

GLUTAMINE SYNTHETASE IN THE NORMAL AND DYSTROPHIC MOUSE RETINA

MATTHEW M. LAVAIL and LIANE REIF-LEHRER. From the Department of Neuropathology and the Howe Laboratory of Ophthalmology, Harvard Medical School, Boston, Massachusetts 02115

Development of glutamine synthetase (GS) activity in the chick embryo retina has proved to be a useful system for studying control mechanisms in animal cells (6, 12, 2, 21). The enzyme activity rises rapidly late in embryonic development (16) and can be induced prematurely both in culture and *in ovo* by a variety of steroids (10, 11, 7). Piddington and Moscona (9) have shown that the time of GS development coincides with structural maturation in the chick retina; in addition, they have reported that steroids which induce GS activity in the retina concomitantly induce these morphological changes. An obvious implication is

the possible existence of a connection between the appearance of GS and the morphological changes.

To investigate this possibility and to attempt to determine the role of GS in the developing retina, it would be advantageous to localize the enzyme within the retina. At present, however, there is no histochemical technique for identifying the site of GS activity in tissues. Utilizing a partially inbred strain of mice with inherited retinal degeneration and normal littermate controls, we have now determined that GS probably is distributed throughout the retina. We have also shown that the parallel between GS development and final

structural and functional maturation in the retina exists in the mouse.

MATERIALS AND METHODS

Animals of postnatal ages ranging from 1 to 30 days were used in this study. Mice homozygous for the fully penetrant, autosomal recessive gene, retinal degeneration (*rd/rd*) and their normal littermates (*+ /rd*)¹ were decapitated and their eyes were removed. Whenever possible, at least three animals of each genotype were examined per age group. The neural retinas were dissected away from the pigment epithelium which remained with the choroid and sclera as previously described (4) except that no bathing medium was employed. One or one and a half retinas from each animal were immediately frozen and stored for enzyme assay. The remaining tissue from the eyes of each animal was fixed in a glutaraldehyde-formaldehyde mixture, postfixed in osmium tetroxide, and embedded in an Epon-Araldite medium; semithin sections of each specimen were then examined with the light microscope. In a few cases, whole eyes were prepared for histological examination without removing the retinas.

Retinas were placed in 1.5 ml of H₂O and allowed to lyse at room temperature for approximately 30 min.² Samples of the lysate (0.25–1.0 ml) were used to assay for GS activity by a modification (13) of the procedure of Thorndike and Reif-Lehrer (20). Results are given as optical density (OD) at 500 nm, either per mg protein (specific enzyme activity) or per retina, as indicated. Further samples of this same lysate (0.1, 0.2, or 0.4 ml) were used to determine protein content by the method of Lowry et al. (5). These protein values were then used to calculate specific enzyme activities as well as mg protein per retina.

RESULTS AND DISCUSSION

Retinas of both the dystrophic and control animals appear to develop normally until about the tenth postnatal day when cell migration and histogenesis are complete and when photoreceptor outer segments are beginning to form (Fig. 1, a and b,

¹ Inherited retinal degeneration (*rd/rd*) occurs in various strains of laboratory mice. "Dystrophic" is used for lack of a more descriptive term, but the pathology is evident before the retina is fully differentiated (19). Animals used in the present study are congenic with C57BL/6J. R. L. Sidman. 1968. *Mouse News Letter*. 39:24. They will be more fully described in a subsequent report.

² GS in the chick embryo retina appears to be stable for at least 72 hr at 37°C under culture conditions (14).

arrows). In the normal animals, outer segment elongation continues up to about the 20th day (Fig. 1, c and e). In the diseased animals, however, there is a progressive loss of receptor cells such that by the 20th day only a single row of photoreceptor nuclei is present (Fig. 1 d and f), while the rest of the retina remains intact.

