Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 4175–4179, July 1980
Developmental Biology

Induction of translatable mRNA for dopa decarboxylase in *Drosophila*: An early response to ecdysterone

(steroid hormone action/cell-free translation/immunoprecipitation)

GREGORY P. KRAMINSKY*, WILLIAM C. CLARK*, MARK A. ESTELLE*, R. DANIEL GIETZ*, BECKY A. SAGE†, JOHN D. O'CONNOR†, AND ROSS B. HODGETTS*‡

*Department of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9, Canada; and †Department of Biology, University of California, Los Angeles, California 90024

Communicated by Orville L. Chapman, April 28, 1980

ABSTRACT **Ecdysteroid titer and dopa decarboxylase (aromatic-L-amino-acid carboxy-lyase, EC 4.1.1.28) activity were determined throughout the life cycle of *Drosophila melanogaster*. Five peaks in the amount of hormone were observed, which preceeded five dopa decarboxylase peaks by times ranging from 5 to 58 hr. Late in the third instar the hormone and enzyme maxima are nearly coincident. The increase in enzyme activity observed at this time is paralleled by an increase in translatable dopa decarboxylase mRNA. To obtain evidence that ecdysterone induces the appearance of this mRNA we made use of the temperature-sensitive *ecd*^{1} mutant. Garen *et al.* [Garen, A., Kauvar, L. & Lepesant, J.-A. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5099–5103] have shown that when third instar mutant larvae are kept at 29°C, the ecdysteroid titer remains low. In such larvae we show that the normal increase in dopa decarboxylase activity fails to appear, and no translatable dopa decarboxylase mRNA can be detected. Exogenous feeding of ecdysterone to these larvae results in a rapid synthesis of dopa decarboxylase in the epidermal cells. In addition, a parallel increase in translatable dopa decarboxylase mRNA occurs, which may be a primary response of these target cells to ecdysterone.**

The enzyme dopa decarboxylase (DDC; aromatic-L-amino-acid carboxy-lyase, EC 4.1.1.28) is found in both nerve and epidermal cells of the Diptera and catalyzes the conversion of dopa to dopamine. This molecule may function as a transmitter in nerve cells (1), whereas in the epidermal cells dopamine is required for the hardening and darkening of cuticular structures (2).

DDC activity in the epidermal cells of larvae is under control of the steroid hormone ecdysterone. Early work on the blowfly, *Calliphora*, showed that DDC activity could be induced by injecting ecdysterone into larval abdomens isolated from endogenous hormone by ligation (3). Karlson proposed that direct action of the hormone on the DNA induced transcription of the mRNA for DDC (4). Fragoulis and Sekeris (5) have isolated mRNA from puparia in which both ecdysterone and DDC levels are maximal (6). This RNA was shown to direct the synthesis of DDC *in vivo*, whereas translation of RNA from a mid-third-instar larval sample did not. The observed correlation between the presence of the hormone at pupariation and the apparent increase in the amount of DDC-specific mRNA at this stage is consistent with Karlson's model. However, a causal relationship between the two has not been demonstrated.

We are interested in the regulation of DDC in *Drosophila*. This is a favorable system in which to investigate problems of steroid hormone action because DDC is amenable to genetic manipulation. Genetic analysis of DDC in *Drosophila* has led to a cytological localization of the structural gene (7) and the recovery of mutants (8). In addition, the recent purification and characterization of the enzyme (9) have provided the basis for the present work, in which the hormonal control of DDC at pupariation is examined. We make use of the temperature-sensitive mutant, *ecd*^{1}, isolated by Garen *et al.* (10). When mid-third instar larvae of this mutant are transferred to the restrictive temperature (29°C), they are unable to produce ecdysterone and fail to pupariate. However, pupariation can be induced at the restrictive temperature if such larvae are fed ecdysterone (10). This latter observation has allowed us to study events in the epidermal cells shortly after exposing the target tissue to the hormone.

MATERIALS AND METHODS

Collection and Staging of Organisms. Wild-type (Canton-S) *D. melanogaster* organisms were raised in constant darkness at 25°C on trays of media (11) spread with live yeast. For ecdysteroid and DDC developmental profiles, eggs were collected over 2-hr intervals and the organisms were resynchronized over 2-hr periods as newly hatched larvae, white puparia, and newly eclosed adults.

