A DIALYZABLE INDUCER FOR THE GLUTAMOTRANSFERASE OF CHICK EMBRYO RETINA*

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In the chick embryo retina the enzyme, glutamotransferase (GTF) is not detectable until the 17th day. At this time the enzyme appears and rapidly reaches a very high level (Rudnick and Waelsch, 1955). This enzyme activity can be made to appear much earlier if the retina is removed from the embryo and cultured in a standard medium (Moscona and Hubby, 1963; Kirk and Moscona, 1963; Moscona and Kirk, 1965; Reif and Amos, 1965).

We and other investigators (Moscona and Kirk, 1965; Reif and Amos, 1965; Kirk, 1965) have obtained evidence which suggests to us that the appearance of the enzyme in vitro is consistent with induction or derepression. In this report, we would like to present the evidence which we have obtained for an "inducer", which appears to be a substance

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of relatively low molecular weight[1].

Methods and Materials.

Retinas were removed from the embryo at 10 days of development. Each retina was cultured in 5 ml of Eagle's basal medium with 10% calf serum in tightly stoppered 50 ml Erlenmeyer flasks at 37°C. The flasks were shaken on a rotary shaker (70-72 RPM) for the culture period (usually 1-5 days). All analyses were done on the sample obtained by mild sonic disruption of the saline-washed retina in 2 ml of 0.06M phosphate buffer (pH 7.4-7.6). Enzyme activity was determined by the method of Waelsch (1955), using a 2-hour incubation[2], and protein by the method of Lowry (1951). Incorporation studies refer to C^{14}-leucine incorporated into cold TCA-precipitable protein. All values of enzyme activity and counts incorporated are reported as specific activity, i.e., per unit weight of protein[3].

Materials were dialyzed for 24 or 48 hours at 4°C in 8 or 20mm

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1. There is a possibility that we may also be dealing with a demonstrable repressor component. The main evidence for this is based on the fact that in many experiments the addition of components of fresh medium to OCM was appreciably less effective in inducing enzyme than changing the medium. Also supporting this idea is the finding that retinas are less responsive to inducer during the first 24 hours of culture than after 2 or 3 days.

2. Product formation in this assay is linear for at least 3 hours at 37°C.

3. Units of enzyme activity are the Klett readings at 540 mμ (i.e., a unit of optical density) divided by the μg protein in the sample. Ten day retinas assayed shortly after excision give values between 0.010 and 0.020. Therefore 0.015 has been subtracted from all values presented here as a zero point.

dialysis tubing (Union Carbide Corp.) pre-boiled in distilled water for 20 minutes.

The dialyzable fraction was prepared as follows: The water dialysate (H_2O/serum = 10/1) of the serum was lyophilized to dryness. The residue was taken up in a small quantity of water or Hanks balanced salt solution (BSS). This reconstituted material was then used in place of serum in the medium of the test samples.

Results and Discussion.

An early observation that enzyme activity in retinas decreased or remained constant after 3 or 4 days of incubation led us to use medium collected from such cultures (= OCM) to start cultures of fresh retinas. No enzyme activity appeared in the latter cultures even by 72-96 hours, when the activity in cultures begun in fresh medium had already reached a maximum. In contrast, protein synthesis, as measured by incorporation of C^{14}-leucine, was about the same in fresh medium and in OCM.

The OCM proved an excellent test system, since enzyme activity could be induced at will from a uniform null baseline simply by changing to fresh medium, or by adding concentrated media components (Reif and Amos, 1965) (Fig. 1). Similar test situations could be achieved by starting cultures in 1% or 3% calf serum medium. One disadvantage of these lower levels of serum, is that the retinas have a greater tendency to deteriorate, as evidenced by a disintegration of the tissue. OCM, though it appears to have no inducer, supports the tissue very well, presumably by virtue of having 10% serum. Although OCM proved to be the best medium for induction experiments, it is time consuming to prepare. An