The protein content of the normal retinas remains more or less constant after about the 12th day (Fig. 2 A). In contrast, the dystrophic retinas display a rapid loss of protein which not only parallels the loss of receptor cells but is in agreement with crude measurements of the decrease in thickness of the diseased retinas compared to their normal counterparts at late stages (e.g., compare Fig. 1, e with f).

The developmental pattern of GS activity in the normal and dystrophic mouse retinas is illustrated in Fig 2 B. In both cases, little, if any, enzyme activity is seen before day 9. In the subsequent 5 days, both types of retinas exhibit a 15-fold rise in activity. After about day 14, GS activity in the normal retina continues to rise at the same rate for about a week. The enzyme activity in the dystrophic retinas may also rise slightly, but is essentially constant for the remainder of the period studied.

It seemed that a comparative study of GS activity in the normal and diseased retinas at a time when the photoreceptors in the latter have completely degenerated would reveal whether or not the enzyme was in fact localized in the photoreceptor cells. Since we have shown that a large difference in protein content would exist between the two types of retinas at such an age, it was pertinent to compare specific enzyme activities, i.e., OD 500 nm per mg protein.

Several possibilities exist: (a) If GS activity were confined to receptor cells, specific activity in dystrophic retinas would decrease as the visual cells were lost, and the ratio of the specific activities of control/dystrophic retinas would become greater than 1. (b) If receptor cells contained no GS, then the specific activity in the diseased retinas would be expected to rise more rapidly as receptor cells are lost than that in control retinas, and the above ratio would drop below 1. (c) If the distribution of GS in the retina is such that its concentration in the visual cells is equal to that in the rest of the retina, the specific activity should be the same in diseased and control retinas at every age and the aforementioned ratio would equal 1.