Extraction of Poly(A)-RNA. Wandering larvae were collected, washed, and then transferred to a bag sewn from nylon bolting cloth. The bag was then passed several times between the rollers of a hand-operated wringer, which ruptured the larvae and allowed the epidermis to be collected. This procedure effected a 3-fold increase in the specific activity of DDC over that found in whole larvae. In an attempt to determine the extent to which such mass-isolated epidermis is contaminated with other tissues, late third instar larvae were fed [^{35}S]methionine for 13 hr. Epidermis was then prepared as above or by careful hand dissection, and homogenates of the two preparations were subjected to $NaDodSO_4$ gel electrophoresis. The results indicated a close correspondence in the patterns of the labeled proteins so that it would appear that the mass isolation procedure yields epidermis relatively free of other tissues.

Approximately 5 g of larval epidermis were homogenized at 4°C in 50 ml of extraction buffer [100 mM NaCl/2 mM $MgCl_2$/1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid/0.5% $NaDodSO_4$/10 mM Tris·HCl, pH 7.4] and centrifuged at 12,000 × *g* for 15 min, and the supernatant was extracted with 2 vol of phenol/chloroform/isoamyl alcohol (50:49:1, vol/vol). The aqueous layer was removed and reextracted twice as above and once with chloroform/isoamyl alcohol (24:1, vol/vol). The final aqueous layer was brought to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: DDC, dopa decarboxylase.

‡ To whom reprint requests should be addressed.

0.2 M NaCl and 2.5 vol of 95% ethanol was added. The RNA was precipitated for at least 8 hr at −20°C, collected by centrifugation, and dissolved in buffer (10 mM Tris·HCl/0.05% $NaDodSO_4$, pH 7.4). The solution was brought to 0.2 M NaCl, and the RNA was reprecipitated as above and finally dissolved in 10 mM Tris·HCl/0.05% $NaDodSO_4$, pH 7.4.

Poly(A)-RNA was isolated by chromatography on oligo(dT)-cellulose (12). Approximately 0.25 g of oligo(dT)-cellulose (Type 7, P-L Biochemicals) was equilibrated in application buffer (10 mM Tris·HCl/0.5 M NaCl/0.5% $NaDodSO_4$, pH 7.4), and packed into a plastic syringe. The RNA to be applied was made up to 0.5 M NaCl and 0.5% $NaDodSO_4$, heated at 65°C for 5 min, quick cooled, and applied to the column at room temperature. Poly(A)-RNA was eluted with 10 mM Tris·HCl/0.05% $NaDodSO_4$, pH 7.4, the eluate was made up to 150 mM sodium acetate (pH 5.5), and the RNA was precipitated with 2.5 vol of 95% ethanol. The precipitated RNA was dissolved in distilled water and stored at −45°C.

Translation of Poly(A)-RNA. Rabbit reticulocytes were prepared and lysed according to Hunt and Jackson (13). The lysate was rendered mRNA dependent prior to use (14) by treatment with micrococcal nuclease (P-L Biochemicals). The translation reaction mixture contained 50% nuclease-treated lysate, 10 mM Hepes/KOH (pH 7.7), 100 mM potassium acetate, 0.5 mM magnesium acetate, 10 mM creatine phosphate, 25 μM amino acids (minus methionine), and [^{35}S]methionine, added as described in the figure legends. The reactions proceeded at 30°C for 60 min. Total incorporation was measured after decolorization (15) of samples fixed on filter paper discs prepared according to Mans and Novelli (16). The remainder of each reaction mixture was made 10 mM in methionine and used for immunoprecipitation and analysis on $NaDodSO_4$/polyacrylamide gels.

Preparation of Antibody. Monospecific antiserum (raised in 12- to 16-week-old male San Juan rabbits) against purified DDC was prepared previously (9). Control serum was collected from the same rabbits 1 week prior to the first injection of antigen. Ammonium sulfate was added to the sera at 45% of saturation and the precipitates were collected by centrifugation, resuspended in 10 mM sodium phosphate, pH 7.5, and dialyzed overnight against the same buffer. The dialyzed sera were loaded onto columns of DEAE-cellulose (Whatman DE32) and the fractions containing IgG (17) were collected and concentrated.

