CORRESPONDENCE BETWEEN GLUTAMINE SYNTHETASE ACTIVITY AND DIFFERENTIATION IN THE EMBRYONIC RETINA *IN SITU* AND IN CULTURE

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INTRODUCTION

The development of glutamine synthetase (GS) in the neural retina of chick embryos has recently been employed in studies on the control of specific biosynthesis in differentiating cells (Kirk and Moscona, 1963; Moscona and Kirk, 1965). Retinal GS activity is characterized by a sharp increase that begins about the 16th day of embryonic development, continues until after hatching, and coincides with the general period of functional maturation of the retina (Rudnick and Waelsch, 1955; Rudnick, 1959; Lindeman, 1947). This rapid increase in GS activity can be reproduced *in vitro*, in organ cultures of retina from 16-day embryos (Moscona and Hubby, 1963; Kirk and Moscona, 1963; Moscona and Kirk, 1965); furthermore, it can be elicited precociously in cultures of retinas from early embryos, resulting in the premature enhancement of this aspect of retinal differentiation (Moscona and Kirk, 1965). These findings made it of interest to determine in further detail whether increases in retinal GS activity, in the embryo and in culture, coincided with other changes characteristic of differentiation in the retina. Since, in the embryo, histological development progresses differently in different areas of the retina (Weysse and Burgess, 1906; Coulombre, 1955; Shen *et al.*, 1956), we looked for differences in the distribution of GS activities and for possible topographical and chronological correspondences between enzymatic and cytologic changes. The distribution patterns of GS activity were mapped throughout retinas from chick embryos, chicks, and young adults; these maps were compared with regional differences in the structural maturation of retinal photoreceptor cells, as indicated by the development of the paraboloid bodies in the visual processes. In addition, the appearance of these structures was examined in retinal cultures with precociously elicited high GS activities to determine whether histological development was also enhanced.

MATERIALS AND METHODS

Neural retinas were obtained from White Leghorn chick embryos, 8th day to hatching, and at various intervals thereafter. The eyes were removed intact into sterile Tyrode's solution and cut through he optical axis into quarters; the nasal and temporal halves were separated through the middle of the fibrous band on the lower temporal surface of the eyeball (Fig. 1) and each half was bisected along the optical axis into upper and lower quarters. The pecten was cut out and discarded since it contains little or no GS activity (Rudnick, 1959). Each quadrant of neural retina was then detached from the pigmented epithelium,[1] lifted out, separated from vitreous humor, and divided into four sectors (Fig. 2). Thus, the retinas could be consistently subdivided into 16 sectors of known original position for precise mapping of structural and enzymatic changes *in situ* and following cultivation *in vitro*.

For organ culture, sectors of retina from 12-day-old embryos were either floated on rafts of siliconized lens

[1] In eyes of 17- to 20-day embryos these layers adhere firmly to each other in the equatorial area.

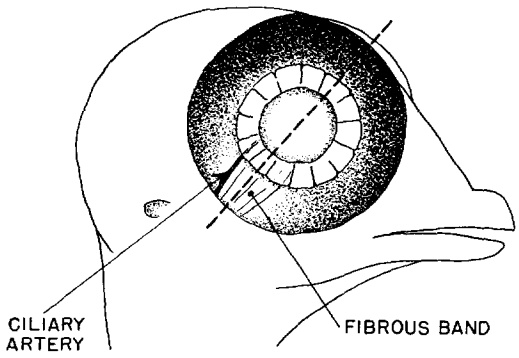


FIGURE 1 The plane (broken line) of separation of the chick embryo eye into temporal and nasal halves.

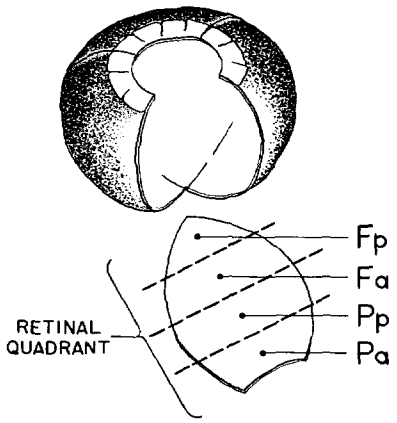


FIGURE 2 Subdivision of a retinal quadrant into pupillary (P), fundic (F), anterior (a), and posterior (p) sectors.

