

ACTINOMYCIN-D ENHANCEMENT OF GLUTAMINE SYNTHETASE ACTIVITY IN CHICK EMBRYO RETINAS CULTURED IN THE PRESENCE OF CORTISOL

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ABSTRACT

Cortisol can prematurely induce glutamine synthetase activity in the chick embryo retina. Under appropriate conditions, this effect can be enhanced by addition of low levels of actinomycin D; this enhancement is reversibly inhibited by cycloheximide. The magnitude of the effect is a function of time of exposure to hormone as well as antibiotic and is also a function of the age of the embryo; within the limits of the present study it did not appear to be a function of actinomycin-D concentration. The data are discussed in terms of current ideas of possible control mechanisms in animal cells.

INTRODUCTION

The premature appearance of glutamine synthetase can be elicited in the chick embryo retina by cortisol (1-2) or related steroids (3-4) both in culture and *in ovo* (5). A further enhancement of the enzyme activity can be brought about by actinomycin-D treatment, and in some cases by interruption of protein synthesis by cycloheximide treatment. These modifications of steroid induction have led to consideration of a model proposed recently by Tomkins et al. (6). The present study provides data which further contribute to this assessment.

MATERIALS AND METHODS

Retinas were removed from embryos (12 days of development unless otherwise specified) and were cultured, harvested, and analyzed as previously described (1, 3).

The general experimental procedure was as follows: Retinas were excised and incubated at 37°C in

Eagle's Basal Medium (Grand Island Biological Company, Grand Island, N. Y.) supplemented with 5% commercially dialyzed fetal calf serum (GIBCO) for about 17 hr. Some of the cultures were then treated with cortisol (0.5 or 0.1 $\mu\text{g}/\text{ml}$), or cycloheximide 2 $\mu\text{g}/\text{ml}$, or both. Where appropriate, a radio-labeled amino acid was also added. Cultures were then returned to the incubator. This treatment period is referred to as stage I and lasted for 5-7 hr in most cases. At the end of stage I, some retinas were harvested (this group included those which had received radioactive amino acid); others were washed as described below and returned to fresh medium with or without actinomycin D (0.2 $\mu\text{g}/\text{ml}$ unless otherwise noted) and, in most cases, tritiated uridine. This was referred to as stage II and was most commonly about 17 hr in duration.

Where washing is indicated, the retinas were washed in the following way: Medium was poured off and the last drops were suctioned away with a sterile Pasteur pipet attached to a vacuum line. 5 ml of fresh

medium were added and removed, after gentle swirling, by decantation and suction. A second 5 ml of fresh medium were added, and the cultures were placed in a gyrotory water bath shaker (37°C, 68-72 rpm). After 20 min, this medium was removed as above and a third 5 ml wash was added and immediately removed. A final 5 ml of fresh medium were then added containing actinomycin D and/or radioactive precursor as indicated, and the flasks were replaced in the incubator for the times indicated.

At the end of the experiment, retinas were washed quickly with 10 ml of cold water containing the nonradioactive uridine (20 µg/ml) or the amino acid (20 µg/ml) used in the experiment. The retinas were then permitted to lyse in 2 ml water (3) and were assayed in a manner similar to that described by Thorndike and Reif-Lehrer (7). The modifications are shown below.¹

Modified Assay

Reagent	Molarity	ml added per sample	Molarity in final incubation mixture
1. Glutamine	0.4	0.40	0.103
2. Sodium acetate	1.7	0.06	0.066
3. NH ₂ OH	2.0	0.01	0.013
4. MnCl ₂	1.0	0.01	0.006
5. KCl	1.0	0.01	0.006
6. ATP	0.01	0.01	6.5 × 10 ⁻⁵
7. Sodium arsenate	0.5	0.04	0.13

1 ml of retinal lysate is added to a mixture of items 1-6, giving a total volume of 1.5 ml. The sodium arsenate is then added, giving a final incubation mixture of 1.54 ml. The tubes are then shaken in a 37°C waterbath for 30 min. After cooling in an ice bath, the color is developed by addition of a mixture of 0.5 ml of 0.1 M HCl, 0.5 ml of 15% (by weight) trichloroacetic acid, 0.5 ml of 7% (by weight) FeCl₃ in 0.004 M HCl. This gives a total of 3.04 ml of solution which is readily read on a Bausch and Lomb Spectronic 100-8.

