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The Age of Dependency of Enzymes in Reactive Glia.* (28302)

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Morphologic histochemical studies have indicated that a marked and sustained increase in activity of a number of oxidative enzymes occurs in reactive glia(1-3). Since this phenomenon resembles adaptive enzyme synthesis in bacteria(4) and inductive enzyme synthesis in mammalian tissues(5,6), a search for possible inducing factors responsible for this increase in enzyme activity appeared warranted. Previous work has shown that during central Wallerian degeneration gliosis occurs despite an unaltered blood-spinal cord barrier, and suggested that substances inducing gliosis or their precursors may pre-exist in normal central nervous system tissue(7). Because major alterations in the composition of central nervous system tissue are known to occur during development(8), examination of glial reaction at various stages of development appeared profitable. This study will demonstrate that reactive gliosis is strongly dependent on factors associated with maturation and is indeed absent in the newborn animal.

Methods. Holtzman Sprague-Dawley rats were employed. Animals were divided into 2 experimental groups:

Group A. Animals 0, 5, 10, 15, 20, 25, 30, 40, 50 and 70 days old were utilized as well as a control group of adult animals 150-180 days old. A total of 132 animals were employed. In each age group 6 animals were sacrificed and served as normal controls, while in 6 other animals the spinal cord was transected immediately above the lumbar enlargement. Operated animals were killed 96 hours following cord transection and the cervical cord was removed for examination. Experimental animals and their controls were of the identical age at time of autopsy.

Group B. A total of 66 animals of the same ages as in Group A were employed. In each age group 6 animals received intracerebral implants of paraffin introduced by trocar. Animals were killed 96 hours after operation and the brain removed for examination. In each case the undamaged cerebral hemisphere served as a control.

Following removal, specimens 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 procedure for demonstration of triphosphopyridine nucleotide

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TABLE I. Age and TPNH Diaphorase Activity of Reactive Glia in Wallerian Degeneration.

Age in days*	Posterior column Myelin content†	Glial TPNH diaphorase‡ Normal posterior column	Wallerian degeneration
0	0+	1+	–
5	0+	1+	1+
10	1+	2+	2+
15	3+	3+	3+
20	5+	4+	4+
25	7+	4+	4+
30	8+	4+	4+
40	9+	2+	4+
50	10+	2+	4+
70	11+	1+	4+
150–180	11+	1+	4+

* Age refers to age at time of sacrifice.
† Myelin content estimated by Luxol Fast Blue stained sections: 0, no demonstrable myelin; 1+ to 11+ progressively thicker myelin sheath.
‡ Enzyme activity estimated by formazan deposition after 10 min incubation. 5+ (maximum formazan deposition), very deep blue; 4+ to 1+ progressively lighter blue.

(TPNH) diaphorase was carried out utilizing 2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-(3, 3'-dimethoxy-4,4' biphenylene) ditetrazolium chloride (Nitro-BT), 0.5 mg/ml, and TPNH 1.5 mg/ml made up in modified Krebs 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 luxol fast blue or H & E. Studies of the spinal cord were performed on the posterior white column since in this region a relatively pure selection of degenerating fibers is sharply demarcated from adjacent tissue.

Results following cord section are summarized in Table I. Low TPNH diaphorase activity is demonstrated in the glial cells of the normal posterior column in newborn animals. With the onset of myelination, at about 10 days, there is a sustained increase in TPNH diaphorase activity which persists until 50-70 days of age(9). At the completion of myelination diaphorase activity decreases to levels comparable to the newborn animal. Prior to 10 days of age there is no demonstrable increase in glial TPNH diaphorase activity within the area of Wallerian degeneration (Fig. 1), while in mature animals a marked response can be demonstrated (Fig. 2). During the period of active myelination glial cells in the area of Wallerian degeneration display a level of enzyme activity comparable to the physiologically high level of activity seen in the normal. Thus, prior to 40 days of age any increase in TPNH diaphorase activity which might be anticipated as secondary to Wallerian degeneration is obscured by the pre-existing level of activity.