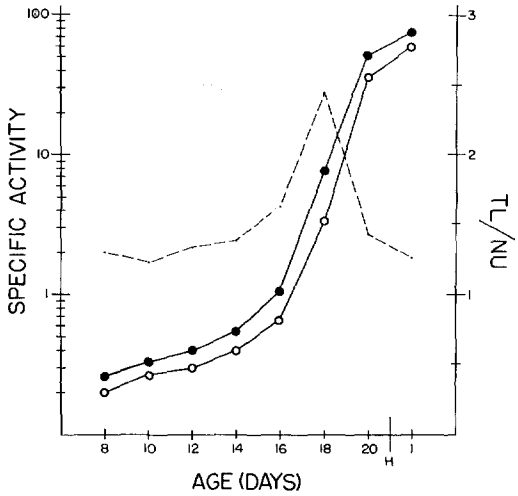
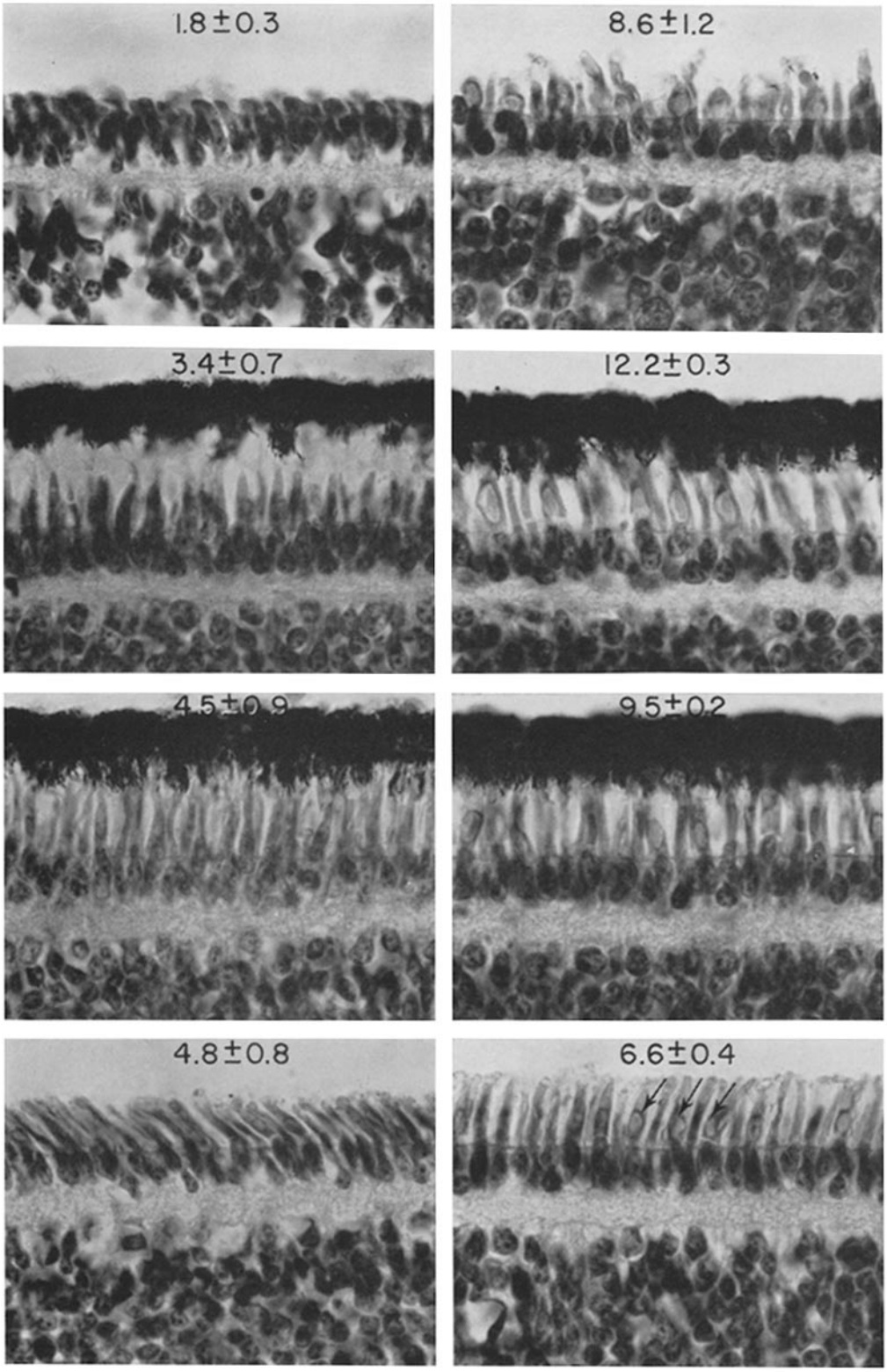


