength scanning mechanism and the recorder.

Digital scanning circuits also permit computer control of the scan mechanism by the SpectroSystem/100.

*Forward beam infrared operation* is possible out to the wavelength range limit with no compromise in instrument specifications. Samples are illuminated with monochromatic light so there is no danger of destroying sensitive samples by exposing them to excessive heat or light.

*Optimum parameters indicators* are essentially go/no go meters which reconcile resolution and signal-to-noise ratio with scanning speed to match recorder pen response. With these meters the operator can easily set the pen period, gain, and scan speed to yield a fully resolved spectrum in the shortest possible time.

*High intensity source* in the visible-near IR range puts out as much as 40 times the energy of conventional tungsten lamps. The tungsten-halogen lamp power level is continuously variable from 70 to 115 volts.

*Pen period control* was formerly an accessory to Cary spectrophotometers. This control allows setting pen periods of 1, 5, or 25 seconds to improve the S/N level in energy limiting sample conditions.

*A new wide range slidewire* is a money-saving feature of the Model 17. This slidewire combines five ranges of absorbance and transmittance in one unit, and also offers zero suppression of up to one absorbance in steps of 0.1 absorbance. Even more important, this new slidewire is convenient to use and saves the operator time because it eliminates the need to change slidewire assemblies manually.

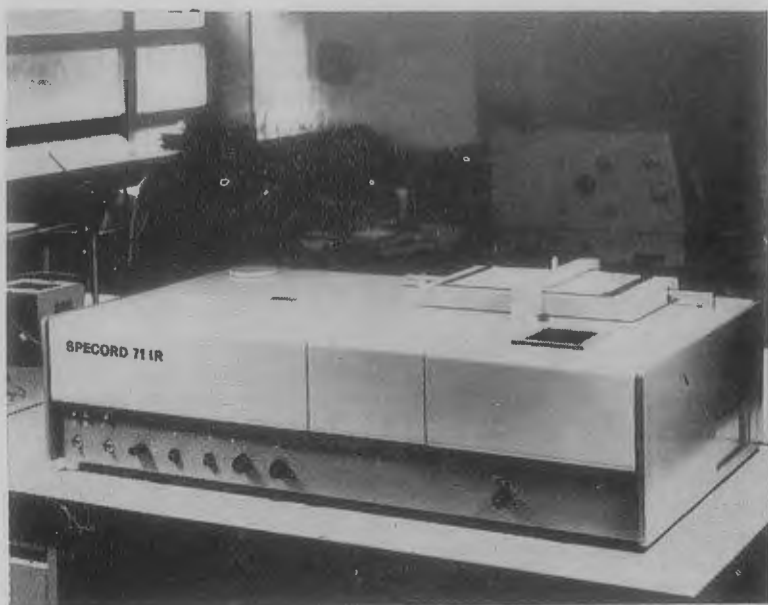
*A new Cary-built strip chart recorder* has been incorporated in the instrument to preserve the high performance of the monochromator and photometric system on a recorded chart. An automatic range change feature effectively doubles chart width to 20 inches.

Further details from:—

Advertising Department, Cary Instruments, 2724 South Peck Road, Monrovia, California 91016, U.S.A.

## SPECORD 71 IR AND SPECORD 72 IR RECORDING SPECTROMETERS

Two new double-beam spectrophotometers for absorption spectral analysis in the infrared region have been developed by VEB Carl Zeiss JENA.



SPECORD 71 IR with NaCl prism for wavenumbers from 4600 to 65  $\text{cm}^{-1}$  and

SPECORD 72 IR with KBr prism for wavenumbers from 1100 to 400  $\text{cm}^{-1}$ .

Operating on the double-beam optical-null principle, both instruments scan sample transmittances (between 0 and 100%) and record them against wavenumbers on chart sheets of 150 mm x 275 mm ruled area. The SPECORD 71 IR and 72 IR instruments extend the range of small recording spectrophotometers started with the well-known SPECORD UV-VIS.

Four wavenumber scales (1 to 8 sheets per total range), four scanning speeds between 4.4 and 44 min/sheet, continuously variable slit programme (ratio 1 : 4), step-by-step gain control at a 1 : 10 ratio and selection between 4 time constants permit to carry out analytical tasks under optimal scanning conditions.

Fixed-wavenumber scans of transmittance vs. time are possible without any special attachment.

Routine work is facilitated by automatic reset and by facilities for selected-range scanning and automatic scan repetition.

The sample compartment (170 mm overall light path) is spacious enough to accept any of the many different sampling accessories to the UR 10 and UR 20 Infrared Spectrophotometers.

Details from VEB Carl Zeiss, Carl Zeiss Strasse, Jena, German Democratic Republic.



**CIRCULATING THYROXINE (T<sub>4</sub>)**

- Sample preparation
1. Resin extraction
  2. Washing
  3. Elution
  4. Drying
  5. Proceed to ashing



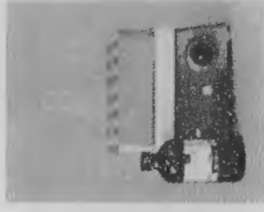
**PROTEIN BOUND IODINE (PBI)**

- 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



**ASHING**

(Note—no pre-treatment of sample required for Total Iodine test. Ashing is first step.)

- A. Chloric-Chromate sample ashing at 170C for 20-30 minutes. (Complete ashing is monitored by appearance of reddish precipitate.)



**COLOR DEVELOPMENT**

B. Ceric arsenite color developing system for 20 minutes at 56C.



**READ-OUT**

C. Read at 400 to 460 nm (microns)



Thyroid Profile Set and Procedures eliminate time-consuming methods, costly additional equipment and provide accurate, reproducible and more sensitive readings.

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