Results are reported as specific activity of glutamine synthetase, i.e., OD 500 nm/mg protein. Each experimental point is an average of three to five samples.

For retinas which had been treated with a radiolabeled precursor, another sample of lysate (0.5 ml) was treated with 0.1 ml of cold trichloroacetic acid,

¹ The assay as published is unnecessarily sensitive for retinas which have been highly induced for many hours and more cumbersome to carry out for experiments with large numbers of samples. It should be noted that the techniques are basically similar.

50% by weight in water (100 gram %). The resulting precipitate is collected by a 15 min centrifugation at 2500 rpm at 4°-8°C in an International centrifuge (International Equipment Co., Needham Heights, Mass.). The precipitate is washed with 1 ml of cold 5% (by weight) trichloroacetic acid and again centrifuged. After decanting off the supernatant liquid, the tubes are allowed to drain upside down on an absorbing surface. The precipitate is then dissolved in 2 ml of 0.2 M ammonia solution. Aliquots (0.5 ml each) of the resulting solution were used for determining radioactivity and protein, as previously described (1). Results are reported as cpm/mg protein.

Cortisol was from Sigma Chemical Co. (St. Louis, Mo.); cycloheximide from Nutritional Biochemicals Corporation (Cleveland, O.); actinomycin D was obtained through the courtesy of Merck, Sharp, & Dohme (Rahway, N. J.). The latter was kept in the dark and stored at 4°C in aqueous solution.

Radioactive materials were obtained from New England Nuclear Corp. (Boston, Mass.).

RESULTS AND DISCUSSION

Retinas incubated overnight in a noninducing medium and then treated with cortisol and cycloheximide (stage I), develop glutamine synthetase activity subsequent (stage II) to the removal of the steroid and antibiotic. The enzyme activity which is elicited during stage II can be augmented nearly threefold by arresting RNA synthesis with actinomycin D during this latter incubation.

In a series of such experiments, the presence of actinomycin D during stage II increased the specific activity of the enzyme by two- to fourfold compared to that obtained in the absence of the antibiotic.

The presence of cortisol in stage I appears to be necessary to obtaining the maximal stimulatory effect of actinomycin D, although an effect is sometimes seen in the absence of the steroid. In this latter case, the results are quite variable and the effect is small. In a series of experiments in which cycloheximide alone was present in stage I, actinomycin D added during stage II gave at most a twofold stimulation in enzyme activity. Table I gives the results of some typical experiments. The concentration of actinomycin D (0.2 µg/ml) used in these experiments usually inhibited uridine incorporation by 70-80% and inhibited incorporation of amino acids into cold TCA-precipitable protein by about 60%. The cycloheximide (2 µg/ml) inhibited amino acid incorporation by greater than 90%.

A phenomenon, sometimes referred to as

TABLE I
Enhancement of Glutamine Synthetase Activity in the Presence of Actinomycin D

Experiment No.	Glutamine synthetase (specific activity)					
	+Cortisol			-Cortisol		
	+Act	-Act	Ratio +Act/-Act	+Act	-Act	Ratio +Act/-Act
1	1.3	0.55	2.4	0.40	0.23	1.7
2	0.51	0.27	1.9	0.13	0.13	1.0
3	0.75	0.31	2.4	—	—	—
4* ‡	0.77	0.39	2.0	0.26	0.17	1.6
5	1.3	0.35	3.7	—	—	—
6 ‡	0.73	0.42	1.7	0.22	0.17	1.3
7	—	—	—	0.19	0.20	0.95
8 §	—	—	—	0.34	0.20	1.7

Specific enzyme activity = OD 500 nm/mg protein.

Cortisol = 0.5 µg/ml except * = 1.0 µg/ml.

Cycloheximide = 2.0 µg/ml.

Actinomycin D (Act) = 0.2 µg/ml except § = 10 µg/ml.

Stage I = 5 hr except ‡ = 7 hr.

Uridine incorporation in most of these experiments was about 90% inhibited in the Act samples compared to controls.

superinduction by actinomycin D, has been reported in a number of animal cell systems (6, 8). The results reported here demonstrate the existence of an analogous phenomenon in the chick embryo retina system. This apparent enhancement of hormonal induction by actinomycin D has been explained by Reel and Kenney (8) as an inhibition of enzyme degradation and more recently by Tomkins et al. on the basis of inhibition of formation of a repressor which would otherwise inactivate the messenger RNA for the induced enzyme (6).