FIGURE 3 Retinal GS activity in the lower temporal (TL) quadrant (closed circles) and upper nasal (NU) quadrant (open circles) during the embryonic period. Differences between the means are statistically significant ($P \leq 0.01$) from the 10th day to hatching. Broken line represents the ratio of TL to NU values and indicates the relative increase of GS activity in the "high" (TL) quadrant at each age examined.

paper over 3 ml of liquid culture medium in embryological watch glasses, or placed directly on culture medium gelled with 1 per cent agar (these explants were more suitable for histological examination). The culture medium (Kirk and Moscona, 1963) consisted of 10 per cent horse serum (sterile, unfiltered) in Tyrode's solution, with penicillin-streptomycin; it was changed every 48 hours. All cultures were gassed

FIGURE 4 Cross-sections through retinal sectors from the "low" (NU) and the "high" (TL) quadrants of an 18-day chick embryo, showing differences in photoreceptor development and corresponding GS specific activities (noted on each illustration). The pigmented epithelium adheres firmly in the equatorial areas of the 18-day retina (Figs. *b*, *c*, *f*, *g*). Stained with hematoxylin and Biebrich scarlet. Explanation of symbols: N, nasal, T, temporal half; U, upper, L, lower quarter; P, pupillary, F, fundic eighth; a, anterior, p, posterior sixteenth. × 600.

(*a*) NUPa sector. Short visual cell processes.
(*b*) NUPp sector.
(*c*) NUFa sector.
(*d*) NUFp sector.
(*e*) TLFp sector. Increase in size of paraboloid bodies (arrows).
(*f*) TLFa sector.
(*g*) TLPp sector. Largest paraboloids.
(*h*) TLPa sector.


1.8±0.3
8.6±1.2
3.4±0.7
12.2±0.3
4.5±0.9
9.5±0.2
4.8±0.8
6.6±0.4

with 5 per cent CO_2-air mixture and incubated at 37°C.

For enzyme assay, freshly dissected or cultured retinal sectors were washed in plastic tubes with cold Tyrode's solution, frozen, and lyophilized. The samples were suspended in 0.01 M phosphate buffer (pH 7.1), disrupted with ultrasound, and assayed for GS activity using the glutamotransferase reaction based on determination of glutamohydroxamic acid (GHA), according to the method of Rudnick, Mela, and Waelsch (1954) modified by Kirk and Moscona (1963; Kirk, 1965). Protein was determined according to Lowry *et al.* (1951). Specific GS activity was calculated as micromoles of GHA formed per hour, per milligram of protein.

For histological demonstrations of glycogen, sections of tissue fixed in Gendre's fixative (Rabinovitch *et al.*, 1954) at 4°C for 18 hours were stained by the periodic acid-Schiff method, using appropriate diastase-treated controls.

This report is based on enzyme assays and histological examinations of retinas from 96 embryos and 237 cultures.

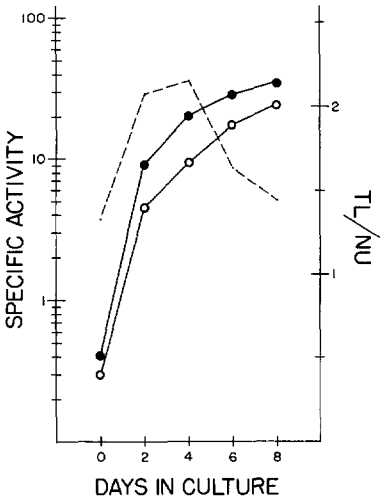


FIGURE 5 GS activity in the lower temporal (TL) quadrant (closed circles) and upper nasal (NU) quadrant (open circles) of organ-cultured retinas from 12-day embryos. Differences between the means are statistically significant ($P \leq 0.01$) through the 6th day of culture. Broken line represents the ratio of TL to NU values and indicates the relative increase of GS activity in the "high" (TL) quadrant at each age examined.

RESULTS

RETINAL GS ACTIVITY IN THE EMBRYO: In each of the retinal quadrants, GS activity increased at a slow rate to the 16th day of incubation, rose rapidly to hatching, and reached a plateau in the early posthatching period. The detailed GS activity patterns of the quadrants differed characteristically (Fig. 3). Throughout the embryonic period, GS activity was highest in the lower temporal (TL) quadrant and increased fastest there until the 18th day of development. The lowest activities were in the upper nasal (NU) quadrant, which also typified the activity patterns in the other "low" quadrants (lower nasal and upper temporal). More detailed localization, using sectors (sixteenths of whole retina) of retinal quadrants from 18-day embryos, showed that GS activities were lowest at the pupillary border in the NU ("low") quadrant, and highest between the