Immunoprecipitation of Labeled DDC. Heat-killed *Staphylococcus aureus* was used as a solid-phase immunoadsorbent to precipitate labeled DDC. Immunoprecipitation reactions were carried out essentially as described by Kessler (18). Protein samples from cell-free translation mixtures or crude epidermal extracts containing 3–20 × 10^5 cpm were diluted 1:1 with 150 mM NaCl/5 mM EDTA/50 mM Tris·HCl/0.02% sodium azide, pH 7.2 (NET) + 0.5% Triton X-100. The suspension was incubated with 36 μg of preimmune IgG for 2 hr at 4°C. Fifty microliters of *S. aureus* suspension (10%) was added, and after incubation for 30 min the cells were removed by centrifugation at 2000 × *g* for 10 min. This preadsorption step was repeated twice to minimize nonspecific binding of radioactivity during the subsequent immunoprecipitation. The supernatants were mixed with 25 μg of anti-DDC IgG, or preimmune IgG in the case of the control, and incubated at 4°C for 12 hr. Fifty microliters of *S. aureus* was added, and the cells were collected after a 30-min incubation period. The cells were washed five times with NET + 0.05% Triton X-100, the final suspensions were transferred to fresh tubes, and after centrifugation the cells were resuspended in 100 μl of reducing buffer (3% $NaDodSO_4$/5% 2-mercaptoethanol/10% glycerol/62.5 mM Tris·HCl, pH 6.8, wt/vol/vol/vol). These suspensions were boiled for 5 min, and cell debris was removed by centrifugation at 4500 × *g* for 15 min. The supernatants, containing released proteins, were loaded directly onto $NaDodSO_4$/polyacrylamide gels for analysis.

RESULTS

Ecdysteroid Titer and DDC Activity During Development. The data in Fig. 1 show that during the life cycle of *Drosophila* five peaks of DDC activity appear. These occur at times when cuticular structures must be hardened. Five maxima in ecdysteroid titer are also evident, and these occur prior to the DDC maxima in every case. However, the intervals between the hormone and enzyme maxima vary considerably. At pupariation the two peaks are virtually coincident, whereas the titer of ecdysteroids rises to a maximum well before DDC ac-

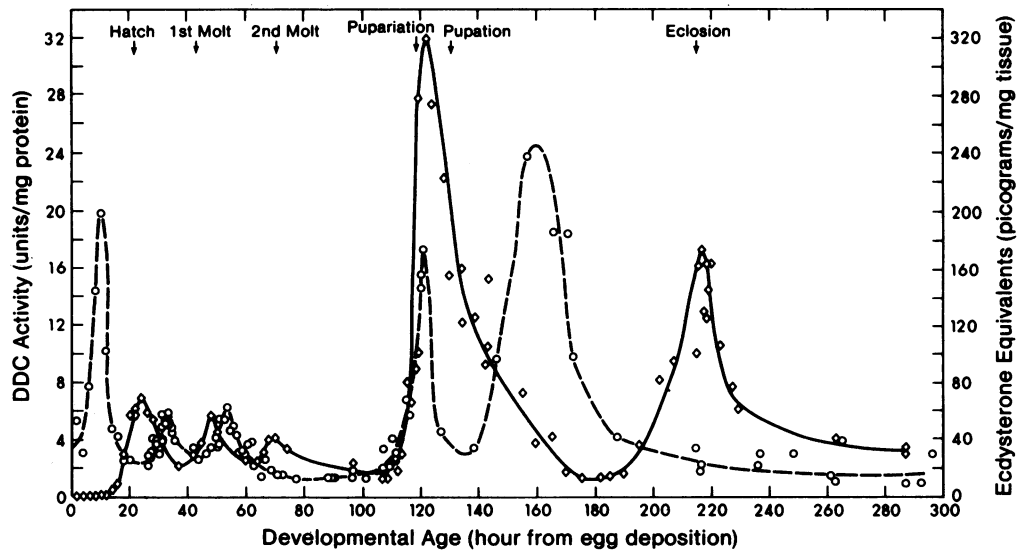

FIG. 1. DDC activity (△) and ecdysteroid titer (○) during the life cycle of the Canton-S strain of *D. melanogaster* at 25°C. Enzyme activity was determined by the $^{14}CO_2$ microdiffusion method (9) on crude extracts prepared by homogenizing whole organisms, at a concentration of 50 mg/ml, in 50 mM Tris·HCl/1 mM phenylthiourea (pH 7.4, 4°C) and centrifuging at 29,000 × *g* for 10 min. The supernatants were assayed in triplicate and the average values are plotted. The ecdysteroid titers were determined as described (19). A portion of the hormone data has been replotted from Hodgetts *et al.* (19).

tivity appears at the other stages. In the following we examine the mechanisms that lead to the appearance of DDC activity at pupariation.

