DEVELOPMENT OF GLUTAMINE SYNTHETASE ACTIVITY IN CHICK EMBRYO RETINA CULTURES IN THE ABSENCE OF ADDED HYDROCORTISONE

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This report concerns an unexpected finding with respect to the development of glutamine synthetase (GS) activity in chick embryo retinas in culture. We have found that an appreciable rise in the enzyme activity can occur, under certain conditions, in the absence of any additives previously thought essential to the appearance of such activity.

In ovo, a large increase in GS activity appears in the chick embryo retina on about the 17th day of development (1). As has been previously reported (2,3) this enzyme activity can be prematurely elicited in cultured retinas by addition of appropriate factors to the maintenance medium. For example, calf serum (CS), horse serum or human serum added to the medium, stimulate an appreciable level of GS activity within 24 hours (2,4). In contrast, fetal calf serum, especially if dialyzed, does not usually elicit this rapid response, although some activity has previously been shown to appear in cultures kept for 3 or 4 days (3). However, the most dramatic effects are observed if one of a number of steroids is added to medium

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containing dialyzed fetal calf serum (dFCS) (5-8). In this case, enzyme activity increases rapidly and reaches a level equal to or greater than that attained in CS medium. Hydrocortisone (HC), the steroid commonly used to elicit GS activity has been shown to be active in the transcription step of this process (5,9). This report presents evidence that the HC, is in fact, not required for rise in GS activity but may perhaps merely accelerate a process which is already programmed to occur in the retina at an early age.

Methods and Materials

The technique for culturing the retinas and for the various assays has been reported in detail elsewhere (5). Dialyzed fetal calf serum was purchased from Grand Island Biological Company, Grand Island, New York. Such serum has been dialyzed for 48 hours at 4°C against 3 changes of ten times its volume of normal saline. The concentration of serum in the medium was 5% (v/v). Preincubated eggs were purchased from Cobb Farms, Littleton, Mass. or Spafas Farms, Norwich, Conn.

Results and Discussion

Retinas from 10 day old chick embryo left in culture in dFCS medium for longer than 4 days continue to respond to HC with development of a high level of GS activity within 24 hours after treatment. The unexpected finding has been that such 10 day retinas left in culture for 8 or 9 days developed a fairly high level of GS activity even in the absence of HC or

sera known to be active in this respect*. Moreover, at about this time the retinas generally reach a state in which 24 hour HC treatment led to only little (one to twofold) enhancement of enzyme activity level compared to the untreated controls. Interestingly, using embryos of various ages, if one assumes that 1 day in culture is equivalent to 1 day in ovo, this loss of effectiveness of HC seems to occur at about the time when the rise in enzyme activity normally occurs in ovo.

The specific enzyme activities developed by retinas from various age embryos cultured in dFCS medium unsupplemented by steroid, CS, etc. are given in Table I; also given in the table are the values for retinas treated with HC for 24 hours prior to harvest.

Piddington and Moscona have reported a study of 24 hour responses of retinas, in culture, to HC as a function of embryo age (11). In general agreement with his findings, retinas from 8 day embryos do not respond as well to HC in the first 24 hours in culture as do ones from 10 day, or older, embryos. It should be noted from Table I, however, that these younger retinas develop as high or higher GS activity as older retinas, after several days in culture. This is true both for their response to HC as well as their response in the absence of steroid.

It seems tempting to conclude that the sequence of events which finally leads to the appearance of GS activity are already preprogrammed in the retina prior to the 8th day of development. At this time, however, the machinery which makes

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*It should be noted that retinas cultured from the time of excision in Tyrode's solution, containing 10% horse serum, develop an increasing level of GS activity with time in culture (10) as would be expected in the presence of any "active" additive.

Table I GS ACTIVITY IN CHICK EMBRYO RETINAS IN CULTURE

Retina Explanted on Day	Total Days of Development[a]											
	9		10		11		12		13		14	
		+HC[b]		+HC		+HC		+HC		+HC		+HC
8[d]	0	0					6	14				
8[c]	0	6									9	31
10[d]					1	13						
12									3	33		
13											2	26
14[d]												
	15		16		18		19		20		22	
		+HC		+HC		+HC		+HC		+HC		+HC
8[d]			25				39	51			66	
8[c]					24	29						
10[d]			10				19	23				
12							32	62				
13			9	22			29	52				
14[d]	7	25			29	59					62	

Legend For Table I

Experiments were done several times with retinas from each age embryo. In addition, eggs from two different sources and at least two different lots of dFCS were used to test the generality of the phenomenon. The results in the table are for a typical experiment from each age group. Values within an experiment are averages of 2 to 5 retinas and are ($O.D._{500}$/mg protein) x 100.

a. Sum of days in ovo plus days in culture.
b. HC added 24 hours prior to harvest.
c. Because of the small size of 8 day retinas, 2 retinas per flask were used in this experiment.
d. 0.001 ug/ml HC; all other experiments are 0.01 ug/ml HC.

GS messenger-RNA (m-RNA) has not yet fully matured to the point where HC can effectively act to catalyze this process. One or two days subsequent (either in ovo or in culture), the mechanism has progressed to the point at which HC can perhaps now accelerate some rate-limiting step, (for example, one connected with m-RNA synthesis) or so stabilize m-RNA being made, that high levels of enzyme activity can develop rapidly. Left without HC, m-RNA perhaps accumulates slowly with gradual increase in enzyme activity over many days. A number of alternative explanations, for example, a possible non-specific loss of control mechanism due to longer times in culture, seem unlikely since the phenomenon occurs at about the same number of days of total development regardless of the time in culture. This and other factors will be further discussed in a future publication.

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