contamination of lysine-containing peptides in the more-electropositive $1D_1$ peptide.

The replacement of aspartic acid by glycine would explain the difference in electrophoretic behavior of transferrin C and transferrin D_1 at alkaline *p*H. Transferrin C would have a greater negative charge and therefore would migrate more rapidly to the anode. In view of the great genetic variability in human transferrin, it is possible that other amino acid changes also exist between transferrin C and transferrin D_1. However, the one reported here is sufficient to explain the observed difference in mobility.

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Molt and Intermolt Activities in the Epidermal Cells of an Insect

Abstract. *In the larva of the butterfly* Calpodes ethlius, *molt and intermolt syntheses by the epidermis are each preceded by a phase of RNA synthesis. Endocuticle deposition and the secretion of wax are not controlled only by the molting hormone when they take place during the intermolt period.*

In the fifth-instar larva of *Calpodes ethlius* (Lepidoptera, Hesperiidae), two molting activities of the epidermis, wax secretion and endocuticle deposition, recur during the intermolt period. Some endocuticle is deposited throughout the stadium, but the rate of deposition is low until about 2½ days after the fourth to fifth molt, 2 days after feeding has begun. The fifth stadium lasts about 7 days, during which time an endocuticle about 7 to 8 times as thick (70 to 100 μ) as that in the fourth stadium (10 to 14 μ) is laid down. This massive reserve of material is resorbed at the end of the stadium during the construction of the pupa (*1*). The deposition of endocuticle after feeding is not unusual in insects (*2*).

We have studied the control of the deposition of this endocuticle autoradiographically, using tritiated glucose as a marker for chitin and tritiated tyrosine for the protein component. Glucose is incorporated in clearly defined bands, presumably in the chitin component of the lamellae (Fig. 1*a*). Larvae in the middle of the stadium continue to deposit cuticle next to the epidermis even when the nerve cord has been cut to induce starvation (Fig. 1*b*). Larvae with the head ligated go on to pupate but do not deposit layered endocuticle (Fig. 1*c*). The nutritional state and the amount of ecdysone are thus adequate for pupation, but some stimulus for the deposition of endocuticle during the intermolt period is missing. The deposition of endocuticle during the intermolt period may thus be similar to the secretion of wax in this period, which has been found to require the presence of both the prothoracic gland and some factor from the head for its continuation (*3*). We may conclude that the epidermis is influenced in its syntheses during the intermolt period by factors in addition to the molting hormone and nutrition.

If the molt and intermolt phases of synthetic activity in the epidermal cells are causally independent, one might expect two periods of preparation on the part of a cell. We obtained evidence for this hypothesis from autoradiographic studies of the rates of RNA synthesis at different times in the stadium, using tritiated uridine (10 μc/gram-weight of *Calpodes* larva, in 0.1 ml Ringer solution) as a marker. The solution was injected into the haemocoel; 4 hours later the larvae were fixed and sections were prepared for autoradiography. Control sections incubated in ribonuclease showed little incorporation of tritium. Most of the tissues showed that some tritium was incorporated, maximum incorporation being related to molting, but the epidermal cells which secrete endocuticle and the cells which later also secrete wax showed two peaks of activity in each stadium. Figure 2 shows the changes in rate of RNA synthesis in the molt-intermolt cycle. There is a peak just before the molt toward the end of the fourth stadium, a peak just before the intermolt period at about a day after ecdysis, and another peak shortly before pupation. The pupa deposits little if any intermolt endocuticle and it shows no increase in RNA synthesis before the intermolt period. We shall refer to the peak before molting as the premolt peak and the peak preceding the intermolt period as the preintermolt peak. The pattern of RNA synthesis is not attributable to the synthetic activity associated with nuclear division. The number of labeled nuclei after the incorporation of tritiated thymidine for 4 hours reaches a broad maximum toward the end of the stadium. These results suggest that the preparation on the part of a cell for the syntheses involved in molting is not