Tomkins et al. have proposed a model for enzyme induction in mammalian cells (6). According to their hypothesis, there is an inducible phase of the cell cycle during which the structural gene is continuously transcribed into enzyme messenger RNA, which is, in turn, translated into product enzyme. Concurrently, a repressor is produced at a constant rate which inhibits messenger translation and promotes messenger degradation. According to the Tomkins model, superinduction by actinomycin D is explained on the basis of loss of a labile protein repressor which is

translated from a messenger RNA with a very short half-life. Thus, both the messenger RNA and the protein must be continuously synthesized in order to maintain an effective level of repressor. This model would predict that actinomycin D given to uninduced cells should cause an increased basal level of enzyme activity. Peterkofsky and Tomkins (9) did, in fact, find a twofold increase in basal tyrosine aminotransferase level with 2.0 µg/ml actinomycin D in the absence of inducer. We have not observed such an effect in freshly cultured retinas from 12-day-old embryos in the case of glutamine synthetase (Table II). However, a possibly analogous effect is illustrated in Table I. Here a small but significant superinduction is sometimes seen if cortisol is omitted from stage I of the usual type experiment, i.e., after 1 day in culture. It could be conjectured that this is, in fact, the same effect but that, in primary cultures, some time is required for the tissue to become acclimated to the culture conditions; acclimatization time would of course not be required for cells in serial culture such as the hepatoma cells.

Moscona et al. (10) did report induction of glutamine synthetase in the retinal system within 24 hr in the absence of added steroid and without any time to adjust to culture conditions. This was accomplished either by addition of 10⁻³ M cytosine

TABLE II
Effect of Actinomycin D on Basal Glutamine Synthetase Level

Treatment*	hr	Glutamine synthetase activity			
		-Cortisol		+Cortisol	
		+Act -Act	Uridine incorporation inhibition	+Act -Act	Uridine incorporation inhibition
Immediately after ex-plantation	7	1.2	63	0.37	83
	7	1.1 ‡	85	—	—
	16.5	1.1	88	0.18	67
	16.5	1.4 ‡	97	—	—
After 29 hr in culture	18	1.2	—	0.68	—
	18	1.1 ‡	—	0.34 ‡	—

* Cortisol and/or actinomycin D as appropriate. Specific activity = OD 500 nm/mg protein.

Cortisol = 0.5 µg/ml.

Actinomycin D = 0.2 µg/ml except ‡ = 10 µg/ml.

TABLE III
Actinomycin D Effect as a Function of Concentration

Experiment No.	Concentration of actinomycin D	Specific activity ratio	Inhibition of uridine incorporation
		$\frac{+Act}{-Act}$	
I	0.2	2.4	77
	2.0	2.2	76
II	0.2	2.3	67
	2.0	2.1	66
III	0.2	2.3	69
	5.0	2.3	89
IV	0.2	2.2	69
	5.0	2.3	89
V	0.2	1.3	49
	10.0	1.5	81
VI	0.2	1.4	51
	10.0	1.9	77
VII	0.2	2.2	87
	10.0	1.8	99

Specific activity as in Table I.

All data based on stage I \rightarrow wash \rightarrow stage II experiments.

0.5 $\mu\text{g/ml}$ cortisol and 2.0 $\mu\text{g/ml}$ cycloheximide used in stage I in all experiments.

arabinoside or by treatment with extraordinarily high concentrations (200–500 $\mu\text{g/ml}$) of cycloheximide, followed by treatment with high concentrations of actinomycin D (10 $\mu\text{g/ml}$). It is difficult to interpret these results, especially in the case of cycloheximide. We have previously reported that the retinas may not recover completely from cycloheximide treatment even at 1 $\mu\text{g/ml}$ (1). In addition, as Moscona et al. themselves point out, not only protein synthesis, but 40% of RNA synthesis is blocked by these high concentrations of cycloheximide (10).