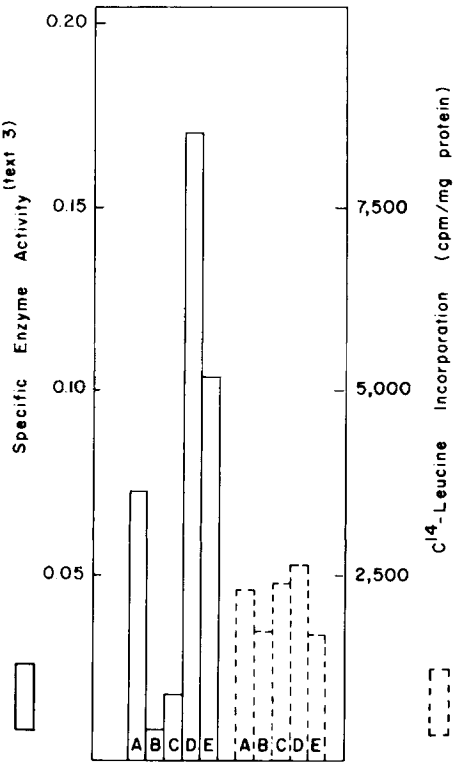

Figure 1. Relative effects of different media on enzyme induction compared to effect on protein synthesis. Cultures in : A) 10% CS medium; B) OCM; C) changed to 0% medium after 1 day in OCM; D) changed to 10% CS medium after 1 day in OCM; E) 10% of fresh CS added after 1 day in OCM. All samples were harvested after 3 days in culture; each value is an average of three samples.

alternative test system was suggested by the report of Moscona and Kirk (1965) that no enzyme activity appeared in retinas cultured with fetal calf serum (FCS) instead of adult serum. We have since found that FCS does permit induction of a low level of enzyme if the cultures are maintained for as long as 96 hours; in such medium, as in OCM, protein synthesis is usually about equal to that in adult serum (Fig. 2). In an early attempt to distinguish between appearance of an inhibitory substance and absence or depletion of an inducer, the effect of addition of components of the medium,

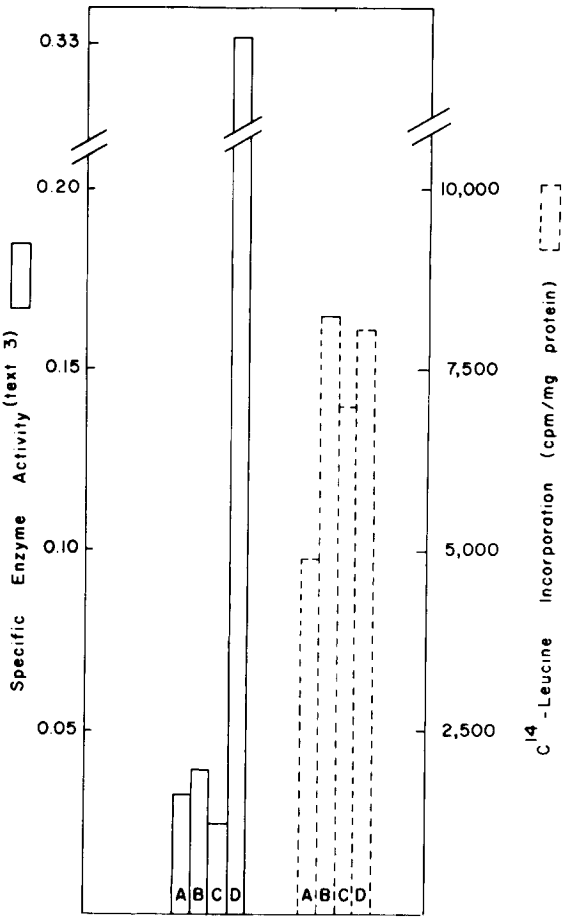

Figure 2. Comparison of enzyme induction and protein synthesis in fetal and adult serum. A) harvested after 1 day in 10% FCS medium; B) harvested after 3 days in 10% FCS medium; C) harvested after 1 day in 10% CS medium; D) harvested after 3 days in 10% CS medium.

individually or in combinations, was compared to the effect of changing medium. The only component whose addition to OCM enhanced enzyme activity was calf serum (CS) (Fig. 3). Indeed, dialysis of OCM against CS or fresh 10% CS medium resulted in restoring the inducer activity to OCM (Reif and Amos, unpublished results). In contrast, OCM dialyzed against serum free medium (0% medium) remained ineffective. Moreover, 0% medium,