Results following intracerebral implantation of paraffin pellets are summarized in Table II. Alterations of glial TPNH diaphorase activity in the developing cerebrum are more easily observed since relatively large areas are myelin-poor, and thus glial cells in such areas do not display the physiological increase in activity expected during myelination. No increase in enzyme activity or morphologic evidence of glial reaction was seen adjacent to the implant in animals operated on at 10 days of age or earlier (Fig. 3). A definite increase in glial TPNH diaphorase activity was observed in animals that received intracerebral implants at 15 days of age, and a marked response was seen in the reactive glia of older animals (Fig. 4).

Discussion. These results clearly demonstrate that the morphologic and enzymic alterations of early gliosis are not produced by the usual stimuli in the newborn rat. Al-

TABLE II. Age and TPNH Diaphorase Activity of Reactive Glia in Rat Cerebrum.

Age in days†	Glial TPNH diaphorase* Normal glial cell	Reactive glia adjacent to paraffin implant
0	1+	1+
5	1+	1+
10	1+	1+
15	1+	2+
20	1+	4+
25	1+	4+
30	1+	4+
40	1+	4+
50	1+	4+
70	1+	4+
150–180	1+	4+

* Enzyme activity estimated as in Table I.
† Age at time of operation.

ternative explanations which are immediately apparent are an inability of the immature glial cell to respond, an absence of the postulated inducing substances, or some combination of the two factors.

On the basis of this work alone it is not possible to choose between these alternative hypotheses. However, when considered in the light of the following observations one alternative becomes attractive. Marked alterations in rat cerebral composition occur with onset of myelination, at 10 days of age(8).