We find that, under appropriate conditions, the superinduction effect is manifested at low (0.2 $\mu\text{g/ml}$) concentrations of actinomycin D. Increasing the concentration of actinomycin D up to 10 $\mu\text{g/ml}$ does not usually change the specific enzyme activity obtained (Table III and Fig. 1) even though uridine incorporation is inhibited to a greater extent (about 90%) at this higher level

of antibiotic. This is in contrast to results reported recently by Moscona et al. (11) in the same system. The latter workers found that high concentrations of actinomycin D (10 $\mu\text{g/ml}$) gave rise to the superinduction, whereas concentrations of 0.2 $\mu\text{g/ml}$ suppressed the increase of glutamine synthetase activity by 70–90% of controls. For an explanation of this effect, two endogenous regulators having different susceptibilities to the antibiotic were proposed. Since the superinduction effects we observe do not appear to change with increasing actinomycin-D concentration, it is not necessary to invoke the two regulators in our case.

Glutamine synthetase appears to be a relatively stable enzyme with a relatively long half-life in the chick embryo retinal system. We have previously reported that retinas treated first with cortisol for 24 hr and then with cycloheximide for various periods of time show little, if any, decrease in enzyme activity at 72 hr (1). The present experiments again indicate little loss of enzyme in this system by 48 hr (Fig. 1). Moscona et al. also have findings supporting this view; they have reported that glutamine synthetase has a half-life longer than 20 hr (11). The stability of retinal glutamine synthetase is, therefore, quite different from that of tyrosine aminotransferase of the rat liver (12) and from the hepatoma cells in which tyrosine aminotransferase turns over rapidly (6). In rat liver, the half-life of tyrosine aminotransferase has been estimated to be 1.7–3.5 hr (12). Martin et al. have estimated a half-life of 4–6 hr for tyrosine aminotransferase in hepatoma cells (13). Although the former workers found that cycloheximide served to stabilize the enzyme in the liver, the latter did not find this to be the case in cultured cells. More recently, Gerschenson et al. (14) did find enzyme stabilization by cycloheximide in cultures of normal liver cells. In the chick embryo retina system, little difference is seen in the glutamine synthetase activity profile over a 48 hr period in the presence and absence of cycloheximide (Fig. 1).

Thus, in the case of retinal glutamine synthetase, it seems difficult to justify the argument that the function of the actinomycin D is to inhibit the production of a substance which degrades the enzyme *per se*. This does not of course exclude the possibility that actinomycin D serves to inhibit the production of a substance which degrades the messenger RNA for glutamine synthetase. It should be kept in mind, however, that in contrast

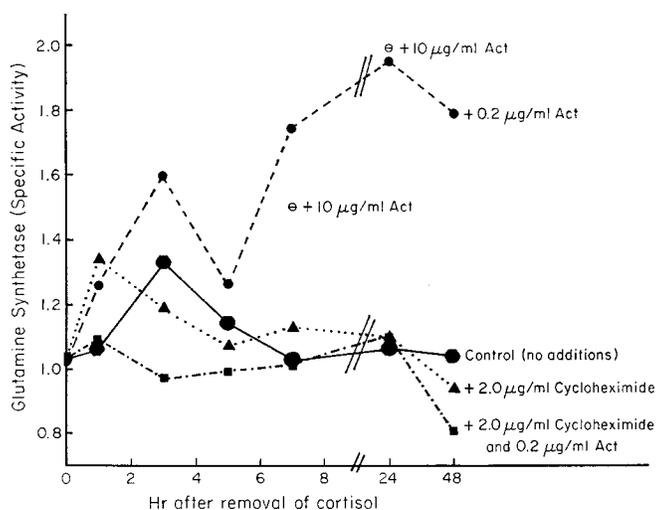


FIGURE 1 Effects of actinomycin D and cycloheximide on glutamine synthetase activity in retinal cultures pretreated with cortisol. All retinas were explanted, and immediately placed in medium containing cortisol. At the end of 18 hr, the retinas were washed to remove cortisol and placed into fresh medium (this equals zero time in the figure) containing the additives shown. Specific activity as in Table I. Cortisol = $0.5 \mu\text{g/ml}$. Cycloheximide = $2.0 \mu\text{g/ml}$. Actinomycin D (Act) = $0.2 \mu\text{g/ml}$ except in the case of the two single points = $10 \mu\text{g/ml}$.

