Glutamine Synthetase: Failure of Induction by Hydrocortisone at 27°C*

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Hydrocortisone addition to chick embryo retinal cells induces the formation of the enzyme glutamine synthetase (GS) (Moscona and Piddington, 1966; Reif-Lehrer and Amos, 1968). Induction requires the synthesis of the specific messenger ribonucleic acid (mRNA) and its translation to make the enzyme protein.

Despite respectable synthesis of protein and of RNA at 27°C compared to that observed at 37°C, very little induction of enzyme is detected at 27°C even when conditions otherwise favoring induction are maintained for as long as 72 hours.

The evidence at hand suggests that the specific mRNA is not synthesized at 27°C, though mRNA formed at 37°C can be translated at 27°C. Moreover, 24 or more hours at 27°C results in an "overshoot" of RNA synthesis when the temperature is raised to 37°C. Enzyme induction proceeds, protein synthesis reattains the 37°C rate after a delay of about eight hours, approximately two hours before the rate of RNA synthesis returns to what is normal at 37°C.

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MATERIALS AND METHODS

Whole retinas were explanted to flask cultures under conditions specified by Reif-Lehrer and Amos (1968). The enzyme activity was determined by the method of Waelsch (1955) with modifications specified previously (Reif-Lehrer and Amos, 1968). The protein was determined by the method of Lowry et al. (1951).

Sucrose (ribonuclease free) was obtained from Mann Research Labs, Inc., New York; actinomycin D, through the courtesy of Merck and Co., Rahway, N.J.; Hydrocortisone, Nutritional Biochemical Corp., Cleveland, Ohio; L-phenylalanine-C^{14} (sp. act. 300 mc/mM), uridine-2-C^{14} (sp. act. 44 mc/mM), and uridine-5-H^3 (sp. act. 20 c/mM), Schwarz Bio-Research, Orangeburg, N.Y.

RNA extraction and sucrose gradient separation were done according to the method of Scherrer et al. (1966). The extracted RNA was centrifuged at 24,000 rpm for seven hours at 4°C through a 5-20% linear sucrose gradient.

RESULTS AND DISCUSSION

When retinas provided with fetal calf serum (10%) and hydrocortisone (2 μg/ml) (minimal concentration for full induction is 0.02 μg/ml) are incubated at 27°C ± 1°C, enzyme induction is markedly curtailed (Table 1). Under these conditions protein synthesis estimated by the incorporation of ^{14}C-labeled amino acid in a 2-hour or 24-hour pulse is approximately 25-30% of that observed at 37°C. RNA synthesis estimated by incorporation of ^{14}C-uridine in 2-hour or 24-hour pulses is also between 21 and 38% of the rate measured at 37°C (Table 2). The curtailment of production of enzyme

Table 1

Enzyme Induction as Function of Temperature of Incubation

	Time after hydrocortisone addition (hours)	22 ± 1°C	27 ± 1°C	32 ± 1°C	37 ± 1°C
Expt. #1	24	< 0.2	0.4	3.0	13.3*
	48	< 0.2	2.0	13.6	26.4
Expt. #2	24	0.5	0.9	3.3	10.3
	48	0.4	< 0.2	8.5	29.4

* Retinas from 11-day chick embryos were explanted to flasks at the temperatures indicated. Hydrocortisone was present (2 μg/ml) from the start.

Enzyme unit = O.D. (Klett)/ μg protein X 10^2. Details of culture conditions (Reif-Lehrer and Amos, 1968).

Table 2

Protein and RNA Synthesis at 27°C and 37°C

		^{14}C-uridine		^{14}C-phenylalanine	
	Time after hydrocortisone addition (hours)	37°C	27°C	37°C	27°C
Expt. #1	24	1125*	311	376	119
	48	1173	300	393	92
Expt. #2	24	686	215	256	97
	48	656	172	182	39

* Conditions of incubation same as Table 1. Cold TCA-ppt cpm/mg protein. Two-hour pulse of ^{14}C-uridine or ^{14}C-phenylalanine.

