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 commercially[1], 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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REFERENCES

Nairn, R. C. (1962). *Fluorescent Protein Tracing*, p. 224. Livingstone, Edinburgh.
——, Ghose, T., Porteous, I. B., and Urquhart, J. A. (1962). *J. clin. Path.*, **15**, 594.
Taylor, K. B., Roitt, I. M., Doniach, D., Couchman, K. G., and Shapland, C. (1962). *Brit. med. J.*, **2**, 1347.

[1]Wellcome 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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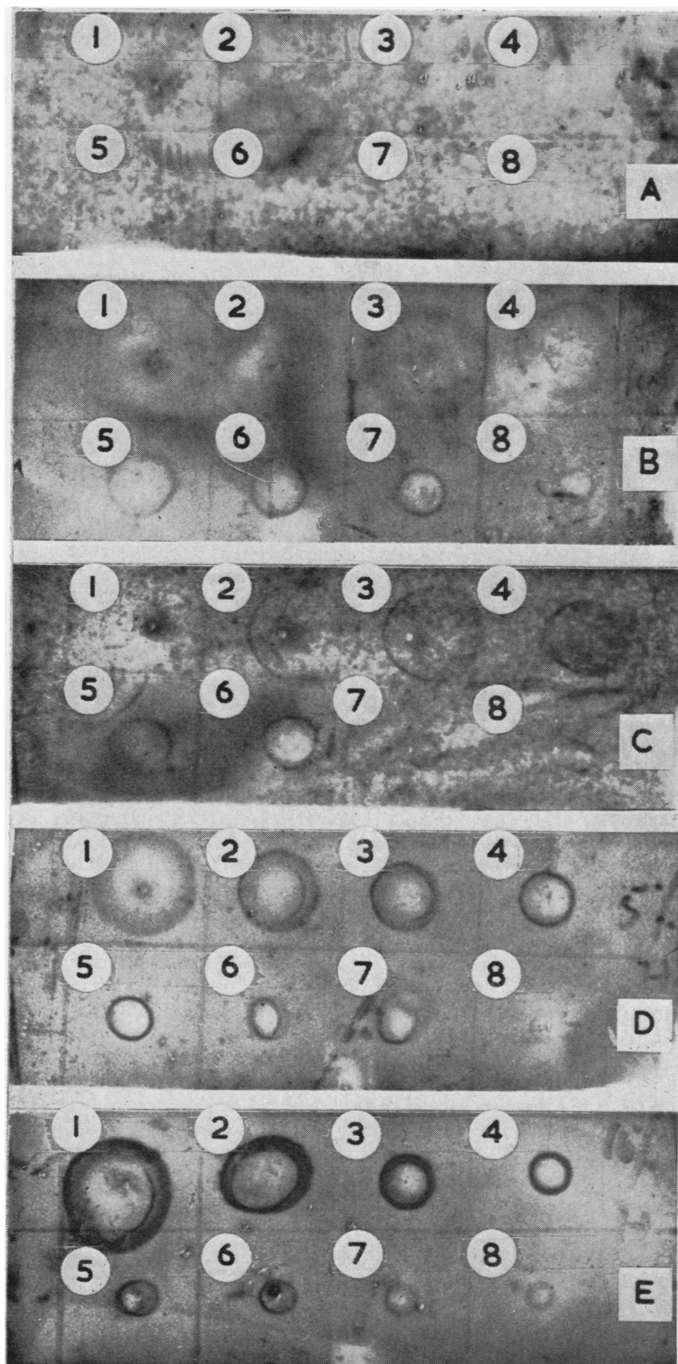

FIG. 1. A, *Pooled normal human serum, 1: 10;* B, C, D, E, *Auto-immune thyroiditis serum, diluted 1: 80, 1: 40, 1: 20, 1: 10 respectively.* 1 *to* 8 *Threefold serial dilutions of human thyroglobulin (starting concentration = 10 mg./ml.).*

was chosen for more extensive investigation, the results of which are reported here.

METHOD

An initial dilution of 1/10 in saline was made of the serum. Ten per cent normal human serum in saline was used to make the following further dilutions of the thyroiditis serum: 1:20, 1:40, 1:80. This had the effect of maintaining a uniform 10% serum level in each of the dilutions made. A 10% solution of normal serum in saline was used as control.

Cellulose acetate strips were prepared as previously described (Feinberg, 1962) and individually saturated with one of the serum dilutions, making five strips in all. The thyroglobulin solution was diluted to 10 mg./ml. and seven further three-fold saline dilutions made therefrom. Using 1 μl. Drummond[1] microcap disposable micropipettes, 1 μl. 'microspots' of each of the antigen dilutions were placed on each of the five lightly blotted, serum-impregnated strips. The strips were immediately placed in a moist chamber and the antigen-antibody interaction allowed to proceed overnight.

The strips were then washed in saline for two hours and stained with 0·002% of nigrosine in 2% acetic acid. Staining of the precipitate could already be seen after some 15 to 30 minutes, but staining was allowed to proceed further for several hours. The strips were removed from the stain, washed in 2% acetic acid for a short time, rinsed in distilled water, and dried.

RESULTS

A positive reaction manifests itself as a ring of stained precipitate. Weak reactions are best seen by transillumination, which is also best for photographing the strips.