Cell-Free Translation of Poly(A)-RNA and Identification of DDC mRNA. The mRNA-dependent reticulocyte system was used to translate poly(A)-RNA obtained from larval epidermis. As shown in Fig. 2 (slots 1 and 2), translation of poly(A)-RNA from late third instar larvae favors production of the smaller polypeptides that are synthesized *in vivo*. However, some polypeptides with molecular weights exceeding 66,000 are synthesized (see also Fig. 5, slots 1–3). A mixture of the polypeptides translated *in vitro* from epidermal RNA obtained from late third instar larvae was subjected to immunoprecipitation. Slot 3 shows a single labeled polypeptide precipitated from the translation reaction. Less of this same product was precipitated from *in vitro* translation reactions primed with poly(A)-RNA extracted from the epidermis of mid-third instar larvae (slot 4). Because the total radioactivity incorporated into protein was the same in both the mid- and late third instar extracts, the concentration of mRNA that directed the synthesis of this species was at a much higher concentration in the latter.

Three pieces of data convince us that the labeled polypeptide seen in slots 3 and 4 of Fig. 2 was the DDC monomer. First, the migration of this polypeptide on $NaDodSO_4$ gels was identical to that of purified DDC. Second, a polypeptide comparable to this translation product can be detected amongst the epidermal proteins of late third instar larvae. This is demonstrated in slot 5. Third, when the extract analyzed in slot 5 was treated with preimmune IgG, no precipitable product similar to that seen in slots 3–5 was detected (slot 6). Similar results were obtained when translation mixtures were treated with preimmune IgG (data not shown).

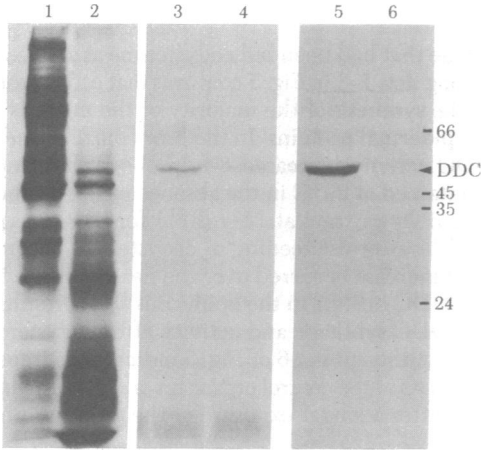

FIG. 2. Immunoprecipitation of DDC from proteins labeled *in vivo* and *in vitro*. Larval proteins were labeled *in vivo* by feeding 50 mg of nearly mature wild-type larvae 40–50 μCi of [^{35}S]methionine (Amersham, 1000–1200 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) in 70 μl of 1% sucrose in small glass petri dishes. After 10 hr the epidermis was prepared, and an extract was made (see legend to Fig. 1). Larval proteins were synthesized *in vitro* in the mRNA-dependent reticulocyte translation system in response to poly(A)-RNA from both mid- and late third instar larvae. RNA was used at 20 μg/ml, [^{35}S]methionine at 1 mCi/ml. Labeled proteins were subjected to immunoprecipitation and analyzed on a $NaDodSO_4$/polyacrylamide gel (20). Fluorographs (21) of the dried gels are shown. Slots 1 and 2 contain the total epidermal proteins labeled *in vivo* and *in vitro*, respectively. Immunoprecipitates from translation reactions programed with poly(A)-RNA from late third instar larvae and mid-third instar larvae are shown in slots 3 and 4, respectively. Slot 5 contains an immunoprecipitate from larval proteins labeled *in vivo*. As a control, *in vivo*-labeled proteins were treated with preimmune IgG rather than anti-DDC IgG (slot 6). Molecular weight markers (Sigma, MW-SED-70) are shown ($\times 10^{-3}$), and the arrow indicates the position of purified DDC electrophoresed on the same gel.

These results, which demonstrate a large increase in the amount of translatable DDC mRNA between mid- and late third instar, are in accord with those reported for *Calliphora* (5). Difficulty in staging the relatively large population of larvae used probably accounts for the low level of DDC message found in the epidermis of the mid-third instar sample. To avoid this problem and to determine whether the appearance of translatable mRNA at pupariation could be a primary response of the epidermal cells to the hormone, the target tissue was exposed to an experimentally effected increase in hormone titer. This was accomplished by using the *ecd*^{1} mutant.

Induction of DDC Activity in *ecd*^{1} Larvae. The experimental protocol we adopted involved growing a large sample of *ecd*^{1} larvae at the permissive temperature until mid-third instar. The culture was then shifted to 29°C and maintained at this temperature. After 2 days the experimental organisms were fed ecdysterone while control organisms were maintained in its absence.

