protein synthesis[10] and N-methyl-alanine, an analogue of alanine which interferes with a large number of biosynthetic pathways[11]. A 10- to 100-fold increase of the resistance towards both substances can be observed (table 2). Suggestions have been made that, in animal cell, such nonspecific larger resistance can be ascribed to changes in membrane permeability[1,2]. This phenomenon is, as we have shown, not restricted to animal cells, but extends also to plant cells. Plant cells contain only a small specific binding activity towards colchicine, and the highest resistance of plant cells has been ascribed to a nonspecific binding component present at the cell surface or in the cell wall[5,12]. In this respect, the observed loosening of the cell aggregates in the resistant cell strains may be due to modifications of the cell wall components and will be correlated with an increase of the nonspecific binding. The observations made by some authors that cytoplasmic microtubules associated with cellulose microfibrils deposition are disrupted by colchicine[13], and that some binding activity is associated with the membrane fraction in plant cells[14] may also lead to the conclusion that resistance is closely associated with modifications of the plasmalemma. The resistance spectrum acquired by colchicine-resistant plant cells can therefore be linked to changes in plasma membrane properties, leading to a decrease in permeability.

1 L. Ling, Can. J. Genet. Cytol. *17*, 503 (1975).
2 P.D. Minor and D.H. Roscoe, J. Cell Sci. *17*, 381 (1975).
3 H.E. Street and R. Stuart, J. exp. Bot. *22*, 96 (1971).
4 T. Murashige and F. Skoog, Physiologia Pl. *15*, 473 (1962).
5 J.W. Hart and D.D. Sabnis, Phytochemistry *15*, 1897 (1975).
6 J.W. Hart and D.D. Sabnis, Curr. Adv. Pl. Sci. *8*, 1095 (1976).
7 F. Meins, Planta *122*, 1 (1975).
8 P. Maliga, G. Lazar, Z. Svab and F. Nagy, Molec. gen. Genet, *149*, 267 (1976).
9 N. Umetsu, K. Ojima and K. Masuda, Planta *125*, 197 (1975).
10 P. Maliga, A. Sz-Breznovits, L. Marton and F. Joo, Nature *255*, 401 (1975).
11 P.J. Lea and R.D. Norris, Phytochemistry *15*, 1897 (1975).
12 J.W. Hart and D.D. Sabnis, J. exp. Bot. *27*, 1353 (1976).
13 J.D. Pickett-Heaps, Devl. Biol. *15*, 206 (1967).
14 Y. Hotta and J. Shepard, Molec. gen. Genet. *122*, 243 (1973).

Histochemical evidence on the accumulation of sulfated proteins in the innermost laticifers of the *Euphorbia marginata* embryo

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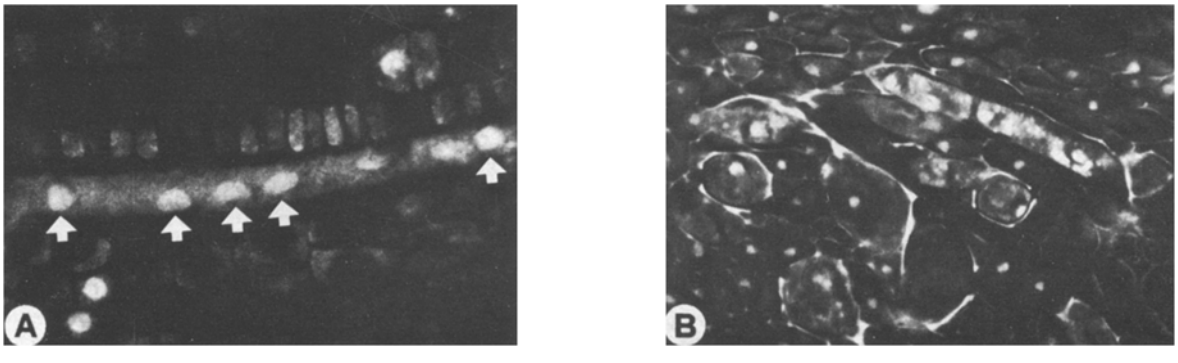
Summary. The presence of proteins containing sulfhydryl and disulfide groups was demonstrated by fluorescent mercurials in the cytoplasm of the innermost embryonal laticifers of *Euphorbia marginata*. The finding is discussed in the context of the role of the embryonal laticifers.

The mature embryo of *Euphorbia marginata* displays a complex latex system morphologically recognizable in vascular, cortical and cotyledonar components[1,2]. During germination a progressively degenerating cytoplasm occurs in the laticifers, and the changing in its content is the first symptom of the lytic-machinery activation for the latex synthesis[3-5]. In this work, some methods normally employed in the detection of animal protein-bound sulfhydryl and disulfide groups[6], have been optimized to evaluate the content in sulfur-containing proteins of the embryonal laticifers, and, consequently, to furnish some indications on the differentiation pattern of these internal secretory structures.

Embryos of *Euphorbia marginata* were fixed in absolute ethanol-acetic acid (3:1), embedded in a butyl-methyl methacrylate mixture and sectioned at 1 µm by a microtome with glass knives. The sections were placed on slides and the plastic medium was removed by a xilene-benzene mixture. To detect SH groups, mercury orange (20 mg dye/100 ml N,N-dimethylformamide) or mercurochrome (20 mg dye/100 ml water) were used. The preparations were placed in the dye solution for 30 min, rinsed in the dye solvent, passed through absolute ethanol, and cleared in xilene. Coverslips were mounted with a free fluorescence medium. The sum of SH and SS groups was demonstrated after reduction by thioglycolic acid[7], and the blocking reactions were made with iodoacetate or N-ethyl maleimide[8]. The optical system used consisted of a Zeiss Photomicroscope II equipped with a HBO 200 W lamp, a reflecting condenser, a 460 nm dichroic mirror, and a series of barrier filters.

When sections were examined by light fluorescence micros-



Innermost laticifers in *Euphorbia marginata* embryo strongly fluorescent by organomercurials: *A* The cytoplasm of a vascular laticifer is positive in the localization of SH plus SS groups (the arrows indicate the nuclei). ×480. *B* Fluorescent laticifers of the latex plexus indicating the presence of SH plus SS groups. ×380.

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.

1 R.C. Scott, Trans. 111. St. Acad. Sci. *61,* 107 (1968).
2 A. Bruni, G.L. Vannini and M.P. Fasulo, Ann. Bot. *42,* 1099 (1978).
3 B.L. Archer, D. Barnard, E.G. Cockbain, P.B. Dickenson and A.I. McMullen, in: The chemistry and physics of rubber-like substances. Ed. L. Bateman Maclaren, London, 1963.
4 P.B. Dickenson, Proc. natl. Rubb. Res. Ass. Jubilee Conf., Cambridge. Maclaren, London 1964.
5 P. Matile, in: The lytic compartment of plant cells, p.78. Springer, Wien/New York, 1975.
6 P. Ganter and G. Jollès, in: Histochemie normale et pathologique. Gauthier-Villars, Paris 1969.
7 D.B. Teiger, A. Farah and H.S. Distefano, J. Histochem. Cytochem *7,* 403 (1957).
8 A.G.A. Pearse, in: Histochemistry, theoretical and applied vol. 1. (Churchill Livingstone, London 1968.
9 R.R. Cowden and S.K. Curtis, Histochemie *22,* 247 (1970).
10 P.G. Mahlberg, Phytomorphology *9,* 156 (1959).
11 T. Hultin, Biochim. biophys. Acta *269,* 118 (1972).

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

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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-