I wish to thank Dr. G. K. McGowan for reading this paper, Miss Joan Little for technical assistance, and the British Empire Cancer Campaign for full financial support.

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The determination of 3-methoxy 4-hydroxy mandelic acid in urine

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A simple colorimetric method for the determination of 3-methoxy 4-hydroxy mandelic acid (V.M.A.) in urine has recently been described by Woiwood and Knight (1961). In this method, urine, after acidification and saturation with sodium chloride, is extracted with ether and the extracted V.M.A. removed into aqueous solution with dilute alkali. After coupling with diazotized para-nitro aniline the azo derivative is extracted into chloroform from which it is re-extracted with sodium hydroxide as a red solution.

Trials in this laboratory have confirmed the suitability of this method for routine use, but we have found it helpful to introduce the following modifications into the technique:—

1 Ethyl acetate is substituted for ether as the first extracting solvent, two successive 10 ml. portions being employed.

2 The combined ethyl acetate extracts are washed with 2 ml. distilled water.

3 Extraction into aqueous solution is brought about by shaking with 25, 15, and 10 ml. portions of 0.01 M phosphate buffer, pH 7.6 (50 ml. 0.2 M KH_2PO_4 + 42.7 ml. 0.2 M NaOH, diluted 1 : 20 with distilled water).

The use of ethyl acetate is safer than ether in the routine laboratory, and the smaller volume of organic solvent makes subsequent handling easier. It was found that variable amounts of acid were being carried over in this first extraction; the washing step was found to remove 60 to 70% of this. The use of a buffer at pH 7.6 for the subsequent extraction avoided any large swing in pH during this step, together with the need for any final adjustment of the pH of the combined extract.

STANDARD CURVE

We have prepared a standard curve giving absolute optical density values for the azo-derivative of V.M.A. in alkaline solution, in order to calculate overall recoveries of quantities of V.M.A. taken right through the procedure. It is shown in Fig. 1 and is prepared as set out on page 389.

The optical density of the red solutions was measured on the Hilger Uvispek spectrophotometer at 510 m μ using 1 cm. cells.

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Tube No.	Standard V.M.A. (5 µg./ml.)	Distilled Water (ml.)	Diazo Reagent (ml.)	N/10 NaOH (ml.)	Equivalent V.M.A. Concentration (µg.)
1	0.0	2.1	0.4	2.5	Blank
2	0.2	1.9	0.4	2.5	4
3	0.4	1.7	0.4	2.5	8
4	0.6	1.5	0.4	2.5	12
5	0.8	1.3	0.4	2.5	16
6	1.0	1.1	0.4	2.5	20

FIG. 1. The standard curve for V.M.A.

RESULTS

Using the modified procedure we have investigated the recovery of various amounts of V.M.A. taken right through the method and the recovery of V.M.A. added to normal urine. The results are given in Tables I and II.

TABLE I
RECOVERY OF V.M.A. IN AQUEOUS SOLUTION

V.M.A. (µg.)	% Recovery
5	94
5	100
10	86
10	92
15	98
15	92
20	91
20	99

TABLE II

RECOVERY OF V.M.A. FROM URINE

Sample	Volume Used (ml.)	V.M.A. Added (µg.)	% Recovery
A	2	10	88
A	2	15	87
B	1	10	98
B	2	10	98

TABLE III

EXCRETION OF V.M.A. IN 24 HOURS IN SUBJECTS EXCRETING NORMAL LEVELS OF PRESSOR AMINES

Number of Cases	Range of V.M.A. Excretion (µg.)
1	2-3
2	3-4
1	4-5
5	5-6
4	6-7
3	7-8
4	8-9
1	9-10
1	10-11
1	11-12
2	12-13

Table III shows the distribution of the results of 25 estimations of the urinary output of V.M.A. in 24-hour samples. All these specimens were from hypertensive patients, and had been submitted for screening for increased pressor amine output; all contained less than 180 µg. noradrenaline/24 hr. by the method of Hingerty (1957). A single case of surgically proved phaeochromocytoma was found to have excreted 29.4 mg. of V.M.A. in 24 hours.

We wish to thank Dr. F. J. W. Lewis, Director of the Pathology Department at Southmead Hospital, for kind support and encouragement. One of us (N.A.S.) is in receipt of a salary grant from the National Spastics Society. Part of the expenses for apparatus have been met from the Ethel Showering Fund.