The DDC activity in *ecd*^{1} mutants transferred to 29°C at mid-third instar remained at the low level characteristic of this stage for several days. By contrast, a significant increase in enzyme activity was demonstrable 4 to 6 hr after the administration of the hormone (Fig. 3). In other experiments increased DDC activity was observed as early as 2 hr after hormone administration. The first puparia began to appear about 16 hr after feeding, at which time the enzyme activity had increased to 50% of the maximum value observed at pupariation during normal development (Fig. 1).

Induction of *de Novo* Synthesis of DDC in the Epidermis of *ecd*^{1} Larvae. Mutant larvae were fed ecdysterone and [^{35}S]methionine at the restrictive temperature and samples were removed at 2, 4, and 8 hr for analysis. The labeled epidermal proteins were compared to those synthesized in larvae fed only on [^{35}S]methionine (Fig. 4*A*). Feeding ecdysterone produced few readily discernible changes in the synthesis of the major classes of epidermal proteins. A similar result has been reported by Lepesant *et al.* (22), who were using a more refined ana-

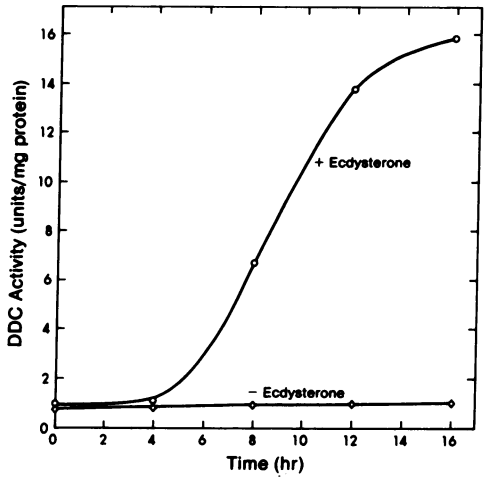

FIG. 3. Induction of DDC activity in *ecd*^{1} larvae fed ecdysterone. Eggs were collected over 4-hr intervals and cultures were maintained at the permissive temperature, 20°C, for 150 hr. These were then transferred to the restrictive temperature, and, after 48 hr at 29°C, 100-mg groups of larvae were transferred to 3-cm glass petri dishes containing 100 μl of a solution of 2.5% ethanol/1% sucrose/± ecdysterone (Sigma, lot no. 18C-04831, 0.5 mg/ml). Crude extracts of larval samples collected at the times indicated were prepared and the DDC activity was measured.

