antigens or a complex of virus with self-components, as hypothesized in accounting for this phenomenon (*29*), could well underlie the tumor regression occurring in chickens genetically resistant to Marek's disease.

In view of the immunological significance of the major histocompatibility complex across species, and the probable nature of immunological resistance to Marek's disease, our finding suggests that the high degree of resistance to herpesvirus tumorigenesis in individual chickens possessing the B^{21} alloallele results from cell-mediated immunity.

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Glia Maturation Factor: Effect on Chemical Differentiation of Glioblasts in Culture

Abstract. *A protein factor from the adult brain increases the concentrations of adenosine 3',5'-monophosphate and S-100 protein in glioblasts in culture. Such changes are correlated with the outgrowth of cell processes.*

The correlation of morphological and chemical differentiation at the cellular level is basic to the understanding of life forms and processes. We recently detected in adult brains a protein factor which is capable of inducing histotypic maturation of glioblasts in culture (*1*, *2*). The morphological transformation results from the retraction of cell bodies and the active extrusion of processes. We now attempt to relate the morphological change with the chemical evidence of differentiation.

Brain cells were dissociated from 17-day fetal rats (Sprague-Dawley) and seeded in Falcon plastic culture flasks as previously described (*2*). After the cells were carried into the second passage, the glioblasts, now free of neuroblast contamination, were exposed to a medium consisting of F_{10} nutrient (*3*), 5 percent fetal calf serum, and 100 μg of the glia maturation factor per milliliter [glia maturation factor was prepared from pig brain by step III of the published procedure (*2*)]. The cells were grown at 37°C in an atmosphere of 5 percent (by volume) CO_2 in air and 100 per-

Table 1. Effect of glia maturation factor on concentrations of cyclic AMP and S-100 protein in glioblasts. Control and experimental cells were paired cultures of glioblasts grown in the absence and presence, respectively, of the factor. Falcon flasks having a surface area of 75 cm² were used. The days indicate the periods after exposure to the factor. For cyclic AMP determination, the incubation medium was decanted from the flasks and the monolayer was rinsed for 2 seconds with ice-cold 0.15*M* NaCl in 0.02*M* tris-HCl, *p*H 7.4 (tris-saline), and immediately mixed with 5 ml of cold 5 percent trichloroacetic acid (TCA). The cells were scraped off with a rubber policeman and homogenized with a ground glass homogenizer. Subsequent steps and cyclic AMP assay were as described by Gilman (*5*). Protein was determined (*6*) in the TCA precipitate after dissolving it in 0.1*N* NaOH. Duplicate flasks were pooled and results from four pools were averaged and presented as mean ± standard deviation (S.D.). For S-100 determination, the monolayer was rinsed twice with ice-cold tris-saline. The cells from two flasks were combined and scraped into 2.5 ml of tris-saline containing 0.1 m*M* EDTA. The cell suspension was homogenized and subsequently sonicated with two 5-second bursts at 50 watts an output. After centrifugation at 30,000*g* for 45 minutes, the supernatant was dialyzed against two changes of tris-saline. The extracted S-100 was assayed by the microcomplement fixation test, using pure S-100 protein and its antiserum prepared by one of us (B.W.M.). Protein was determined (*6*) in the supernatant. Results from four pools were averaged and presented as mean ± S.D.

Days	Cyclic AMP (pmole/mg protein)		S-100 protein (ng/100 μg protein)	
	Control	Experimental	Control	Experimental
0	22 ± 2	22 ± 1	7.6 ± 0.1	7.5 ± 0.1
1	25 ± 3	24 ± 2	5.0 ± 0.1	5.2 ± 0.2
4	24 ± 2	70 ± 13*	6.0 ± 0.7	35.3 ± 0.8*
7	20 ± 5	66 ± 7*	5.4 ± 0.5	50.3 ± 1.2*

*Significantly different from controls, $P < .001$.

cent relative humidity. Feeding was carried out every other day with the medium described above.

The appearance of the glioblasts, which originally resembled an epithelial cell carpet, was transformed in 1 day by the factor into that of a cell net of interconnected, multipolar astrocytes. Prolonged culture of the cells in the presence of the factor led to further extension of the processes so that the picture of a crisscrossing network emerged between 4 and 7 days. Table 1 shows the concentration of adenosine 3′,5′-monophosphate (cyclic AMP) and S-100 protein in these cells. No change could be detected at the 1-day point, but considerable increases in these compounds were observed thereafter. At the 7-day point, the concentrations of cyclic AMP and S-100 in the experimental cells were three times and nine times, respectively, those in the control cells.

