copy, a sharp contrast between reactive and non-reactive sites was obtained: the positive sites, for instance nuclei and the cytoplasm of the innermost laticifers, were strongly stained in orange, while those weakly positive were orange-brown in colour. The negative sites, for instance the cytoplasm of the remnant embryo cells and the outermost laticifers, emitted a weak green fluorescence. The fluorescent emission diminished or was absent when SH groups were blocked with iodoacetate or N-ethyl maleimide.

When the proteins are transferred from aqueous to non-aqueous solvents or vice versa, complex conformational changes occurred, with reflections on stain pattern[9]. In this connection, since the fluorescent images furnished both by mercurochrome (dissolved in a polar solvent) and mercury orange (dissolved in a non-polar solvent) were equivalent, the selectivity of the methods used was confirmed. In the specimens studied, the cytoplasm of the innermost laticifers showed a pattern of staining consistent with the demonstration of SH plus SS groups bounded to proteins. Since, in contrast, the outermost laticifers were non-reactive to the tests used, this constitutes new support for the hypothesis of different functional roles of the laticifers in the embryo. Because of the function which the innermost laticifers have in the formation of the latex system in the seedling, and their rapid differentiation after germination[10], we suppose that the accumulation in sulfated proteins is related to the embryonal pre-activation of the latex biosynthesis. No straight forward explanation for the large amount of sulfated proteins in the innermost laticifers can be developed, if obvious biological significance such as structural, 'on-off' switching activity, and participation in the reduction-oxidation balance during the laticiferous differentiation, are excluded. However, since it is known that the high concentration in disulfide groups is connected with an assemblage stage of ribosomes[11], the hypothesis of a preparatory stage of the innermost laticifers in the latex biosynthesis, appears justified.

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GFA expression in aggregating cultures of rat C6 glioma[1]

A. Bignami, J. Swanson and D. Dahl

Spinal Cord Injury Service, West Roxbury Veterans Administration Medical Center, and Department of Neuropathology, Harvard Medical School, Boston (Massachusetts 02132, USA), 18 January 1979

Summary. Few C6 glioma cells synthesize the astroglia-specific GFA protein in monolayer culture. A uniform population of GFA-positive cells was obtained by aggregating C6 cells in suspension culture, as previously reported for C6 glioma maintained on sponge foam matrices. These results strongly suggest that cell-to-cell interactions promote GFA expression.

In a previous communication[2], it was shown that few C6 glioma cells accumulate the astrocyte-specific GFA protein when grown in suspension (spinner) or monolayer culture, while most cells became GFA positive when the cultures were maintained on sponge foam matrices (organ culture). Quantitation of GFA by radioimmunoassay confirmed these findings[3], and it was later shown that although the concentration of GFA increased in monolayer culture during the stationary stage of cell growth, it never reached the level observed in organ culture[4].

C6 is by far the most extensively studied cell line with respect to specific aspects of glial research[5] and thus could provide a useful tool to investigate the effect of GFA expression on other differentiated glial functions. However, these studies were difficult to conduct in monolayer or spinner culture since only a minority of cells were GFA positive, while the use of supporting matrices severely limited the amount of material available for biochemical analysis.

We now report that the desired effect of organ culture on C6 glioma, i.e. the development of a uniform population of GFA-positive cells, may also be obtained by aggregating C6 cells in suspension culture, without the use of supporting matrices. Aggregatting cultures have provided a powerful tool for developmental studies of dissociated brain cells[6-17], but to our knowledge the method has not been used before to study differentiation in glial cell lines.

Methods. The C6 cell line obtained from the American Tissue Culture Collection was grown in 100 mm^2 Falcon tissue culture dishes at 37.5 °C in an atmosphere of 5% CO_2:95% air; 98% humidity. The medium was MEM with Earle's balanced salt solution (GIBCO) supplemented with MEM vitamins (GIBCO), additional glutamine (0.3 mg/ml) and 15% horse serum (Microbiological Associates). The medium was changed every 3–5 days. The procedures for aggregate cultures were modifications of the original procedure of Moscona[18] as used before for reaggregating culture of dissociated brain cells[8-11]. Confluent monolayers were washed with Versene (GIBCO) and incubated with 0.025% trypsin for 5–7 min. Trypsinization was stopped with plating media, the cells centrifuged at 100 rpm and resuspended in MEM-spinner solution, or MEM with Earle's balanced salt solution supplemented as previously described. Cells were diluted at 2×10^5 cells/ml and 7 ml aliquots were inoculated into 25 ml Erlenmyer flasks, gassed with 99% CO_2 to 5% CO_2 (98% of the cells were viable by trypan blue exclusion). Flasks were incubated at 37.5 °C in a New Brunswick Psychtherm gyratory shaker at 85 rpm. Larger aliquots (10 ml) were incubated at 115–118 rpm. Immunofluorescence studies of cell aggre-

