cloning experiments do not favour such a possibility, it has not definitely been ruled out that 'transformation' of female cells into established strains is actually a process of selecting the sex chromatin negative cells in the original explant.

Whether or not the 2½-year-old negative female amnion culture reported here had become malignant, it did show a very high mitotic rate (74 mitoses per thousand cells in one count), and moderate variation in nuclear size. It may be true that the hypothetical development of cancer in tissue cultures of benign origin is invariably accompanied by a loss of sex chromatin. Since primary explants of female cancer developing *in vivo* have invariably shown sex chromatin *in vitro* when appropriately stained (but not always in tissue section[2]), it seems that at least in this one respect hypothetical malignant change *in vitro* is not identical with cancer development *in vivo*.

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Detection of Thyroid Antibodies using Bentonite Particles

DEMONSTRATION of circulating thyroglobulin antibodies by the gel diffusion-precipitation technique is a simple procedure which can be used for routine laboratory diagnosis of Hashimoto's thyroiditis[1]. Witebsky and Rose[2], Roitt and Doniach[1] and Owen and Smart[3] have also applied Boyden's tannic acid hæmagglutination technique for detection of thyroid antibodies and have demonstrated its greater sensitivity over the precipitation technique. The tannic acid hæmagglutination technique is however in our experience, less easy to adapt as a routine procedure because it is complex in its performance and requires minute attention to detail to produce consistent results. A recent report by Bozicevich *et al.* on the successful use of bentonite particles, coated with human γ-globulin, in the detection of the rheumatoid serum factor prompted us to try this substance sensitized with thyroid antigen in the detection of thyroid antibodies.

Thyroid glands removed at autopsy were stripped of their connective tissue, weighed and then blended with twice their weight of physiological saline in a Kenmix blender for 10 min. The resulting homogenate was spun at 4,000 r.p.m. for 10 min. to remove heavy cell debris and fibrous tissue. The supernatant from this centrifugation was then spun in a high-speed centrifuge at 27,000 r.p.m. for 45 min. at 4° C. The supernatant was removed and stored at −20° C. for use in both precipitation and bentonite sensitization tests.

Bentonite is a native colloidal hydrated aluminium silicate, insoluble and free from gritty particles. 2·5 gm. of bentonite (B.D.H.) are added to 300 ml. of distilled water in a flask and thoroughly shaken to mix. 25 ml. of this mixture is transferred to a universal container which is then centrifuged at 3,000 r.p.m. for 10 min.; the supernatant is discarded and replaced with 25 ml. of distilled water, the container shaken vigorously to mix and then recentrifuged. This process is repeated twice. At the end of the final wash the supernatant is removed and 2 ml. of thyroid antigen is added and mixed. After standing for 1 hr. at room temperature the sensitized bentonite particles are washed twice in physiological saline and finally resuspended in 25 ml. of physiological saline.

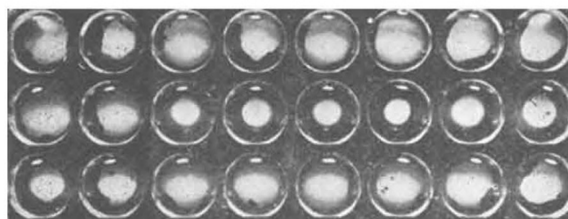

Fig. 1. A positive serum of titre 1 in 250,000, from a case of Hashimoto's disease, compared with two normal sera in rows 1 and 3.

Serial dilutions of the sera to be tested are made, as described by Roitt and Doniach[1], in 'Perspex' agglutination trays. The dilutions range from 1/5–1/2,500,000 and are made with a single pipette using 0·25 ml. volumes and 2 per cent serum saline as a diluent. An antigen inhibition control is included. 0·1 ml. of sensitized bentonite is then added to each dilution and the trays placed in the refrigerator at 4° C. overnight. The pattern of the deposited bentonite particles is read macroscopically and shows the opposite pattern to that encountered with red cell agglutination. Positive agglutination is shown by a small central round button of agglutinated particles and negatives by a thin carpet of bentonite over a wide area of the bottom of the cup (Fig. 1). Weak positives show a central button with a small carpeting around. The end-point is often sharp with no zone of weak positives, and weak positives are only included in the titre if the central button is well defined with little carpeting.

Care must be taken not to jog or disturb the trays after their removal from the refrigerator as this may result in false positives or alteration of titre.

Sensitized bentonite suspensions were used in titrating sera from a variety of cases of thyroid disease and from cases with no thyroid abnormality. In known cases of Hashimoto's disease, with positive precipitin tests, titres up to 1 in 25,000,000 were observed (cf. Owen and Smart[3]). Lower titres of the order 1/5–1/250 were found in a number of other types of thyroid disease. Occasional normal sera gave weak positives at a dilution 1/5. In comparing titres and for controlling the test we use a standard known positive serum and a normal serum together with an antigen inhibition control.

These results suggest that bentonite sensitized with thyroid antigen may be superior to tanned red cells for the detection of thyroid antibodies because of the ease of preparation and the simplicity of the technique.

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