to the situation with tyrosine aminotransferase, induction of retinal glutamine synthetase does not seem to require the continued presence of cortisol. In fact, we have previously reported that cortisol is not required by the system at all, but only serves somehow to hasten the appearance of enzyme activity (15). In cultures devoid of added steroid, glutamine synthetase activity rises to appreciable levels after a time in culture such that the sum of developmental days *in ovo* plus days in culture is 18–19. At the corresponding time, the activity is normally quite high *in ovo*. This rise is unaffected by actinomycin-D treatment during the last 24 hr in culture (Table IV). However, if cortisol is added during these last 24 hr, there is sometimes an additional rise in enzyme activity (15). This additional increase due to cortisol is almost completely inhibited if actinomycin D is added together with the cortisol; no increase in activity due to actinomycin D is seen in the absence of cortisol. But if cortisol is added and actinomycin D is given 5 hr later, there does appear to be some enhancement of the cortisol effect (Table IV).

Figure 2 shows the effect of varying the duration of cortisol-cycloheximide treatment in our experiments. When stage I was varied from 1 to 7 hr, the enhancement by actinomycin D in stage II

was optimal when stage I is 4–5 hr. A composite graph based on data from three such experiments is seen in the figure. The actinomycin D inhibited uridine incorporation by 80–90%. When stage I was 24 hr long, only a very small enhancement by actinomycin D was observed. This is in agreement with earlier work in this laboratory in which a small, but consistent superinduction by actinomycin D was observed in a large number of experiments in which retinas had been treated with cortisol and cycloheximide for 24 hr (L. Reif-Lehrer, unpublished results).

In the experiments in Table V, stage I was 2 and 3 hr, respectively, and stage II varied from 1 to 5 hr with a control taken at the usual 17 hr. Only the control had enhanced activity. Because of the short stage I, probably only a small amount of messenger RNA accumulated before addition of actinomycin D. The controls with a stage II of 17 hr duration do exhibit an appreciable actinomycin D effect, despite the short stage I. These data would seem to indicate that the actinomycin D effect requires some hours to maximally manifest itself. The rate of inhibition of uridine incorporation by the antibiotic during stage II in these experiments is shown in Fig. 3.

As a variation, retinas in culture were treated

TABLE IV
Effect of Actinomycin D on Long-Term Retinal Cultures

Time in culture	Specific activity					
	-Cortisol			+Cortisol		
	-Act	+Act	Inhibition of uridine incorporation	-Act	+Act	Inhibition of uridine incorporation
days			%			%
1	0.15	0.11	79	1.2	0.32	56
2	0.19	0.23	66	0.57	0.25	43
6	0.63	0.56	83	2.6	1.1	79
7	1.1	0.96	71	2.1	1.3	83
7	0.96*	1.0†		1.4*	2.7‡	
9	1.8	1.6	48	1.6	1.6	52

Retinas were treated with cortisol or actinomycin D or both for 24 hr before harvest; radiolabeled uridine was also added during these last 24 hr in culture. Medium of cultures was changed every 3 days.

Specific activities as in Table I.

0.2 $\mu\text{g/ml}$ actinomycin D except as noted below.

0.1 $\mu\text{g/ml}$ tritiated cortisol.

* = 2.0 $\mu\text{g/ml}$ cycloheximide were added during the last 24 hr in culture.

† = the actinomycin D in this case was added 5 hr after addition of cortisol to the control cultures.

‡ = the actinomycin D in this case was added 5 hr after addition of cortisol.