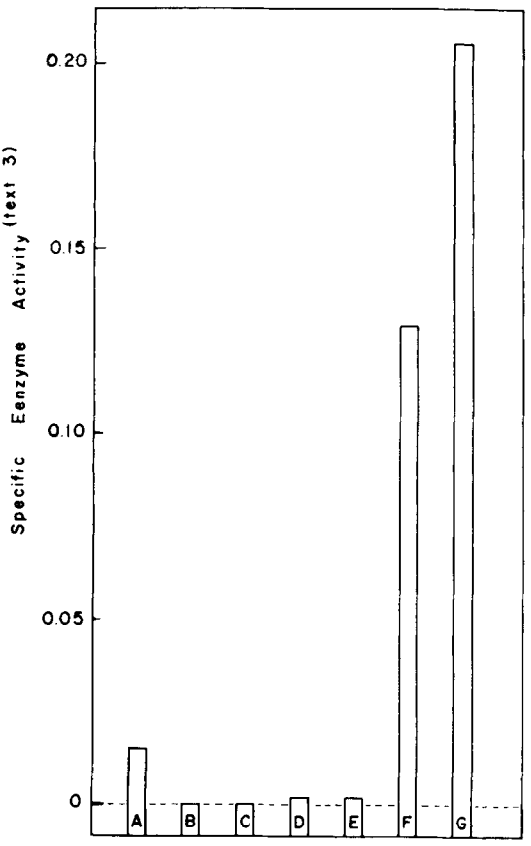

Figure 3. Effect of different media components on enzyme induction. A) harvested after 2 days in OCM; B) harvested after 4 days in OCM. (C through G were treated as indicated, after 2 days of culture in OCM, and were harvested 2 days after treatment. For additions, the amount of the specified substance added is equal to the amount of that substance initially added to make Eagle's basal medium with 10% CS.) C) add $NaHCO_3$; D) add amino acids; E) add vitamins; F) add CS; G) change to fresh 10% CS medium.

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which does not induce enzyme activity, acquires this capacity after dialysis against calf serum (Table I). As a first step toward isolating the inducer, serum was dialyzed against 10 volumes of H_2O or BSS (as described under Methods). The concentrated dialysate proved to contain significant amounts of the inducer; in fact in most cases, as prepared, it was essentially as effective as

TABLE I. ENZYME INDUCTION WITH DIALYZABLE EXTRACT OF CALF SERUM.

Expt. No.	Medium[a]	Untreated cultures		Treated cultures			
		At time[b] of treatment of parallel cultures	At time[b] of harvest of parallel cultures	Add CS[c]	Change to 10% CS medium	Add CS extract[c,d]	Change to 0% medium vs. which CS has been dialyzed
		(Specific enzyme activity[e])					
1	1% CS	0.002	0.023	0.145	0.102	0.070 0.052	0.066
2	10% FCS	0.005	0.078	0.257	---	0.280 0.200	0.262
3	10% FCS	0.036	0.057	0.343	---	0.316 0.269	0.314
4	10% FCS	0.026	0.071	0.213	---	0.272 0.265	0.142 0.217 } g
5	0%	0.000[e]	0.035[f]	0.085	---	---	0.052
6	OCM	0.004	0.019	0.244	0.334	0.321 0.301	0.302 0.327[h]

a) Medium is Eagle's basal medium containing the indicated % of the specified serum.
b) Retinas were treated after 2 days in culture and harvested 2 days after treatment except: in expt. no. 1: treated after 1 day, harvested after 2 additional days; in expt. no. 5: treated at zero time, harvested after 3 days.
c) All additions: 0.5 ml added per 5 ml culture.
d) After lyophilization to dryness, the residue of the water dialysate was taken up in a volume of H_2O (upper value) or BSS (lower value) equal to 1/2 the volume of serum originally dialyzed. The extract for each experiment was prepared from a separate dialysis; several different lots of serum were used.
e) Units are as described in footnote 3 in text.
f) After 3 days in 0% serum medium, the retinas are in a bad state of deterioration and the protein value is very low, thus giving an erroneously high value for specific enzyme activity.
g) Values obtained with 2 different dialysates.
h) Dialysed at 37°C.

whole serum (Table I). The reduced effectiveness of the dialysates when the retinas were incubated in 0% or 1% CS medium probably reflects what was earlier mentioned regarding the poor state of the tissue in such media.

Since the inducer is dialyzable, we conclude that it is of relatively low molecular weight. It is reasonable to assume that the mechanism for the observed induction is analogous to the control mechanisms which have been proposed for induction and repression in bacteria (Jacob and Monod, 1961); indeed, experimental evidence indicates that the effect of the serum (and presumably of the dialyzable fraction) is at the level of messenger RNA (m-RNA) synthesis. Moscona and Kirk (1965) and Kirk (1965) have reported that the m-RNA for GTF is not present in freshly excised 10-day retinas. They have also reported that the formation of enzyme gradually becomes refractory to the effect of Actinomycin D[4]. Kirk found that when no enzyme had been made in FCS medium, the m-RNA for GTF had also not been made. We have confirmed all these findings using OCM as the initial culture medium[5].

We are currently pursuing the isolation and identification of the inducer.

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4. Dr. Sarah Ben-Or (Hebrew University Medical School, Jerusalem) has also confirmed these results (personal communication).

5. A detailed account of these experiments will be published shortly.

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