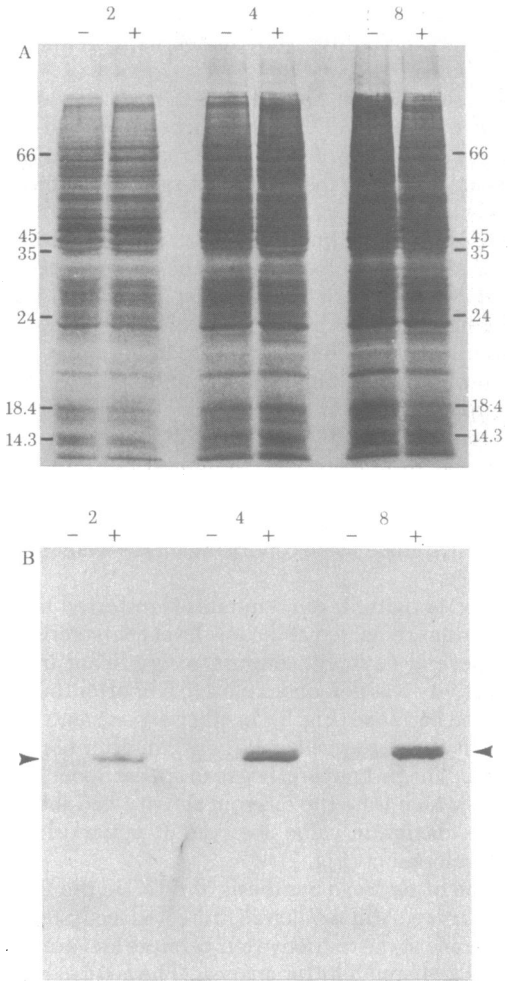

FIG. 4. *In vivo* synthesis of DDC in the epidermis of *ecd*^{1} larvae fed ecdysterone. Larvae were cultured as described in the legend to Fig. 3. Beginning 48 hr from the time of transfer to the restrictive temperature, they were fed [^{35}S]methionine ± ecdysterone for 2-, 4-, or 8-hr periods prior to the preparation of epidermis and extraction of proteins from it. Pairwise comparisons of labeled polypeptides displayed on dried NaDodSO$_4$/polyacrylamide gels are shown (−, controls; +, ecdysterone-fed larvae). The positions of molecular weight markers ($\times 10^{-3}$) are indicated. Also, the position of the internal marker rabbit IgG heavy chain (M_r 53,000) is indicated by the arrow. The subunit molecular weight of DDC is 54,000 (9). (*A*) Autoradiography of total *in vivo*-labeled products. (*B*) Fluorography of the polypeptide immunoprecipitated from the labeled products.

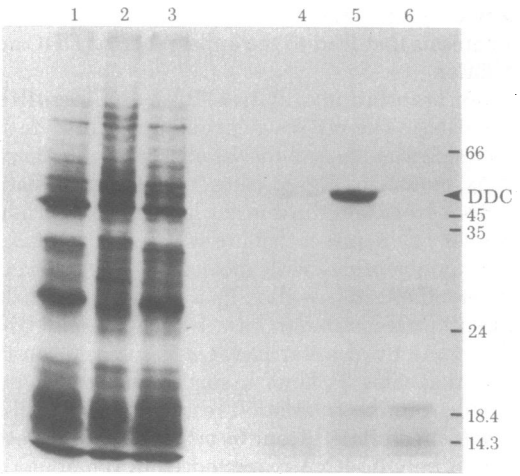

FIG. 5. Induction of DDC mRNA in *ecd*^{1} larvae. Large batches of *ecd*^{1} larvae were raised at 20°C and transferred to 29°C at mid-third instar. After 2 days, 15-g batches were placed in trays containing 7 ml of a solution of ecdysterone at 0.5 mg/ml in 2.5% ethanol/1% sucrose for periods of 2 or 8 hr. As a control, another group of larvae was fed the solution minus ecdysterone. Additional solution was added to the trays when they became dry. Poly(A)-RNA prepared from the epidermis of each group of larvae was used for *in vitro* translation. [^{35}S]Methionine was used in the translation reactions at 1 mCi/ml (1200 Ci/mmol). The fluorogram of the NaDodSO$_4$/polyacrylamide gel shows the total products of translation reactions programed with poly(A)-RNA from larvae fed on ecdysterone for 2 hr (slot 1), 8 hr (slot 2), and the control (slot 3), and the immunoprecipitates from each reaction, respectively (slots 4, 5, and 6). The epidermal DDC activity in each group of larvae was determined and found to be constant in the controls, 1.2 times greater in the 2-hr induced sample, and 7.8 times greater in the 8-hr induced sample.

lytical gel. They found only five new polypeptides synthesized in the fat body of *ecd*^{1} larvae fed ecdysterone.

To determine whether an accumulation of newly synthesized DDC molecules had occurred in larvae fed ecdysterone, extracts of *in vivo*-labeled proteins were subjected to immunoprecipitation. The immunoprecipitates, which were analyzed by NaDodSO$_4$ gel electrophoresis (Fig. 4*B*, + slots), consisted of a single polypeptide with essentially the same molecular weight as the marker, rabbit IgG heavy chains (53,000). The amount of this labeled polypeptide increased significantly during the feeding period. By comparison, this species remained at a very low level throughout the experiment in the larvae not fed ecdysterone (Fig. 4*B*, − slots). The significant increase in labeled DDC observed over the 8-hr period during which ecdysterone was administered correlates well with the increase in DDC activity (Fig. 3).

Induction of Translatable DDC mRNA in the Epidermis of *ecd*^{1} Larvae. Poly(A)-RNA was obtained from the epidermis of *ecd*^{1} larvae that had been fed ecdysterone at 29°C. A comparison among slots 1–3 in Fig. 5 confirms that ecdysterone does not affect the synthesis of the majority of the mRNAs for the prevalent epidermal proteins. In the 8-hr course of the experiment, DDC activity increased 8-fold above that in control larvae maintained at 29°C in the absence of the hormone. As slot 4 of Fig. 5 shows, translatable mRNA for DDC was present as early as 2 hr after the feeding of ecdysterone began, and a pronounced increase occurred over the next 6 hr (slot 5). This increase in DDC mRNA in the epidermis likely results in the increase in DDC synthesis and activity after ecdysterone administration. Although slot 6 of Fig. 5 indicates no translatable mRNA for DDC in the control organisms, a slight amount could be detected after a longer exposure of the fluorograph.

