Enzyme Induction in Immature Glia.* (29879)

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Among the properties of the newborn brain which appear to be quite different from those of adult is the lack of glial reaction to a penetrating wound(1). The present study is devoted to elucidating the factors which result in this distinctive unresponsiveness in the hope that such information might be useful in understanding the more general problem of control of glial proliferation.

Morphologic histochemical studies have demonstrated that a marked increase in activity of a number of oxidative enzymes occurs in reactive glia of the mature rat, rabbit and human(2,3,4). This phenomenon resembles inductive enzyme synthesis in other mammalian tissues(5), a situation in which cells synthesize increased quantities of certain enzymes following exposure to relatively simple organic molecules. Therefore a search for possible specific inducing factors responsible for this increase in reactive glia has been instituted. In contrast to the situation in adult animals previous work has indicated that the morphologic and histochemical features of reactive gliosis were not observed after a penetrating wound of the newborn rat brain. It was felt that the absence of reactive gliosis might be due to immaturity of the glial cell, absence of the inducing agent in immature tissue, ineffective liberation of the inducing agent, or some combination of the 3 factors. Since the appearance of myelin parallels the onset of the mature glial response to injury, it was suggested that the absence of certain substances found in, but not limited to, the myelin sheath might be the determining factor(1). This study will demonstrate that the glial cell of the newborn rat resembles the mature astrocyte in its ability to react, and that the absence of reactive gliosis may be related to 2 factors: low concentration of the inducing factor in newborn rat brain and ineffective liberation from tissue compartments.

Methods. Holtzman Sprague-Dawley rats of both sexes were employed. Within 24 hours of birth each animal received either an intracerebral implant by trocar or a cerebral wound made by a 1 mm blunt probe. The materials implanted were paraffin, newborn brain tissue, adult brain tissue, or adult brain tissue previously boiled in isotonic saline for 10 minutes. Animals were killed 5 and 10 days following the operation and the brain removed for examination. Twelve animals were employed in each group.

Following removal, 6 brains from each group were fixed in formalin-ammonium bromide for Cajal's astrocyte stain, and specimens from the remaining 6 brains were placed on block holders and quick frozen in pulverized dry ice. Sections were cut at 16 μ in a cryostat, mounted on coverslips, thawed at room temperature and air dried. The enzymatic histochemical procedures for demonstration of triphosphopyridine nucleotide (TPNH) and diphosphopyridine nucleotide (DPNH) diaphorases were carried out utilizing 2,2′-di-p-nitrophenyl-5,5′-diphenyl-(3,3′-dimethoxy-4,4′-biphenylene) ditetrazolium chloride (Nitro-BT), 0.5 mg/ml, and DPNH or TPNH 1.5 mg/ml made up in modified Kreb's solution buffered at pH 7.4. Control sections were incubated in media lacking the substrate. Following incubation for 10 minutes sections were fixed in buffered formalin, washed and either mounted in glycerine or dehydrated and mounted in Permount. Adjacent sections were formalin fixed and stained with H & E.

Results. Results are summarized in Table I. No increase of TPNH or DPNH diaphorase activity was observed in glial cells adjacent to the probe wound or paraffin implant (Fig. 1,2). Glial cells situated adjacent to implants of newborn brain tissue displayed a moderate increase in diaphorase activity, whereas a marked increase was associated

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with implants of adult brain (Fig. 3, 4). Prior boiling of the adult brain tissue implants did not lessen the response. The glial response demonstrated on Cajal stained sections by an increased number and thickness of processes paralleled the increase of enzyme activity observed in the reactive glia in each experimental group.

Discussion. These results demonstrate that the morphologic and enzymatic changes of reactive gliosis may be produced in the newborn rat by the proper stimulus. Thus, implantation of a small fragment of adult brain tissue will initiate a vigorous glial response,

