Technical methods

Detection of autoantibodies to cytoplasmic and nuclear antigens in freeze-dried thyroid

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Since our recent report of a routine immunofluorescence method for detecting autoantibodies to thyroid colloid antigens (Nairn, Ghose, Porteous, and Urquhart, 1962) we have chanced on a modification of procedure which makes the method also applicable to thyroid cytoplasmic and nuclear antigens.

The preparation of freeze-dried blocks embedded in polyester wax and cutting and staining of sections are the same as for the colloid method. The only variation is in the mounting and preparation of the sections for staining. These are floated in a bath of phosphate-buffered saline (0.01M, pH 7.1) kept at 37°C. to secure flattening, and mounted, while in the saline, on chemically clean slides which are then drained and wiped to remove surplus fluid. The sections are dewaxed immediately with about 2 ml., in drops, of a 3:1 mixture of isopentane and absolute methanol at room temperature. This mixture is not a solution and must not be allowed to separate into its two constituents during use. After wiping off the solvent, the preparations may either be used immediately for fluorescent antibody staining or may be stored for several weeks at 0°C. or lower temperature in a sealed container with desiccant. In either case just before staining, they are rinsed vigorously for 30 sec. in buffered saline at 37°C. and wiped to remove surplus fluid. This final rinsing helps to dislodge any residual colloid from the thyroid acini and, by wetting the section, facilitates reaction between the test sera and thyroid cells.

The isopentane methanol mixture, chosen empirically, provides a saturated solution of each component in the other, making an excellent wax solvent with only minimal fixative properties insufficient to denature the cellular antigens or fix the colloid. Many other solvents and variations of method have been tried without so far improving on the present procedure. In three months' experience with the method we have obtained unambiguous cytoplasmic and nuclear staining with autoimmune sera (Figs. 1 and 2). Polyester wax-embedded thyroid tissue, also used for the colloid study described previously, provided, after more than eight months' storage in a desiccator at -20°C., excellent preparations for cytoplasmic and nuclear as well as thyroglobulin

FIG. 1. *Cytoplasmic staining of freeze-dried human thyroid by autoimmune thyroiditis serum and RB 200-conjugated antihuman-globulin ($\times 140$).*

FIG. 2. *Nuclear staining in a similar preparation to Fig. 1, employing the serum of a patient with systemic lupus erythematosus ($\times 140$).*

and CA2 colloid staining. Sections both for colloid and cellular antigens can with care be prepared on the same slide by mounting at one end the colloid-retaining preparation first and avoiding wetting this during subsequent treatment of the cytoplasmic section; we find it less troublesome to make the two types of preparation on different slides.

Reasonably good correlation has been obtained between staining results by test sera and their complement-

fixation reactions against thyroid microsomal antigen. Positive complement-fixing sera failed to give cytoplasmic staining in only one of six cases and in this the titre was low (1:4); on the other hand positive staining was sometimes obtained with sera which did not fix complement, and these were presumably not false positive reactions because all such sera showed other serological evidence of antithyroid activity. The method sometimes appeared to be less sensitive than the corresponding immunofluorescence techniques with fresh frozen sections (Nairn, 1962). The lack of precise correlation with the complement-fixation and fresh-frozen techniques, which was not always in the same direction, suggests the possibility that some of the cellular autoantigens may have been partially denatured by the processing whilst others, still in active form, were better retained by the mild fixation. The combination of differential fixation with immunofluorescence might usefully contribute to the identification of the diverse antigens in autoimmune processes.

The most important single factor for success with the present freeze-drying method is the suitability of the original thyroid tissue to provide antigenically active microscopical preparations. The thyroid should be hyperplastic to ensure an abundance of cytoplasmic microsomal antigen and a predominance of small acini to retain colloid. If satisfactory embedded tissue blocks were available commercially¹, the technique for cellular and colloid antigens described in this and the previous publication could be used for most routine laboratory investigations of clinically significant autoantibodies. For research studies it might supplement, but until further experience is gained should not replace, the possibly more sensitive techniques using cryostat cut sections. The method for cellular antigens could perhaps also be adopted for other autoimmune systems such as the reaction between gastric mucosa and sera from patients with pernicious anaemia (Taylor, Roitt, Doniach, Couchman, and Shapland, 1962).

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¹Wellcome Laboratories are considering the practicability of marketing these blocks.

Detection of auto-immune antibody and tissue antigens by the 'microspot' technique

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The demonstration by Roitt, Doniach, Campbell, and Hudson (1956) of precipitating antibody to human thyroglobulin in the sera of patients with auto-immune thyroid disease led to the use of precipitation in agar gel as a test for the presence of such antibody (Doniach and Roitt, 1957; Anderson, Goudie, and Gray, 1959). Doniach and Roitt (1957) were able to demonstrate precipitating antibody in 109 of 144 suspected cases of auto-immune thyroiditis by double diffusion in tubes. Anderson *et al.* (1959), employing a similar technique, found precipitin to thyroglobulin in about 70% of their cases and considered its presence as virtually diagnostic; Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson, and Goudie (1961) considered diagnostic serological tests essential to avoid unnecessary destructive surgery or irradiation in cases of overt or suspected thyrotoxicosis. In view of these findings a simple and quick test for circulating precipitating auto-antibody should be useful in the routine clinical pathology laboratory.

Recently I described a 'microspot' test for antigen-antibody interaction in thin agar films (Feinberg, 1961) and its adaptation to and enhanced sensitivity on cellulose acetate membranes (Feinberg, 1962). The antibody for these tests had been induced in rabbits by immunization with hetero-antigens.

The 'microspot' test would have a particular advantage for routine use on clinical specimens because it is simple, sensitive, objective, quickly carried out and read, requires but minute quantities of serum and antigen, and provides a permanent record for the case files. I have, therefore, investigated the suitability of the cellulose acetate 'microspot' test for the detection of spontaneously occurring precipitating auto-antibody of human origin and of the specific antigen thereto.

Auto-immune thyroiditis was chosen for this preliminary investigation because of the well-authenticated occurrence of precipitating antibody in patients suffering from this condition. Sera of two patients with auto-immune thyroiditis and a solution of 40 mg./ml. human thyroglobulin were kindly provided by Dr. I. M. Roitt of the Courtauld Institute of Biochemistry. A preliminary 'microspot' test was carried out on both sera and they were found to contain approximately similar amounts of precipitating antibody to the thyroglobulin. One of these

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