DISCUSSION

Fig. 1 shows an analysis of ecdysteroid levels throughout the entire life cycle of *D. melanogaster*. Qualitatively similar patterns of ecdysteroid titers have been observed for larvae of the cyclorraphan *Sarcophaga bullatas*. (S. L. Wentworth, B. Roberts, and J. D. O'Connor, unpublished observations). Several points warrant discussion. First, an obvious peak in the titer of the hormone occurs about mid-embryogenesis. Interestingly, the ring gland, which is required for ecdysterone production in mature *Drosophila* larvae (23, 24), does not develop until 11–12 hr (25). Because an elevated ecdysteroid titer was seen as early as 6 hr, the production of the hormone during embryogenesis must have a different physiological basis than at pupariation. Ecdysteroids are found in the embryos of a number of insects (26–28) and often prior to the development of the prothoracic gland. Lagueux *et al.* (27, 29) present evidence that such ecdysteroids are derived from polar conjugates deposited in the developing oocyte by the follicle cells of the mother.

Second, ecdysteroid peaks occur in both the first and second larval instars almost exactly midway between the molts. Thus, the transition from cuticle synthesis to degradation that occurs at these times (30) may be triggered by the hormone. Twelve hours after the molt into third instar, a small increase in hemolymph ecdysteroids has been observed (31). However, no switchover to cuticle degradation occurs in third instar and at present we do not know of any developmental correlate to this hormone increase.

The fate of steroid hormones upon entering target cells has been reviewed recently (32, 33); a very early response of these cells appears to be an increase in the amount of translatable mRNA from a specific set of genes (34–36). From this work and the study of *Calliphora* (5), it is apparent that the increase in the amount of translatable mRNA for DDC that occurs just prior to pupariation is correlated with a high ecdysteroid titer. To investigate further the relationship between the hormone and the appearance of DDC mRNA, experimental control over ecdysterone dose was required. This was effected by feeding the hormone to *ecd*[1] larvae maintained at 29°C. The results demonstrate that increased DDC synthesis occurred as early as 2 hr after the target epidermal cells had been exposed to ecdysterone. The cause of the increased DDC production is presumably the observed substantial increase in the amount of translatable mRNA for DDC.

An increase in translatable RNA is not proof that the hormone induced transcription of the gene. Ecdysterone could effect the posttranscriptional modification of the DDC transcript and thereby control its translation. The data on the induction of ovalbumin and conalbumin by estrogen (35) show that in these cases the increase in translatable mRNA was accompanied by a parallel increase in the amount of RNA complementary to a cDNA probe. However, even monitoring the quantity of transcript directly by using a cDNA probe cannot exclude the possibility that a hormone controls the stability of a messenger species produced constitutively in the nuclei of both target and nontarget cells (37).

Ashburner *et al.* (38) have shown that a set of "early" puffs appear in salivary gland chromosomes of *Drosophila* within a few hours of exposure to ecdysterone. Because appearance of these puffs was not inhibited by cycloheximide, they concluded that the activation of these genes was a primary response to the hormone. In contrast, a set of "late" puffs appeared with a lag of at least 3 hr. Because appearance of the late puffs was prevented by the inhibition of protein synthesis, these puffs were viewed as a secondary response to the hormone. An increase in translatable DDC mRNA occurs within 2 hr after exposure to ecdysterone. This, by analogy to the early puffs discussed above, may well represent a primary response of the epidermal cells to the hormone.

Palmiter *et al.* (35) present evidence that the 3-hr delay in the appearance of ovalbumin after estrogen administration is not a secondary response to the hormone. These authors attribute the delay to the traverse time of the hormone–receptor complex from a distant binding site to the structural gene. We feel it is unlikely that this model explains the long delays observed between the appearance of ecdysterone and DDC at stages in the life cycle other than pupariation (see Fig. 1). If indeed the prior appearance of the hormone is required for DDC activity at these stages, the activation of gene expression is more likely a secondary or higher-order effect. Study of the relationship between hormone levels and DDC activity in embryos may shed light on this problem.

We are indebted to Paul Pass for the development of the technique to label DDC *in vivo*. This work was supported by grants from the Natural Sciences and Engineering Research Council Canada (A6477), the National Institutes of Health (NS-08990), and the National Science Foundation (PCM 78-05471).