Due to the uneven quality of the cellulose acetate membrane, some non-specific blotching or speckling may occur on stained strips. However, such artefacts in no way resemble specific reactions and are more disturbing to the photographic plate (Fig. 1) than to the naked eye. They in no way confuse the reading of the test.

In the serum tested, it was possible to detect both antigen and antibody at the limits of dilutions employed, *i.e.*, 1:80 dilution of antiserum and less than 5 μg./ml. thyroglobulin. With the lower concentrations of the immune serum one gets a proxone effect at the higher concentrations of antigen (the reaction area being large

[1]Shandon Scientific Company Limited.

and diffuse), the circle of reaction becoming smaller and more intense with decreasing antigen concentration. The control strip impregnated with the normal human serum is completely negative apart from one atypical spot at the site of application of the highest concentration of antigen.

DISCUSSION

As reported in an earlier paper (Feinberg, 1962), the time of antigen-antibody reaction, washing and staining can be considerably reduced. Taken together with the sensitivity of the test as now shown for precipitating auto-immune antibody, it becomes possible to carry out the entire test—from blood-taking to final result—within a day.

The technique could be considerably simplified by using a single 'microspot' of a 40 μg./ml. concentration of thyroglobulin, which approximates to dilution 6 on the strips in the illustration. This would readily detect auto-immune thyroiditis antibody in sera of patients having 1%, or even less, of the antibody contained in the serum of the patient used in this investigation.

Though the present investigation was restricted to auto-immune thyroiditis, the 'microspot' test should be equally applicable to other auto-immune conditions in which precipitating antibody circulates in the blood plasma, and may be useful in demonstrating such antibody in conditions where it has not yet been found. Such conditions could include rheumatoid arthritis, lupus erythematosus, atopic dermatitis, idiopathic adrenal atrophy, and Sjögren's syndrome. In the last, Jones (1958) found precipitating auto-antibodies to extracts of the lachrymal and salivary glands, though Anderson, Gray, Beck, and Kinnear (1961) subsequently showed these antibodies would react with antigens from a wide variety of tissues. They also demonstrated the presence of auto-antibody to thyroglobulin in some Sjögren sera. The 'microspot' test should also find a use in the demonstration of naturally, or artificially induced, heterophile antibody in man, as well as in the detection of low concentrations of antigens.

SUMMARY

A new specific antigen-antibody precipitation technique, the 'microspot' test, has been applied to the detection of the antibody arising spontaneously in auto-immune thyroiditis. It has been found that the technique can detect low levels of both the auto-immune antibody and the antigen against which it is directed. It is suggested that the technique could usefully be extended to the study of other proven or suspected auto-immune conditions.

I should like to acknowledge the photographic reproductions of Douglas F. Lawson.

REFERENCES

Anderson, J. R., Goudie, R. B., and Gray, K. G. (1959). *Scot. med. J.*, **4**, 64.

The estimation of β amino-isobutyric acid in human urine

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β Amino-isobutyric acid (B.A.I.B.) was identified independently by Crumpler, Dent, Harris, and Westall (1951) and Fink, Henderson, and Fink (1951), and was shown by Fink, Cline, Henderson, and Fink (1956) and Gartler (1959) to be a product of thymine metabolism. Examination of urine from victims of accidental whole body irradiation by Rubini, Cronkite, Bond, and Fliedner (1959) and by Gerber, Gerber, Kurohara, Altman, and Hempelmann (1961) showed significant increase of β amino-isobutyric acid. This suggested that the estimation of B.A.I.B. in urine would be helpful in indicating the extent of cellular damage after massive irradiation. Furthermore Awapara (1957) has shown significant increases of B.A.I.B. after nitrogen mustard therapy in cases of leukaemia, lymphosarcoma, and Hodgkin's disease, and measurement of this metabolite may be a useful index of D.N.A. catabolism in clinical medicine. For this purpose a relatively simple, quick, cheap and reliable method is required.

EXISTING METHODS OF DETERMINING B.A.I.B.

1 The method of Rubini *et al.* (1959) comprises decolorization with charcoal, desalting with resin, and two-way chromatography. After ninhydrin staining the unknown is compared visually with standards.

The advantages are ease, cheapness, and safety. Disadvantages are the impossibility of running large numbers of samples (including standards) at any one time and the use of visual methods of comparison. The problem of separation from urea, which is not stressed by Rubini, is discussed elsewhere.

2 In the high-voltage electrophoresis method of Gartler (1959), after separation and ninhydrin staining the paper is scanned. Advantages are speed and possibly accuracy, but the high cost and unfamiliarity of the

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——, Gray, K. G., Beck, J. S., and Kinnear, W. F. (1961). *Lancet*, **2**, 456.
Buchanan, W. W., Alexander, W. D., Crooks, J., Koutras, D. A., Wayne, E. J., Anderson, J. R., and Goudie, R. B. (1961). *Brit. med. J.*, **1**, 843.
Doniach, D., and Roitt, I. M. (1957). *J. clin. Endocr.*, **17**, 1293.
Feinberg, J. G. (1961). *Nature (Lond.)*, **192**, 985.
—— (1962). *Ibid.*, **194**, 307.
Jones, B. R. (1958). *Lancet*, **2**, 773.
Roitt, I. M., Doniach, D., Campbell, P. N , and Hudson, R. V. (1956). *Ibid.* **2**, 820.