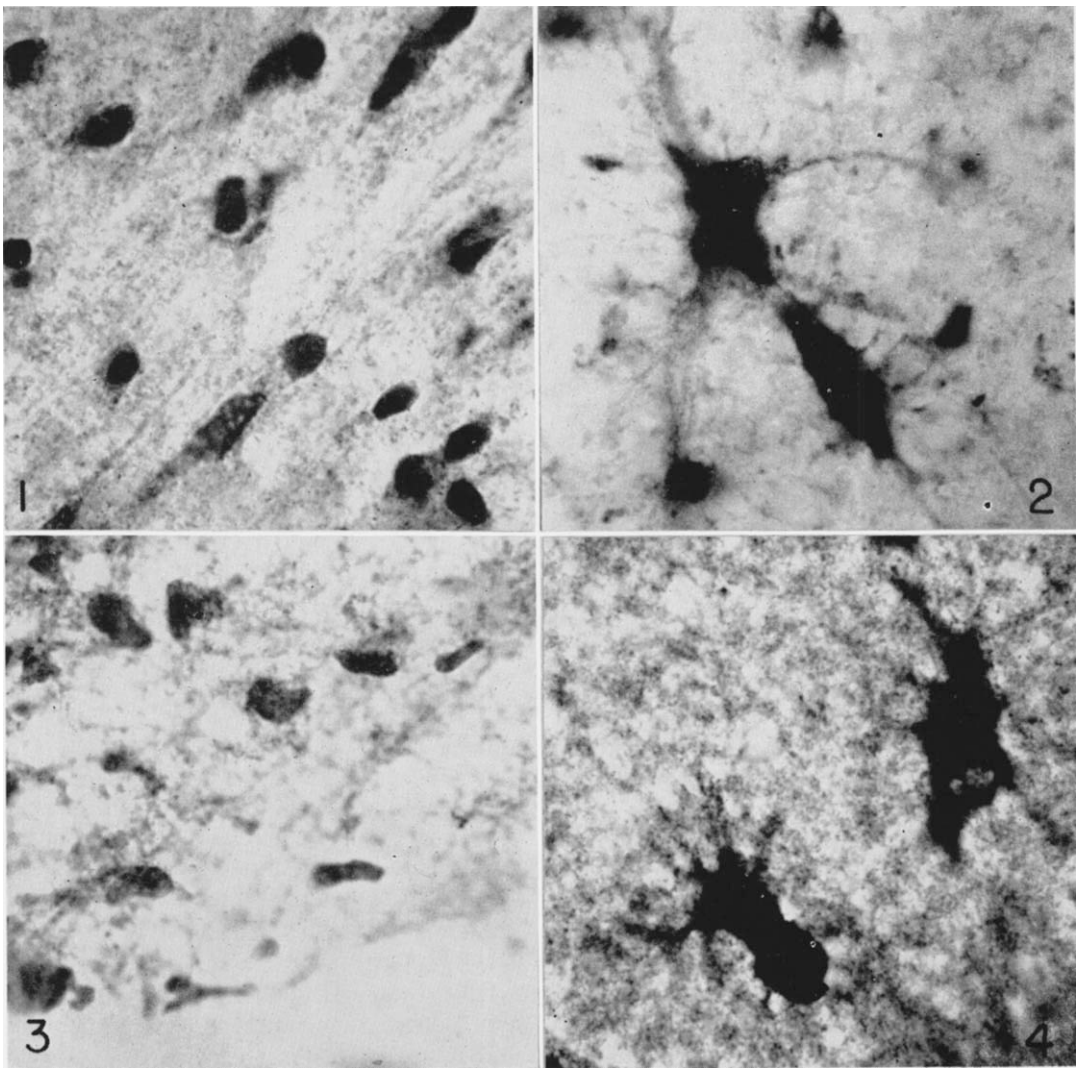

FIG. 1. Posterior column of 5-day-old rat spinal cord in area of Wallerian degeneration. Cord was transected at one day of age. TPNH diaphorase, safranin counterstain. Formazan deposits appear black or darker grey than background. Note faint formazan deposits in glial cells. ×1250.

FIG. 2. Posterior column of adult rat spinal cord in area of Wallerian degeneration 96 hr after cord section. TPNH diaphorase, safranin counterstain. Heavy formazan deposits are contained within 2 reactive glial cells. ×1250.

FIG. 3. Area immediately adjacent to cerebral paraffin implant in 4-day-old rat. Paraffin was implanted on day of birth. TPNH diaphorase, safranin counterstain. Only very faint formazan deposits are contained in glial cells. ×1250.

FIG. 4. Area immediately adjacent to cerebral paraffin implant in adult rat. Paraffin was implanted 4 days prior to sacrifice. TPNH diaphorase, safranin counterstain. Heavy formazan deposits are contained in 2 reactive glial cells. ×1250.

That one observes no glial reaction prior to 10 days of age suggests that the absence of reactive gliosis in the immature animal may be secondary to the absence of specific inducing agents rather than simple glial immaturity. In adult rats reactive gliosis occurs early in Wallerian degeneration, a situation in which components of the axone and myelin sheath alone are liberated. Reactive gliosis is also seen in human multiple sclerotic plaques and following cerebral-spinal fluid exchange in the cat(9), two lesions in which the myelin sheath alone is destroyed. Thus it is suggested that some component contained in, but not limited to, the myelin sheath may be implicated as the inducing factor responsible for reactive gliosis.

Conclusions. Studies of experimental central nervous system lesions in the immature rat indicate that the morphologic and enzymic alterations of reactive gliosis are not observed prior to 10 days of age. The absence of reactive gliosis in the newborn animal may be due to immaturity of glial cells, absence of specific inducing agents, or some combination of the two factors. It is suggested that absence of certain inducing substances found in, but not limited to, the myelin sheath may be the determining factor.

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Immunologically Privileged Sites in Studies of Polyoma Tumor Antigens. (28303)

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The production of tumors by cells inoculated into appropriate animals is the only universally accepted criterion of oncogenic transformation. Yet, in the case of studies on *in vitro* transformation by tumor viruses, a serious limiting factor in showing the oncogenic properties of the transformed cells is their immunological rejection by the inoculated host as the animal reacts to the "foreign" cell antigen contained in the cells(1,2). To overcome this resistance, usually large numbers of cells must be inoculated and some procedure used, such as whole body X-irradiation or cortisone therapy, to reduce the host's immunological competence. The same limiting factors also reduce the sensitivity of biological tests for antigens in these virus-induced tumors where animals immunized with viral or tumor materials are tested for resistance to tumor challenge, since large numbers of cells frequently must be used to produce tumors in the control animals.

It has been well established that certain organs represent "immunologically privileged" sites in the sense that homografts and even heterografts are either not rejected at all or are rejected less efficiently when the "foreign" cells are inoculated into these areas. The brain, anterior chamber of the eye, the testes, and, in the case of the hamster, the cheek pouch have been shown to be such immunologically privileged sites. The purpose of the experiments reported here was to find whether the use of these areas could increase