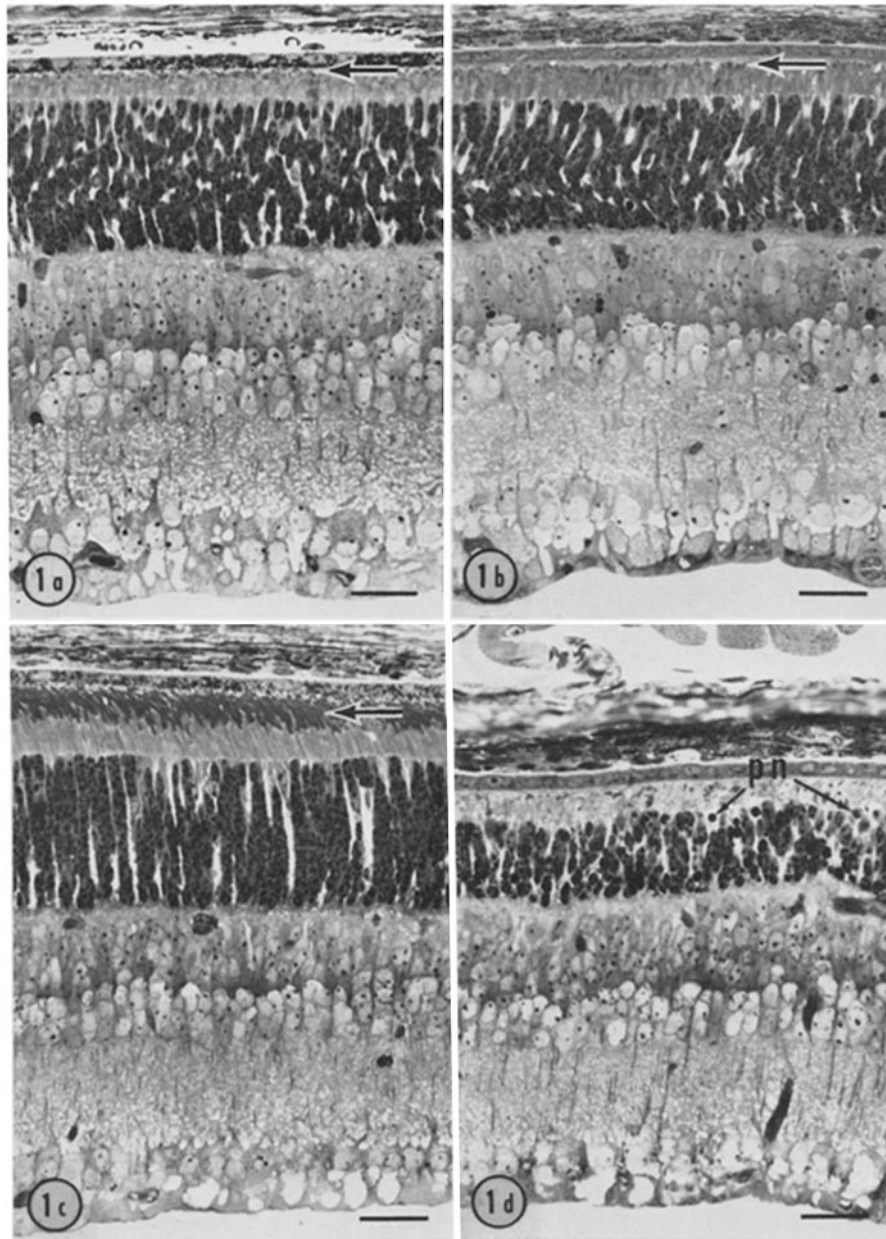
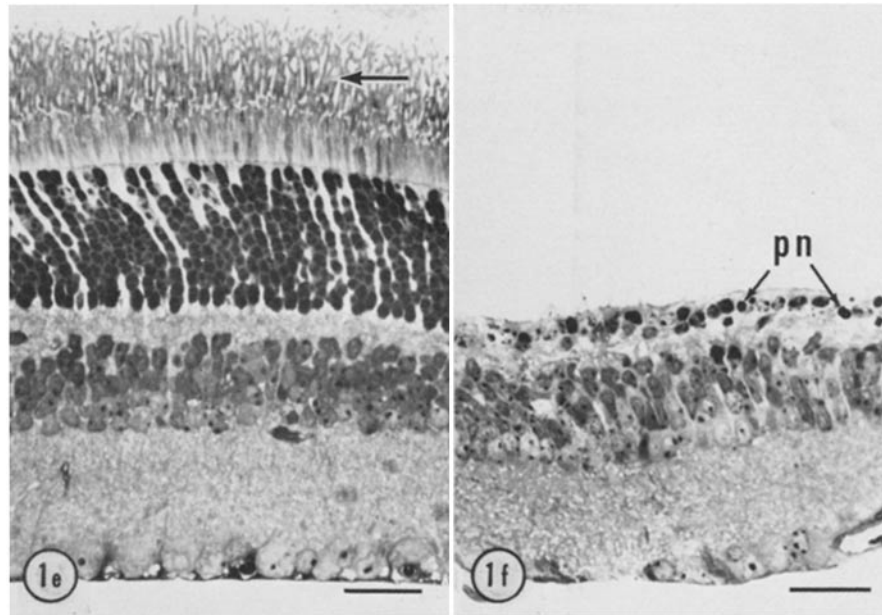


FIGURE 1 Micrographs of normal and dystrophic retinas from littermate pairs of mice of different ages. 1a and 1b, postnatal day 10. Normal (1a) and dystrophic (1b) retinas appear identical. Outer segments of photoreceptor cells are just beginning to develop (arrows). 1c and 1d, postnatal day 13. Rod outer segments in the normal retina (1c, arrow) have grown appreciably, whereas in the dystrophic retina (1d,) developing outer segments are not evident, and the visual cell layer is reduced to four to five rows of photoreceptor cell nuclei, some of which are pyknotic (*pn*). 1e and 1f, postnatal day 20. Outer segments in the normal retina (1e, arrow) have reached their adult length. In the dystrophic retina (1f) the visual cell layer is virtually absent; only a single row of pyknotic nuclei (*pn*) remains. (1a-1d), intact retinas are illustrated. 1e and 1f, retinas are shown removed from the pigment epithelium as in preparation for enzyme assay. Toluidine blue. Bars represent 25μ . $\times 355$.