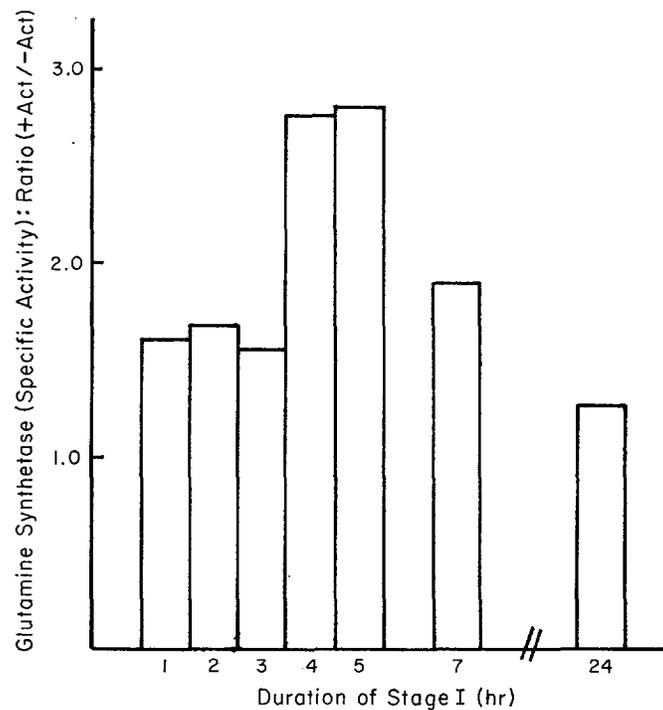


FIGURE 2 Enhancing effect of actinomycin D as a function of duration of stage I. Specific activity as in Table I. Stage II = 17 hr. Cortisol = 0.5 $\mu\text{g/ml}$. Cycloheximide = 2.0 $\mu\text{g/ml}$. Actinomycin D = 0.2 $\mu\text{g/ml}$.

TABLE V
Actinomycin D Effect as a Function of Time

Duration		Glutamine synthetase specific activity Ratio $\frac{+Act}{-Act}$
Stage I	Stage II	
hr	hr	
2*	2	1.1
2	5	1.0
2	17	1.8
3	1	1.1
3	3	1.0
3	17	1.6

Specific activity as in Table I.
Cortisol = 0.5 $\mu\text{g/ml}$ except * = 2.0 $\mu\text{g/ml}$.
Cycloheximide = 2.0 $\mu\text{g/ml}$.
Actinomycin D = 0.2 $\mu\text{g/ml}$.

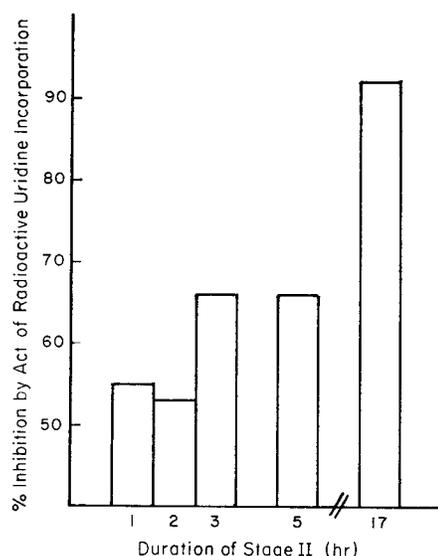


FIGURE 3 Rate of inhibition of uridine incorporation by actinomycin D in stage II. Stage I = 2 or 3 hr. Actinomycin D = 0.2 $\mu\text{g/ml}$. Cortisol = 0.5 $\mu\text{g/ml}$. Cycloheximide = 2.0 $\mu\text{g/ml}$. Incorporation measured as cpm/mg protein. % inhibition = $100 - 100$ (incorporation in presence of actinomycin D divided by incorporation in absence of actinomycin D). The data in this figure were obtained from the experiments shown in Table V.

with 0.5 $\mu\text{g/ml}$ cortisol for 18 hr beginning immediately after explantation. At the end of this time, the cortisol was washed out and the retinas were put into fresh medium either with no additions or with 2.0 $\mu\text{g/ml}$ cycloheximide or 0.2 $\mu\text{g/ml}$

TABLE VI
Effect of Cycloheximide on Actinomycin D Enhancement of Glutamine Synthetase Activity

Condition	Glutamine synthetase specific activity Ratio $\frac{+Act}{-Act}$
No cycloheximide	2.5
+ Cycloheximide	1.4
* +Cycloheximide for 7 hr, then harvest	1.2
‡ +Cycloheximide for 7 hr, then wash and put into fresh medium for the remaining 17 hr	2.3

Specific activity as in Table I.
Retinas were not preincubated, but were immediately placed into medium containing 0.5 $\mu\text{g/ml}$ cortisol upon explantation. After 20 hr, the retinas were washed in the usual way and placed into fresh medium, fresh medium with 0.2 $\mu\text{g/ml}$ actinomycin D, or fresh medium with actinomycin D and 2.0 $\mu\text{g/ml}$ cycloheximide and harvested after 24 hr except for *: harvested after 7 hr and ‡: washed again after 7 hr to remove cycloheximide, replaced into fresh medium, and harvested with the other flasks at the end of the 24 hr period.

actinomycin D or both. A pronounced enhancement by actinomycin D (but not by cycloheximide) compared to controls not treated with either drug became apparent at 7 hr. This effect was still high even at 48 hr (Fig. 1) in contrast to the experiments in which cortisol was omitted from the culture medium for the first 17 hr. However, here as before, use of 10 $\mu\text{g/ml}$ actinomycin D did not increase this effect.