Cyclic AMP and the glia-specific protein S-100 (*4*) were chosen in this work as the chemical indicators of glioblast maturation. This study shows that although chemical differentiation does not precede or accompany the initial morphological change (at the 1-day point), where cell retraction is the main feature, it is correlated with the long-term morphological effect (4- and 7-day points), where process elongation is the major event. While the exact relationship between chemical and morphological differentiation in the glioblasts requires further investigation, the data reported here strongly suggest that they are parallel events triggered by the same maturation factor.

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Glial-Neural Interaction Demonstrated by the Injection of Na^+ and Li^+ into Cortical Glia

Abstract. *Injection of Na^+ or Li^+ into cortical glia evokes glial depolarization, discharge of adjacent neurons, and vascular pulsation. The effects can be explained by the extrusion of K^+ from glia after cation injection, glial swelling, and the slow removal of the cation from glia. The data suggest that the reduced rate of reuptake of K^+ into Na^+-loaded glia results in epileptiform firing of neurons, and support the hypothesis that glia function to buffer the environment of neurons.*

Neuronal discharge evokes depolarization of glia that is mediated by K^+ released from neurons (*1*). It has been suggested that during depolarization the glia take up K^+ to maintain the constancy of the neuronal environment by a process termed spatial buffering (*2*). The spatial buffering hypothesis predicts that generalized glial depolarization would interfere with glial uptake of K^+. It has also been proposed that disturbance of glial uptake of K^+ in areas of injury could be a contributing cause in the generation of epileptic neuronal discharge (*3*). These hypotheses were investigated by injecting Na^+ and Li^+ into glia with the objective of electrochemically displacing the intracellular K^+ and depolarizing the glia so that the effect on the activity of adjacent neurons could be studied.

Intracellular recordings in the motor cortex of cats anesthetized with pentobarbital were obtained with beveled micropipettes (tip resistances, 15 to 30 megohms) filled with 0.5 to 3*M* solutions of NaCl, LiCl, $LiCO_3$, or KCl. Glia were identified by a stable membrane potential of −95 to −70 mv, the presence of slow depolarizations of 2 to 4 mv during electrocortical spindle bursts, and the absence of spike and synaptic potentials on penetration (*4–6*). Iontophoretic injection of ions was carried out with current pulses of $0.1 - 1 \times 10^{-8}$ amp and 100 msec duration (*7–9*).

Single injections of Na^+ into glia with a 1×10^{-8} amp pulse (*10, 11*) evoked glial depolarizations of 7 to 30 mv in 90 percent of over 200 glia (Figs. 1 and 2). Repolarization had a duration of 3 to 20 seconds in different cells, and had an irregular course, marked by small depolarizing shifts of potential, quite different from the smoothly decaying repolarization that glia exhibit after neural activity evoked by thalamocortical stimulation (*4*). When two injections were made at intervals greater than 20 seconds, depolarizations of approximately equal amplitude were evoked. The rate of repolarization after the second injection was often more rapid than after the initial injection (Fig. 2B). However, if injections were made at shorter intervals the depolarizations were initially additive. A train of injections produced an incrementally increasing series of depolarizations for the first two to four pulses of the train, followed by a plateau of depolarization, and then repolarization despite continued injection (Fig. 1, third injection). Glial responses to Li^+ injection were similar to those evoked by Na^+. The depolarization was generally larger, and repolarization was more rapid. Repolarization was followed by hyperpolarization of 2 to 5 mv in some glia.

In Na^+- and Li^+-injected glia we recorded, through the glial membrane, the high-frequency discharge of neurons during the period of glial depolarization. Neuronal spike discharges, generally of low frequency, have occasionally been recorded in uninjected glia (*4, 12*). In contrast, an intense multiunit discharge composed of spikes of different amplitudes was detected, by means of auditory and oscilloscopic monitoring in all glia depolarized by cation injections. Subsequent injections evoked more intense and larger discharges than the initial injection (Fig. 2A). The spikes were as large as 20 mv in some cases and could be resolved by the ink-writing pen of the oscillograph. When the spikes were of large amplitude, the cessation of neural firing was often accompanied by an abrupt return of the glial membrane potential to preinjection levels (Fig. 2A, middle trace). This suggests that the glial depolarization recorded after cation injection may be in part mediated by K^+ or other substances released by active adjacent neurons.

Repetitive Na^+ and Li^+ injections also evoked a marked increase in the vascular pulsations that are recorded as small os-