A routine immunofluorescence method for detecting autoantibodies to thyroid colloid

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The theoretical and practical aspects of demonstrating autoantibodies to thyroid by immunofluorescence have been discussed elsewhere (Nairn, 1962). The principle of the method is simple: the sera of patients with suspected autoimmune thyroiditis are applied to sections of human thyroid and any interaction of thyroid antigen with specific autoantibody is detected by means of a fluorescent anti-human-globulin conjugate.

Unfixed fresh frozen sections of thyroid are employed for identifying the complement-fixing anti-cytoplasmic autoantibody, but these are inadequate for identifying anti-colloid activity as thyroid colloid is poorly retained in unfixed sections. To preserve colloid without denaturation in frozen sections, Balfour, Doniach, Roitt, and Couchman (1961) employed a methanol-fixation procedure which has usually given satisfactory results in our hands but suffers from certain disadvantages. In particular, stocks of thyroid tissue blocks, even when kept at -76°C ., deteriorate rapidly and require replacement about every three weeks; fresh microscopical sections are required on every occasion testing is carried out and there is always an element of uncertainty about the colloid fixation or about the suitability of a new thyroid gland which may, in any case, be difficult to obtain at the appropriate time. Balfour *et al.* also employed freeze-substitution of thyroid tissue but we find the method unsuitable for routine use: colloid seems often to be lost into the dehydrating solvent.

The above objections have been overcome by using vacuum freeze-dried thyroid embedded in polyester wax and sectioned with an ordinary microtome. Such preparations provide an effective substrate for repeated autoantibody testing over many months.

TECHNIQUE

Fresh surgical specimens of thyrotoxic glands provide the best material as their small acini retain colloid well during processing; adequate pre-operative medical treatment of the donor ensures that colloid will be abundant. Representative blocks of thyroid (about 2 mm. cubed) on small strips of aluminium foil are plunged into a mixture of liquid nitrogen and isopentane at about -180°C . They are then quickly transferred to a tissue freeze-drying apparatus and dried *in vacuo* at -35° to -40°C . With our equipment, based on that of Pearse (1960), drying takes 48 hours whereupon the blocks are vacuum-embedded at 38°C . in polyester wax (400 polyethylene

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FIG. 1. Freeze-dried section of thyroid treated first with serum from a patient with Hashimoto's disease and then with RB 200-conjugated anti-human-globulin. The presence of anti-thyroglobulin activity in the patient's serum is indicated by the flocculent staining pattern ($\times 190$).

glycol distearate) mixed with 10% cetyl alcohol (Steedman, 1960).

The wax blocks are stored at 0°C . or at a lower temperature in sealed containers with a few crystals of silica gel as desiccant and to date, after six months' storage, they have shown no loss of antigenic potency. Sections (5μ) may be made at any time; their preparation is facilitated by cooling the specimen in a stream of cold air from a block of dry ice held in a kitchen strainer above the microtome (Sidman, Mottla, and Feder, 1961) and by keeping the knife cold with a dry ice trough (Pearse, 1960). The sections are dry mounted on slides warmed just sufficiently to melt the wax, which may then be dispersed by a few drops of absolute methanol. The dewaxed sections are ready for use or they may be stored in the same manner as the wax blocks and, as far as our experience goes (six months), their antigenicity is unimpaired by such storage. Slides prepared in this way can also be kept in desiccators in a cryostat cabinet and used for mounting fresh frozen sections additional to the freeze-dried section already in place. Staining multiple microscopical preparations for autoantibodies on one slide is a very convenient time-saving procedure for the simultaneous detection, for example, of anti-colloid and anti-cytoplasmic activity (Doniach, personal communication). Cytoplasmic staining cannot be obtained in the freeze-dried sections prepared by the present method as the antigen is inactivated by the processing.

Fluorescent staining of the microscopical preparation is accomplished by applying a drop of the patient's serum and leaving for 30 minutes in a damp atmosphere at room temperature. The slide is then washed in phosphate-buffered saline (pH 7.1) for 10 minutes after which anti-human-globulin labelled with fluorescein isothiocyanate or lissamine rhodamine B (RB 200) is applied for 20 minutes and the preparation washed again in buffer for 10 minutes and mounted in buffered glycerol. Permanent

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.

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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,