FIGURES 1e-1f See legend under Figs. 1a-1d. Bars represent 25μ . $\times 430$.

TABLE I
A Comparison of Normal and Dystrophic Mouse Retinas at Postnatal Day 18

	Wet weight	Protein	OD	GS specific activity*
	mg	μ g	500 nm	
Normal (+/rd)†	2.4 ± 0.4	362 ± 31	1.87 ± 0.21	5.2 ± 0.4
Dystrophic (rd/rd)§	1.4 ± 0.2	192 ± 11	1.04 ± 0.19	5.5 ± 1.0
Ratio, normal/dystrophic	1.7	1.9	1.8	0.95

All values are calculated on a per retina basis.

* Glutamine synthetase specific activity = OD_{500nm}/mg protein.

† Average of eleven retinas.

§ Average of nine retinas.

Uniform distribution of GS throughout the retina would be a special case of this type. (d) If there exists any nonuniform distribution of GS in the retina which, however, does not fulfill the equality requirement in case c, one might expect to obtain results which differ, but not dramatically, from those in c. This would be the most difficult case to interpret.

Table I shows the results of an experiment in which the normal and dystrophic retinas from two litters of 18-day-old mice were analyzed. It can be seen that the ratios of normal to dystrophic retinas with respect to wet weight, protein content, and enzyme activity are about 1.8 and are in close agreement with each other as well as with the morphological findings presented in Fig. 1,

e and f. The ratio of specific activities is very close to 1, and these data, therefore, most closely fit case c above; that is, that GS is present both in the photoreceptor cells and in the rest of the retina. Rudnick (15) found a similar distribution of GS in the chick embryo retinas, despite considerable experimental variability, by assaying dissected frozen sections of the tissue.

Fig. 2 C shows the developmental pattern of specific enzyme activities in normal and dystrophic retinas. The ratios of the values at each age are very close to unity and again support the conclusion stated above.

Another possible interpretation is that GS exists entirely in one or more of the cells of the inner