1. Dewhurst, S. A., Croker, S. G., Ikeda, K. & McCaman, R. E. (1972) *Comp. Biochem. Physiol. B* **43**, 975–981.
2. Sekeris, C. E., Karlson, P. & Congote, L. F. (1971) in *The Action of Hormones*, ed. Foa, P. P. (Thomas, Springfield, IL), pp. 7–19.
3. Karlson, P. & Sekeris, C. E. (1962) *Biochim. Biophys. Acta.* **63**, 489–495.
4. Karlson, P. (1963) *Perspect. Biol. Med.* **6**, 203–214.
5. Fragoulis, E. G. & Sekeris, C. E. (1975) *Eur. J. Biochem.* **51**, 305–316.
6. Shaaya, E. & Sekeris, C. E. (1965) *Gen. Comp. Endocrinol.* **5**, 35–39.
7. Hodgetts, R. B. (1975) *Genetics* **79**, 45–54.
8. Wright, T. R. F., Bewley, G. C. & Sherald, A. F. (1976) *Genetics* **84**, 297–310.
9. Clark, W. C., Pas, P. S., Venkataraman, B. & Hodgetts, R. B. (1978) *Mol. Gen. Genet.* **162**, 287–297.
10. Garen, A., Kauvar, L. & Lepesant, J.-A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5099–5103.
11. Nash, D. & Bell, J. B. (1968) *Can. J. Genet. Cytol.* **10**, 82–90.
12. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
13. Hunt, T. & Jackson, R. J. (1974) in *Modern Trends in Human Leukemia*, eds. Neth, R., Gallo, R. C., Spiegelman, S. & Stahlman, F. (J. F. Lehmanns, Munich, Germany), pp. 300–307.
14. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
15. Woodward, W. R., Ivey, J. L. & Herbert, E. (1974) *Methods Enzymol.* **30**, 724–731.
16. Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53.
17. Andres, R. Y. (1976) *Eur. J. Biochem.* **62**, 591–600.
18. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
19. Hodgetts, R. B., Sage, B. & O'Connor, J. D. (1977) *Dev. Biol.* **60**, 310–317.
20. Kikuchi, Y. & King, J. (1975) *J. Mol. Biol.* **99**, 645–672.
21. Chamberlain, J. P. (1979) *Anal. Biochem.* **98**, 132–135.
22. Lepesant, J.-A., Lepesant, J. K. & Garen, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5570–5574.
23. Vogt, M. (1943) *Naturwissenschaften* **31**, 200–201.
24. Bodenstein, D. (1944) *Biol. Bull.* **86**, 113–124.
25. Poulson, D. F. (1965) in *Biology of Drosophila*, ed. Demerec, H. (Hafner, New York), pp. 168–274.
26. Kaplanis, J. N., Dutky, S. R., Robbins, W. E., Thompson, M. J., Linquist, E. L., Horn, D. H. S. & Galbraith, M. N. (1975) *Science* **190**, 681–682.
27. Lagueux, M., Hirn, M. & Hoffmann, J. A. (1977) *J. Insect Physiol.* **23**, 109–119.
28. Bullière, D., Bullière, F. & de Reggi, M. (1979) *Wilhelm Roux Arch. Entwicklungsmech. Org.* **186**, 103–114.
29. Lagueux, M., Hetru, C., Goltzene, F., Kappler, C. & Hoffmann, J. A. (1979) *J. Insect Physiol.* **25**, 709–723.
30. Mitchell, H. K., Weber-Tracy, U. M. & Schaar, G. (1971) *J. Exp. Zool.* **176**, 429–443.
31. Berreur, P., Porcheron, P., Berreur-Bonnefaut, J. & Simpson, P. (1979) *J. Exp. Zool.* **210**, 347–352.
32. Yamamoto, K. R. & Alberts, B. M. (1976) *Annu. Rev. Biochem.* **45**, 721–746.
33. Schrader, W. T. & O'Malley, B. W. (1978) in *Receptors and Hormone Action*, eds. O'Malley, B. W. & Birnbaumer, L. (Academic, New York), Vol. 2, pp. 189–224.
34. Chan, L., Means, A. R. & O'Malley, B. W. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1870–1874.
35. Palmiter, R. D.,.Moore, P. B. & Mulvihill, E. R. (1976) *Cell* **8**, 557–572.
36. Killewich, L. A. & Feigelson, P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5392–5396.
37. Davidson, E. H. & Britten, R. J. (1979) *Science* **204**, 1052–1059.
38. Ashburner, M., Chihara, C., Meltzer, P. & Richards, G. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 645–662.