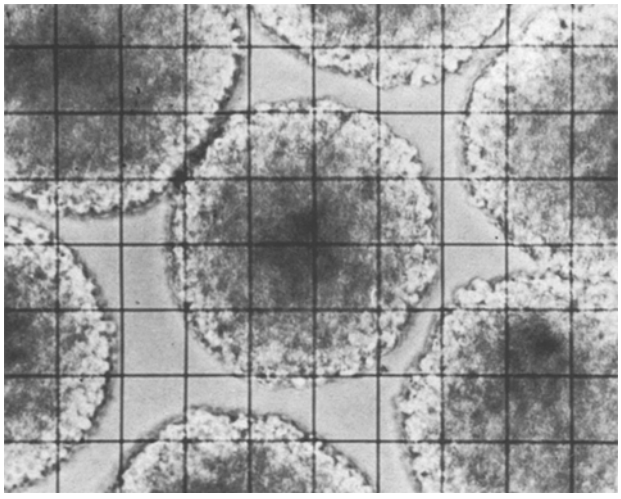

Fig. 1. Aggregates of C6 cells after 3 days in culture. The sides of the square measure 0.1 mm.

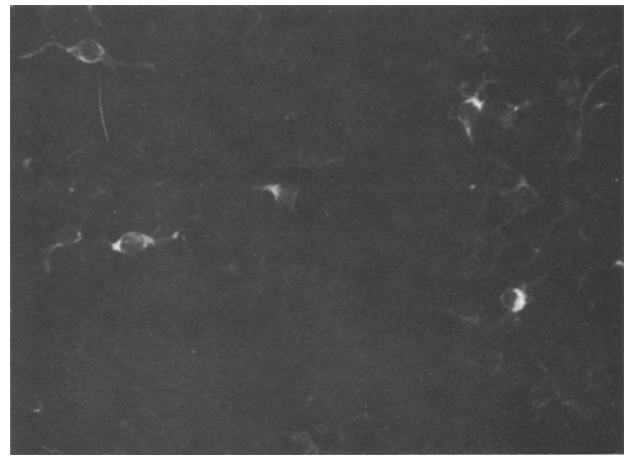

Fig. 3. Air-dried confluent monolayer of C6 cells stained by the indirect immunofluorescence method with GFA antiserum after 1 week in culture. A few cells with processes are GFA positive. × 245.

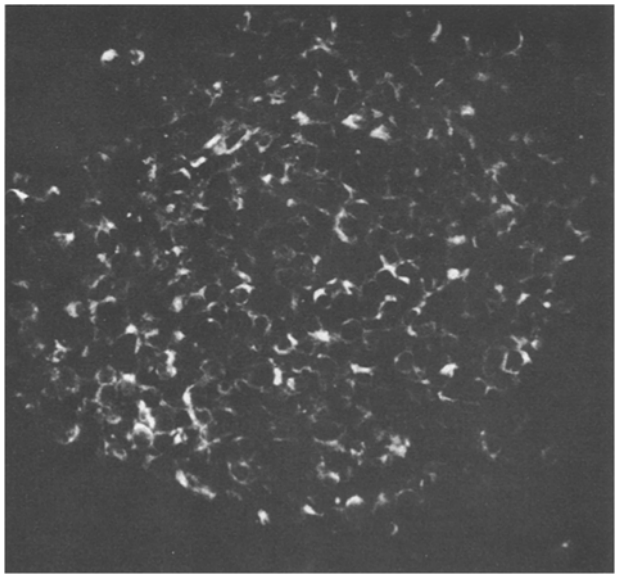

Fig. 2. Cryostat section of C6 aggregate stained by the indirect immunofluorescence method with GFA antiserum. Most of the cells are GFA positive × 250.

gates were conducted as described before[10]. The GFA antisera were those reported in a previous publication[19]. Preimmunization sera and immune sera absorbed with GFA served as controls. All sera were used at 1:40 dilutions.

Results and discussion. Round aggregates formed after 3 days in culture. They were rather uniform in size with a diameter of approximately 0.4 mm (figure 1). In hematoxylin-eosin stained sections, the cells appeared uniformly distributed with a rim of cuboidal cells at the periphery. It is interesting to note that under similar conditions murine neuroblastoma formed irregular clumps of cells rather than tight aggregates[20].

Most of the C6 cells in the aggregates showed intensely perinuclear immunofluorescence with GFA antisera (figure 2). Figure 3 shows a confluent monolayer of C6 cells stained with the same method. In agreement with quantitative data on GFA concentrations during different phases of cell growth[4] more cells were positive in confluent monolayers compared with nonconfluent ones. However, a majority of cells still did not stain. It is interesting to note that most GFA positive cells in monolayer culture developed long cell processes, while processes were rarely demonstrable in reaggregating culture and the same also appears to be true for organ culture (see figure 1, a by Liao et al.[4]).

It was previously suggested that cell-to-cell contact occurring in the organ culture system might represent the major factor responsible for the increased production of GFA protein[2]. Our observations on C6 glioma in aggregate culture support this hypothesis. The high yield of the method may facilitate the biochemical analysis of the C6 cell line in conditions favoring the production of GFA protein.

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