Table 3. Translation and Transcription of the Specific mRNA for GS at 27°C

	Preincubation time at 37°C	Act. added (2 μg/ml)	Further incubation for 24 hr. 37°C	Further incubation for 24 hr. 27°C	Final enzyme activity units*	Units after shift to 27°C -- % of 37°C: units	Units after shift to 27°C -- % of 37°C: %
Expt. #1	4	-	-	-	4.8		
	24	-	-	-	21.4		
	-	-	-	+	2.2		
	4	+	+	-	15.8	11.0	100
	4	-	-	+	8.2	3.4	31
	4	+	-	+	11.7	6.9	63
Expt. #2	4	-	-	-	2.8		
	24	-	-	-	18.9		
	-	-	-	+	1.4		
	4	+	+	-	6.5	3.7	100
	4	+	-	+	5.0	2.2	59
Expt. #3	24 hours at 37°C				17.6		
	24 hours at 27°C				0.9		
	24 hours at 27°C, then 24 hours at 37°C				13.9		
	24 hours at 27°C, then 24 hours at 37°C with Act. D				2.5		

Conditions for incubation of retinas same as in Table 1. Where indicated preincubation represents the immediate explanting of the cultures.

* Enzyme unit as in Table 1.

at low temperature might at first glance be expected if the reduced rates of RNA and of protein synthesis are both applicable to glutamine synthetase. It was found, however, that the specific mRNA for GS made at 37°C was translated 30-60% as well at 27°C as at 37°C (Table 3, Expts. 1 and 2). Moreover, synthesis of enzyme subsequent to the shift to 27°C is not reduced when actinomycin is present suggesting that even in its absence no additional mRNA for the enzyme is synthesized at 27°C. This evidence further establishes that transcription initiated at 37°C is interrupted by lowering the temperature, implying that all mRNA synthesis may be virtually halted. Additional support for the tentative conclusion that GS mRNA synthesis is effectively stopped (or very rapidly destroyed before it can initiate protein synthesis) at 27°C is found in the failure of cells maintained for 24 hours at 27°C to demonstrate any accumulation of the specific mRNA translatable at 37°C (Table 3, Expt. 3).

When retinas incubated for 24 hours or longer at 27°C are transferred to 37°C the initiation of enzyme synthesis proceeds as on primary incubation, at 37°C. RNA synthesis, estimated by 2-hour pulses of ^{14}C-uridine incorporation into TCA-insoluble material* accelerates rapidly to reach a peak at 6-8 hours and returns to the 37°C rate at approximately 10-12 hours (Fig. 1). The same general picture is obtained if protein synthesis is 97% inhibited by puromycin or cycloheximide at the time the cells are transferred to 37°C (unpublished results).

Analysis of RNA Synthesized at 27°C. Analysis by sucrose gradient centrifugation of the RNA synthesized at 27°C (Figs. 2a and 2c) revealed that heterogeneous RNA (Soeiro, Birnboin and Darnell, 1966) and modest

*Not more than 5% of uridine incorporated can be accounted for in DNA under these conditions (unpublished results).

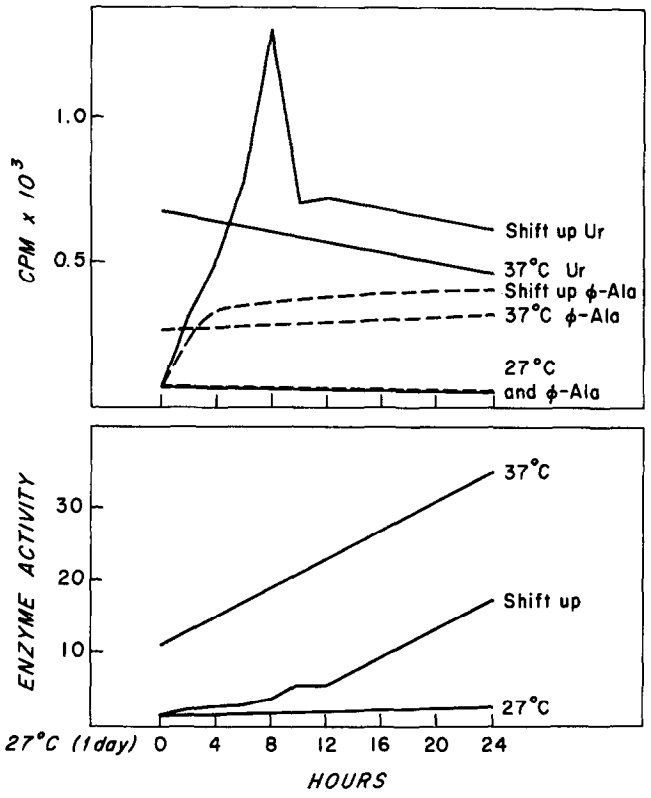

Fig. 1. Enzyme induction, RNA and protein synthesis following the shift from 27°C to 37°C. Retinas were explanted directly to standard medium containing 2 μg/ml of hydrocortisone at 27°C or 37°C. The experiments were started after 24 hours of such incubation.

