the damage of the periventricular structures. An additional advantage of this method is the simplicity and unexpensiveness of the technical tools as compared to the methods using pressure transducers and recording devices[8,9].

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## Immunogenicity of agarose-immobilized immune complexes[1]

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*Summary.* A novel method is described for production of heterologous antisera to a specific tumor-associated murine antigen by immunization with agarose-trapped immune complexes.

Preparation of high titer heterologous antisera to specific tumor-associated antigens is complicated by the use of crude tissue extracts as immunogen in which the relative concentration of the antigen is quite low. Where the tumor-associated antigen is present in moderate quantities, reasonable quality antisera can be obtained by immunization with unfractionated tissue extracts and appropriate serum absorptions. Such antisera have been used to precipitate antigen from solution and the immune complexes, in turn, employed as immunogens to elicit the formation of a more highly specific antiserum[2]. The limiting factors, however, of sufficiently absorbed precipitating antibody, low concentrations of specific antigen in crude tissue extracts and difficulties in the efficient processing of minute precipitates combine to make effective immunization with solution-precipitated immune complexes a prodigious task.

A simple method for the preparation of high titer antisera to a specific tumor-associated antigen, murine $\gamma$-FA[3-5], is described. This procedure should be applicable to studies of diverse tissue antigens.

*Methods and results.* Immunization of rabbits with saline extracts of a mouse fibrosarcoma, antiserum absorption and subsequent identification of an antigen common to tumor, fetal and adult splenic tissue, termed $\gamma$-FA, has been described[3-5]. Radial immunodiffusion plates[6] contained 0.1 ml of absorbed anti-$\gamma$-FA serum and 2.5 ml of 1% agarose (w/v) in Beckman B-2 buffer, pH 8.6. Antigen wells (3.7 mm in diameter) were cut into the agarose gel, filled with 7 µl of the 10,000 × g supernatant fraction of a $10^{-3}$ M Tris, pH 7.5, homogenate of normal adult mouse spleen and the plates incubated at 37 °C for 72 h to allow for precipitin ring formation. The agarose slabs were then dialyzed with stirring against daily 200-ml changes of phosphate-buffered saline (PBS) for 3 weeks at 4 °C in order to remove unbound protein. The use of a 1% agarose gel facilitated this removal while antigen-antibody complexes remained trapped within the gel matrix. After dialysis, sections of gel containing precipitin rings were cut out, passed several times through an 18-gauge needle and frozen at −20 °C in twice the volume of PBS. On day 1, 14 and 21, 1 ml of agarose-immobilized immune complexes was emulsified in 1 ml of complete Freund's adjuvant and inoculated s.c. into a New Zealand white rabbit (on day 21 incomplete adjuvant was substituted for complete adjuvant). 22 days later the rabbit was bled and 1-ml aliquots of the antiserum inoculated i.p. into each of several adult C57 mice for in vivo absorption. After 24 h, the mice were bled and the antibody activity of the absorbed antiserum compared with that of the original anti-$\gamma$-FA serum. Clearly, the antiserum to $\gamma$-FA-anti-$\gamma$-FA immune complexes possessed all the precipitin specificity of the original anti-$\gamma$-FA serum but at a much higher titer (table) and, unlike the original antiserum, yielded a positive indirect immunofluorescence test[4] on methanol-fixed rat hepatoma cells (figure). This antigen was previously thought to be synthesized only by in vivo propagated tumor cells[4] and the present data, therefore, provide the 1st direct evidence for production of $\gamma$-FA by transformed cells. Moreover, reten-

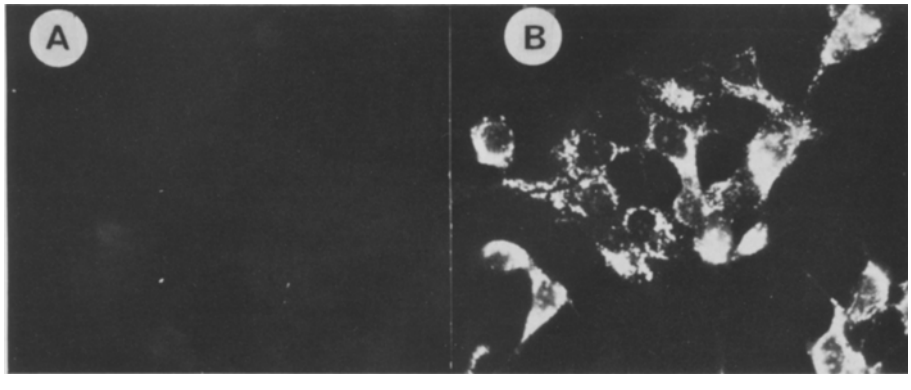


Fig. 1. Indirect immunofluorescence test of in vitro propagated rat tumor cells isolated from a transplanted hepatoma[7]. *A* Original anti-$\gamma$-FA serum (1:20); *B* anti-$\gamma$-FA immune complexes (1:60). UV light microscopy, BG12-53/44 filters, Zeiss Photoscope.

Table 1. Comparative precipitin tests[a]

| Test antigen | Original anti-γ-FA (undiluted) | Anti-γ-FA immune complexes (1:30)[b] |
|---|---|---|
| Meth A serum[c] | + | + + + |
| Meth A tumor[d] | + | + + + + |
| Normal serum[e] | – | – |
| Adult spleen[f] | + | + + + + |
| Viscera[g] | – | – |

[a] Hyland Immuno-Plates, pattern 'D'. Preliminary experiments indicated the precipitin line which formed upon interaction of the original anti-γ-FA serum with a saline extract of adult mouse spleen and that which formed by interaction of anti-γ-FA immune complexes with the same splenic tissue extract was one of identity. [b] Calculated dilution after in vivo absorption. [c,d] Serum and saline extract of tumor tissue obtained from a mouse bearing a transplanted 3-methylcholanthrene-induced fibrosarcoma. [e,f] Obtained from normal adult mice. [g] Individual saline extracts of adult mouse liver, kidney, brain, heart, lung, testes and small intestine pooled from several mice.

tion of antibody activity after in vivo absorption in mice (which have γ-FA-positive spleens) suggests that γ-FA is not a cell surface antigen.

Preparation of a high titer anti-γ-FA serum by immunization with agarose-trapped immune complexes will facilitate future in vitro studies of various aspects of cellular transformation. In addition, the method described should be adaptable to diverse tissue antigen systems.

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*Brighton, 13–20 September 1980*

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