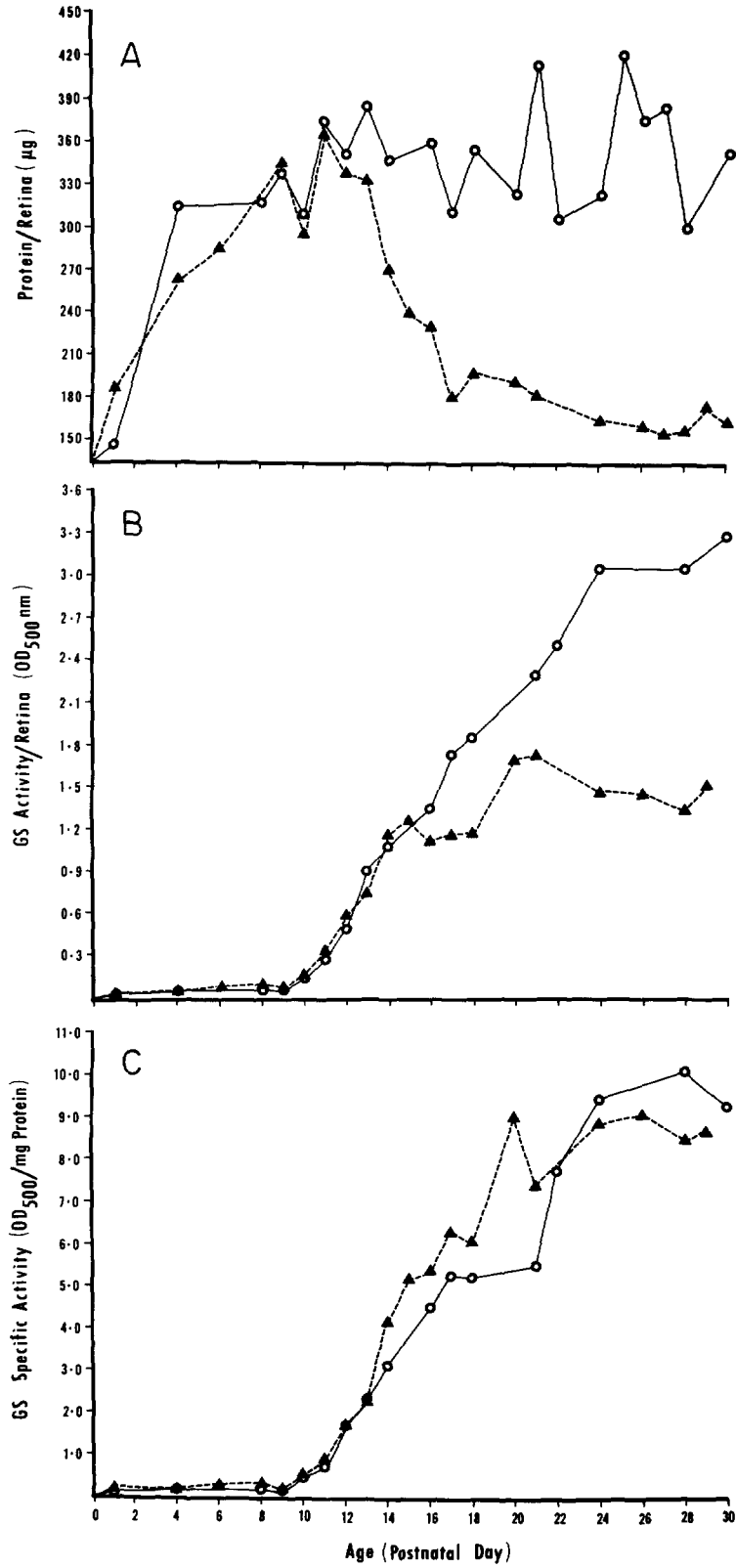


FIGURE 2 (A) Protein content, (B) glutamine synthetase activity, and (C) specific glutamine synthetase activity in normal (open circles) and dystrophic (solid triangles) mouse retinas as a function of age. Each point is the average of data available at that time point and is frequently five to ten retinas between days 10 and 18.

retinal layers and is not present in the photoreceptor cells. This, however, seems less likely; to be consistent with the data, it would require the decay of GS activity in the inner cells to occur at the same time and rate as the loss of protein due to degeneration of the photoreceptor cells.

GS activity in the chick retina rises rapidly around the 17th day of embryonic development (2) and coincides with final stages of photoreceptor cell differentiation (4). We have demonstrated that in the developing mouse retina a similar but less sharp rise in GS activity occurs also concomitant with functional maturation of the tissue.³ In the retinas of both species, final synaptogenesis (17, 8), the appearance of electrical activity (22, 3), and the formation of photoreceptor outer segments (1, 8) occur during the time of the initial rapid increase of GS activity in the tissue. It seems unlikely that the sharp rise in enzyme activity in the mouse retina between days 10 and 15 (Fig. 2 B) is dependent upon outer segment production, since the rise is essentially the same in both normal and dystrophic retinas. In the latter case, no mature outer segments are formed, and indeed there is a marked degeneration of the visual cell layer.

Since the genome of the mouse is better defined than that of any other vertebrate (18), and since mutants are readily available, the mouse retina may prove to be uniquely profitable for study of the function and genetic control of GS as well as other enzymes.

SUMMARY

In the retinas of normal mice and of littermates with inherited retinal degeneration, glutamine synthetase activity increases to more than 15 times the basal level between postnatal days 10 and 14. Beyond this age, the two types of retinas differ in morphology, protein content, and enzyme activity. A comparison of the normal and dystrophic retinas at day 18 indicates that glutamine synthetase is not confined to the photoreceptors and probably is distributed throughout the retina.

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³ A similar parallel between the development of GS activity and structural maturation has been seen in the normal rat retina. G. J. Chader, personal communication.

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