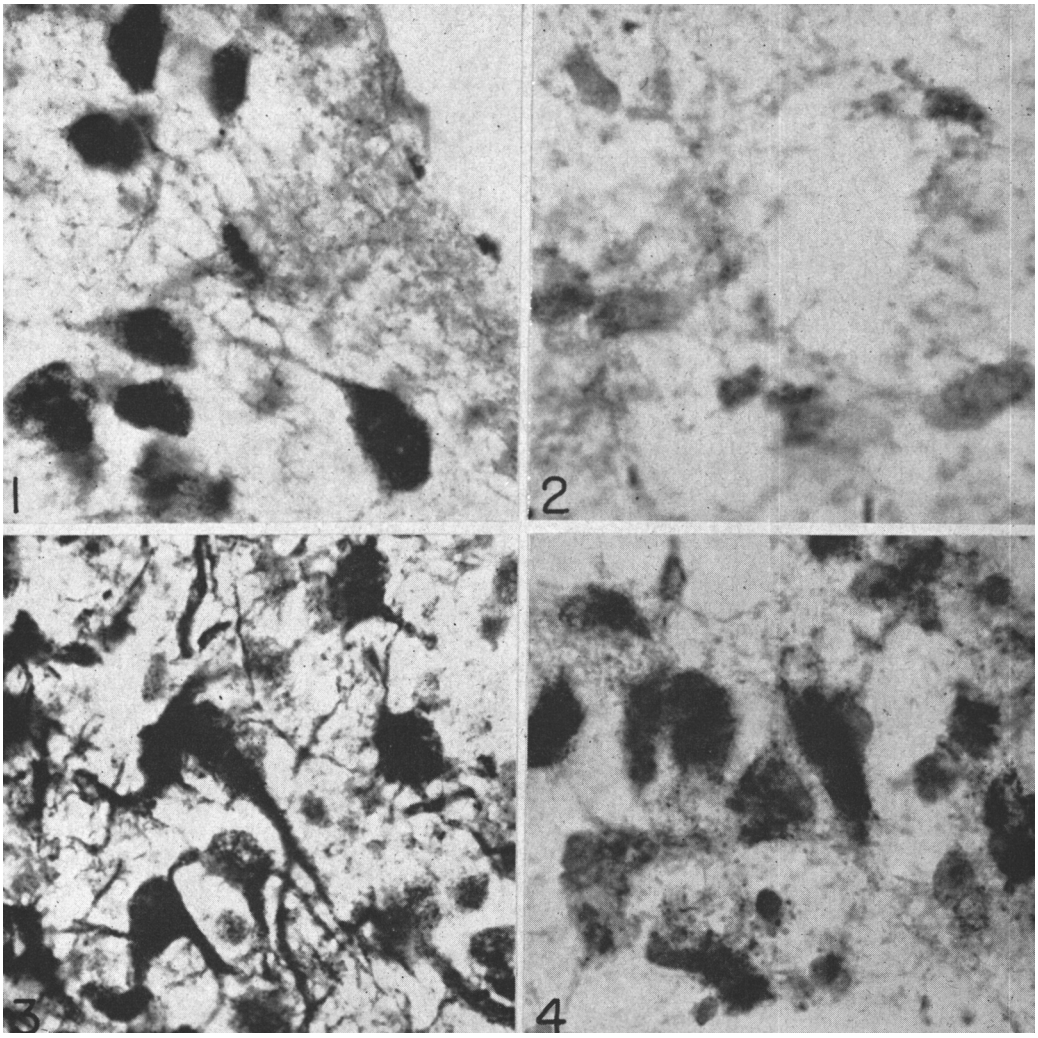

FIG. 1. Area immediately adjacent to a trocar wound in 10-day-old rat. Lesion created on day of birth. Astrocytic processes are slender and there is no evidence of glial reaction. Cajal's gold sublimate. × 1250.

FIG. 2. Area immediately adjacent to a trocar wound in 10-day-old rat. Lesion created on day of birth. DPNH diaphorase, safranin counterstain. Only very faint formazan deposits are contained in glial cells. × 1250.

FIG. 3. Area immediately adjacent to an implant of adult brain tissue in 10-day-old rat. Implanted on day of birth. Note prominent processes of reactive astrocytes. Cajal's gold sublimate. × 1250.

FIG. 4. Area immediately adjacent to an implant of adult brain tissue in 10-day-old rat. Implanted on day of birth. DPNH diaphorase, safranin counterstain. Heavy formazan deposits are contained within a number of reactive astrocytes. × 1250.

TABLE I. Glial Response to Various Stimuli Administered on Day of Birth.

Stimulus	Glial diaphorase activity* 5 days after operation TPNH	DPNH	Glial diaphorase activity* 10 days after operation TPNH	DPNH
None	1+	1+	1+	1+
Trocar wound	1+	1+	1+	1+
Paraffin implant	1+	1+	1+	1+
Newborn brain implant	2+	2+	2+	2+
Adult brain implant	4+	3+	4+	3+
Boiled adult brain	4+	3+	4+	3+

* Enzyme activity estimated by formazan deposition after 10 min. incubation: 4+ maximum deposition, deep blue; 3+ to 1+ progressively lighter blue.

while an implant of paraffin is without effect. The mild but definite response to implants of non-viable newborn brain tissue suggests that the inducing agent is present at a lower concentration, but seems at variance with the lack of response to a simple penetrating wound. Other work, however, has demonstrated that very little tissue necrosis results from a penetrating wound of the newborn brain. Therefore the two situations are not comparable, since a relatively large volume of necrotic tissue is present following implantation of newborn brain tissue. It would seem that the postulated inducing agent is present in newborn brain tissue, and that the failure to incite reactive gliosis may in part be a consequence of failure to liberate the inducer from inactive tissue compartments *via* the mechanism of necrosis.

It is of interest that prior boiling of implanted adult brain tissue does not lessen the inductive effect. The heat stability of the inducing factor excludes certain heat labile compounds in the search for the effective agent, and also excludes the possibility that the reactive astrocytes observed originate from within the implant.

Conclusion. This study clearly demonstrates that astrocytes of newborn rat brain display the morphologic and enzymatic changes of reactive gliosis in response to the appropriate stimulus. The absence of newborn glial response to simple penetrating wounds may be a result of ineffective liberation of an inducing agent present at low concentration.

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Hexosamine-Synthesizing Enzyme in Human Arterial Tissue.* (29880)

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The hexosamine-synthesizing enzyme which was first discovered by Leloir and Cardini(1) in *Neurospora crassa* has subsequently been demonstrated in some mammalian tissues, whereas several tissues do not display any activity(2,3). The presence of a clearly measurable hexosamine synthetase activity in the aorta of young rabbits was observed by Bolognani *et al*(4). This enzyme is an aminotransferase which catalyzes the following reaction: Glucose-6-phosphate + L-Glutamine ⇄ Glucosamine-6-phosphate + Glutamic acid.

Because this enzyme is involved in the biosynthesis of mucopolysaccharides it was considered of importance to study its activity in human arterial tissue. For comparative purposes, a limited number of venous samples was included in the investigation. In addition, enzyme assays were made separately on normal and arteriosclerotic arterial tissue.

Materials and methods. Vascular samples

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