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preparations can be made by drying the stained section in air and then mounting in Fluormount (E. Gurr, Ltd.), though this treatment sometimes causes a reduction in fluorescence.

COMMENT

The freeze-dried sections are equally suitable for identifying the autoantibody against thyroglobulin (Figure 1), and the CA2 antibody first described by Balfour *et al.* (1961) for which immunofluorescence provides the only known method of detection. The technique is also sufficiently reliable for use in reverse, so to speak, for studying the reactions of thyroid biopsy material with autoimmune sera of known specificity.

Because of the excellent preservation of stored sections, there is clearly no necessity for all laboratories interested in the detection of autoantibodies to thyroid to carry out the freeze-drying, embedding, and microtomy themselves. It should be possible to have embedded blocks or mounted sections prepared commercially for routine laboratory use.

This research programme has been supported by grants from the Medical Research Council and the Scottish Hospital Endowments Research Trust. We also wish to thank Dr. Deborah Doniach and colleagues for confirmatory tests with our stored sections in routine applications.

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CORRECTIONS

We regret that in the paper by J. A. Campbell and A. H. Cruickshank on 'Cystadenoma and cystadenocarcinoma of the pancreas' (*J. clin. Path.*, **15**, 432-436) the legends to figures 8 and 9 on page 436 have been transposed.

Dr. Small regrets that there was an error in the third modification of the technique he describes in his paper on 'The determination of 3 methoxy-4 hydroxy mandelic acid in urine' (*J. clin. Path.*, **15**, 388). It should read as follows:—

3 Extraction into aqueous solution is brought about by shaking with 25, 15, and 10 ml. portions of a phosphate buffer, pH 7.6 (50 ml. 0.2 M KH_2PO_4 + 42.74 ml. 0.2 M NaOH, diluted to 200 ml. with distilled water). Dilute this solution 1 : 20 with distilled water for use.

Book reviews

ANTICOAGULANT THERAPY By A. S. Douglas. (Pp. xi + 394; illustrated. 50s.) Oxford: Blackwell Scientific Publications. 1962.

Whenever the subject of anticoagulant therapy is broached there tends to be an immediate thought-transference to the somewhat emotional and contentious question of the long-term prophylaxis of myocardial ischaemia. The protagonists and antagonists assume their prepared positions and vehement pragmatism tends to obscure the existing wide measure of agreement on the hospital treatment of acute surgical and gynaecological venous thrombosis.

This book by Dr. Douglas, acknowledged to be an expert with experience both as a clinician and as a meticulous laboratory worker, provides a valuable and authoritative reference text with practical information superior to any other currently available.

One can find in this volume all the information necessary for the clinical and laboratory surveillance of occlusive venous disease. The selection and use of heparin and the indanediones or coumarins is supplemented by a helpful and succinct review of the relevant underlying physiological processes.

There is a review of the pharmacology and metabolism, so far as the latter is capable of interpretation, of the various anticoagulant preparations and, for those who are taking a renewed interest in the clinical value of heparin, full information concerning its therapeutic administration, spiced with a little bit of history.

The chapter on the therapeutic indications for the use of coumarin and indanedione drugs should be read by all those who accept hospital responsibility for this therapy. The first half refers to short-term treatment upon which there is general agreement.

Dr. Douglas presents the two principal arguments in favour of anticoagulants for acute myocardial infarction but, here, one is moving into deep water. This book is written by a protagonist and those interested might usefully read Professor McMichael's critical review of it (*Brit. med. J.*, **1**; 1812, 1962) in which some of the opposing points are mentioned. Be this all as it may, your reviewer, when he has his coronary occlusion, will insist on receiving heparin followed by phenindione anticoagulant screening for the initial six weeks, unless some fresh and very persuasive evidence to the contrary is forthcoming!

The second half of the chapter on therapeutic indications is devoted to long-term prophylactic therapy—a subject much bedevilled by claims and counter-claims regarding the management of coronary insufficiency.

This text-book appears at a time when fibrinolysin mechanisms are becoming more widely understood and thrombolytic therapy feasible. Hence, the therapeutic approach to thrombo-embolic disease may be altered or expanded during the next five or so years. Dr. Douglas has reviewed the current knowledge of physiological mechanisms in fibrinolysis, as we now understand them, and with advice from his colleague, Dr. G. P. McNicol,