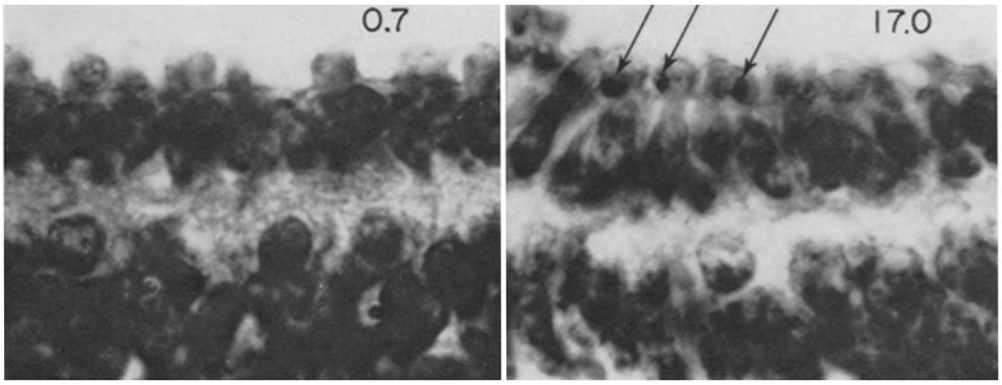


FIGURE 6 Cross-sections through part of the "high" (TLPp) sector of chick embryo retinas with corresponding GS specific activities (noted on each illustration); stained with PAS and hematoxylin. × 1000. (*a*) Normal retina of a 15-day embryo. (*b*) 12-day retina cultured for 3 days: notice glycogen-rich paraboloids (arrows).

pupillary border and the equator of the eye in the TL ("high") quadrant. Activities increased across the fundic region from the "low" to the "high" quadrant of retina.

Histological examination of 18-day embryonic retinas revealed a striking correspondence between the topographical distribution of GS activities and structural differentiation in the visual cells (Fig. 4). The visual processes were shortest in the sector with lowest GS activity and were most developed in the sector with peak activity. The paraboloid bodies were smallest or non-detectable in the area with lowest GS activity, and were strikingly largest and richest in glycogen in the sector with peak GS activity. The size of the paraboloids increased progressively across the fundic region of the retina, in topographical correspondence with the increasing gradient of GS activity.

In other stages of embryonic development there was a similar correlation between GS activity and the size of the paraboloid inclusions and visual processes. Similarly, in posthatching stages, after retinal GS activities had reached a plateau, the activities were generally lowest in the deep fundic regions of the retina where the paraboloids were smallest.

GS ACTIVITY IN ORGAN-CULTURED RETINAS: Retinal quadrants from 12-day embryos cultured on liquid medium were collected at 48-hour intervals and assayed for GS activity. In agreement with previous findings (Moscona and Hubby, 1963; Kirk and Moscona, 1963), GS activity in these retinal explants increased precociously, rapidly for 48 hours and at a slower rate thereafter. The rate of increase was initially fastest in the TL ("high") quadrant; after 96 hours it was more rapid in the other quadrants (Fig. 5).

This precocious increase in GS activity in the explanted 12-day retina was accompanied by cytological changes in the visual cells which normally appear in later phases of retinal differentiation. Histological examination of sectors cultured on gelled medium showed that in the sector with high GS activity (TLPp) numerous paraboloids with heavy deposits of glycogen were present after 3 days *in vitro*; *i.e.*, well in advance of their normal appearance *in situ* (Fig. 6). Elongation of outer segments of the visual processes could not be critically evaluated in cultured material because of distortion artifacts.

REMARKS

In the developing neural retina of the chick embryo, increases in GS activity were found to follow a characteristic pattern that coincided topographically and chronologically with other features of retinal development. GS activity increased fastest in sectors with photoreceptor cells undergoing advanced differentiation, and leveled off with the completion of their development. Similarly, in organ cultures of early embryonic retina in which a precocious increase in GS activity was elicited, there were also premature changes in the photoreceptor cells evidenced by the enhanced appearance of glycogen-rich paraboloids. A correspondence exists, therefore, between both the normal and the precociously enhanced increases in GS activity and the progression of other aspects of retinal differentiation. In establishing this, the present findings add to the interest in mechanisms that control the developmental pattern of this enzyme (Moscona and Kirk, 1965), and in its significance to the program of retinal differentiation and function. The exact nature of these relationships is unknown, at present. The restriction of the histological observations, reported here, to changes in the photoreceptor cells was motivated by their obviousness and does not imply confinement of retinal GS activity to this layer; Rudnick (1963) detected GS activity also in other layers of the retina. However, the detailed vertical distribution of GS activities in the different retinal layers, sectors, and cell types at different embryonic stages remains to be determined in correlation with other developmental changes.

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