Addition of cycloheximide alone had no effect on induced retinas (Fig. 1) but completely abolished the enhancement by actinomycin D when the two antibiotics were added together during the last stage of an experiment (Table VI). In long-term cultures, in which cortisol addition 24 hr before termination of the experiment enhanced glutamine synthetase activity, cycloheximide did reduce this enhancement (Table IV). The cycloheximide effect could readily be reversed by washing.

Another interesting finding in this study is the apparent age dependence of the actinomycin D effect. No enhancement of enzyme activity by actinomycin D was seen in retinas from 9-day eggs; retinas from 10-day eggs seemed to show some

TABLE VII
Age Dependence of Actinomycin D Effect

Experiment	Embryo Age	Specific activity ratio	Inhibition of uridine incorporation
		$\frac{+Act}{-Act}$	
	<i>days</i>		<i>%</i>
I	9	1.1	78
	10	1.8	64
	12	2.4	77
	12	2.3	69
II	9*	0.62	50
	10	1.2	60
	11	1.3	49
	11‡	1.6	81
	13	1.4	51
	13‡	2.0	77
III	8	0.73	83
	9	1.2	84
	10	1.7	83
	13	2.2	87
	13‡	1.8	99
IV	10	1.7	64
	12	2.2	69
	12§	2.3	89
V	9	1.1	78
	12	2.3	77
	12	2.1	76

Specific activity as in Table I.

Stage I = 5 hr.

Cortisol = 0.5 $\mu\text{g/ml}$ except * = 2 $\mu\text{g/ml}$.

Cycloheximide = 2.0 $\mu\text{g/ml}$.

Actinomycin D = 0.2 $\mu\text{g/ml}$ except ‡ = 10 $\mu\text{g/ml}$, § = 5 $\mu\text{g/ml}$, || = 2 $\mu\text{g/ml}$.

effect, although not so much as those from the 12-day eggs used in most experiments (Table VII). It should be noted that the inhibition of uridine incorporation by actinomycin D did not depend on the age of the retina, but was equal at all ages. Martin et al. (13) have reported that in the case of synchronous hepatoma cells, different responses to inducer are observed during mitosis as compared with other phases of the cell cycle. They have proposed that no transcription occurs during the mitotic phase of the cell cycle and that tyrosine aminotransferase synthesis is constitutive at this time if the synchronous cells have been previously induced. In cultures of cells which are not in synchrony, however, maximal tyrosine

aminotransferase synthesis is always inducer-dependent. Both Moscona (5) and Reif-Lehrer (15) have reported that there is a change in the inducibility of glutamine synthetase in chick embryo retinas between days 9 and 10 of development. This is also about the time in retinal maturation when cell division decreases sharply. It is evident that important changes in tissue metabolism are occurring at about this time, and these undoubtedly also account for observed differences in the enhancing effect of actinomycin D as a function of age.

It is apparent from the work of Martin et al. (13) that it is possible to obtain different data from the same system depending on the state of the system at the time of measurement, the difference, in their case, being whether or not the cells are in synchrony. The retinal system, after the 10th day of development, is perhaps more analogous to a system in synchrony; but here there is a mixed population of cells. All the cells may be making glutamine synthetase at the same time and at the same rate. This seems *a priori* unlikely; however, we have evidence that glutamine synthetase is not, at least, confined to a single cell type in the retina. The enzyme may be made at different times and rates in different cell types. There is also the possibility that different cells of the retina may have different sensitivities to steroid and to actinomycin D. We are attempting to answer some of these questions by means of subcellular and cell fractionation studies.

It is difficult, at present, to reconcile all the observed results in the retinal system with any simple, unified control mechanism. By some means, actinomycin D is permitting enhanced translation of available glutamine synthetase message. A sound hypothesis will have to await further information.

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