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amounts of 4S RNA are synthesized. We have not eliminated the possibility that the material in the 4S region represents breakdown products. Virtually no 28S or 16S rRNA was detected even after an 8-hour pulse (Fig. 2c). At 37°C for a like period considerable 28S and 16S RNA accumulated (Fig. 2c). Whether 45S RNA is synthesized at 27°C is unclear since efforts to "chase" the products of synthesis at 27°C at the elevated temperature were so far unsuccessful. Indeed actinomycin appears to promote the breakdown of RNA even at 37°C in the retinal cells (Fig. 2b). Following the shift from 27°C

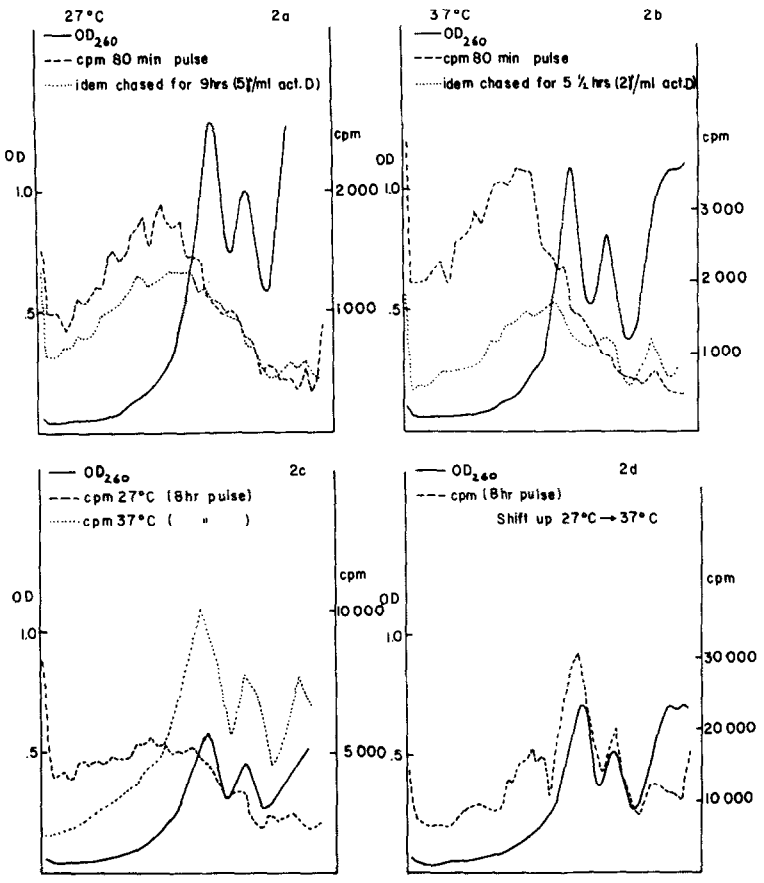

Fig. 2. RNA analysis in sucrose density gradient. Six to ten retinas were pulsed with uridine-5-H^3 (2 μc/ml in 2a; 1 μc/ml in 2b, c and d). RNA extraction and analysis as indicated in Methods. Forty to fifty fractions were separated by the drop collection method. Optical densities and specific radioactivities were normalized for comparisons on the same graph. The top of the gradient is on the right.

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to 37°C the cells synthesize all species of RNA (Fig. 2d). They do appear to accumulate more heterogeneous RNA than cells incubated only at 37°C.

A good deal of attention has been given to enzymatic and non-enzymatic proteins selected for functional configuration at 25-30°C which are non-functional at 37-41°C (Burge and Pfefferkorn, 1966). Relatively little has been said of the possibility that reduced thermal energy can in some instances significantly alter the conformation of a protein to a degree detectable in

its biologic, chemical or physical properties (O'Donovan and Ingraham, 1965; Kirschner et al., 1966).

Three immediate questions are apparent from these data: (1) Is the failure to induce GS mRNA limited to the specific transcription? (2) If so, does the conformation of the hydrocortisone binding site(s) at 27°C play a role? (3) What mechanism for control of RNA synthesis operates to restore the normal rate after the "overshoot"?

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