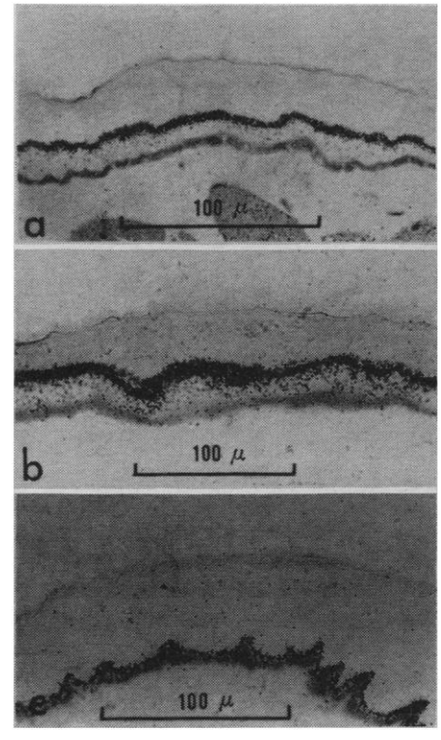

Fig. 1. Autoradiographs showing the uptake of tritiated glucose (dose, 10 μc per gram live weight of larva) and its deposition in the endocuticle after a 12-hour period of incorporation (fixed in 4 percent formaldehyde at *p*H 7). (*a*) Control larva; the silver grains extend in a dense band at a distance from the epidermis. (*b*) Larva starved and quiescent after the nerve cord had been cut between the first and second thoracic segments 24 hours before the beginning of the incorporation period. The incorporation of labeled glucose still occurs at a distance from the epidermis. (*c*) Larva which had had the head ligated 24 hours before the incorporation period. The silver grains lie next to the epidermis and there has been little deposition of endocuticle, although this larva would have gone on to pupate.

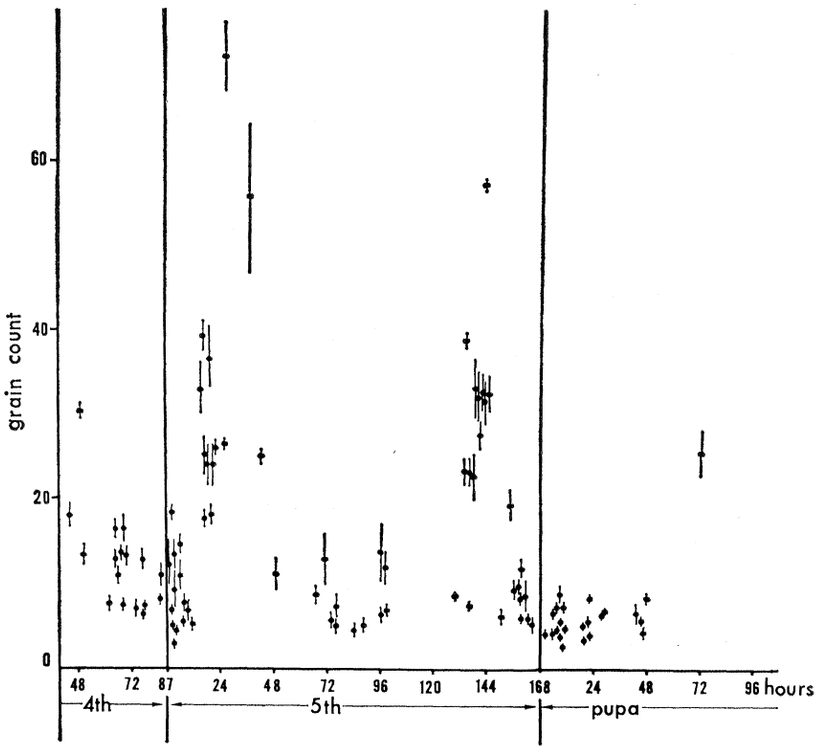

Fig. 2. The synthesis of RNA by the epidermis in the molt-intermolt cycle. Larvae were injected with tritiated uridine (10 μc per gram) for a 4-hour incorporation period at various times in the development of the fourth, fifth, and pupal stages (fixed in 5 percent glutaraldehyde at *p*H 7, as for electron microscopy). Ordinate, grain counts per 100 μ^2; abscissa, mean incorporation time. Each point is the mean of counts from one larva. There are peaks of activity before each molt and before the intermolt in the fifth stadium.

necessarily appropriate for similar syntheses which take place in the intermolt.

The premolt peak of RNA synthesis coincided with the critical period for the operation of the prothoracic gland, which confirmed a similar observation upon *Rhodnius* (*4*). If the premolt peak of RNA synthesis is an immediate result of the action of ecdysone, one may ask what controls the pre-intermolt peak. Experiments are complicated by the fact that newly molted larvae are susceptible to starvation. However, starved larvae made quiescent by severing the nerve cord still show a pre-intermolt peak of RNA synthesis, although the peak is lower than normal. Thus, although starvation has some effect upon the pre-intermolt peak of RNA synthesis, it does not suppress it. Nor is RNA synthesis suppressed by ligating the head or the prothorax. Table 1 shows that after these procedures the rate of incorporation of tritiated uridine still rises as much as in the starved larvae, and in some may even exceed that in control larvae allowed to feed. Some tissues, such as the fat body, responded much like this epidermis; in others, such as muscle, the rate of incorporation was markedly lowered by starvation and ligation. The consistent response of these other tissues makes the results from the epidermis more significant. We may conclude that the synthesis of RNA in this epidermis must be initiated at ecdysis or earlier.

Table 1. Synthesis of RNA during the pre-intermolt peak after operations performed immediately after ecdysis. Twenty-two larvae were operated on within 1 to 1½ hours of molting to the fifth instar. They were injected with tritiated uridine in Ringer solution (dose 10 μc/gram of body weight) after 12, 24, or 36 hours. After 2 hours they were fixed and autoradiographs prepared. The grain density over that part of the epidermis destined to secrete both endocuticle and wax is given per 100 μ^2. Incorporation of tritiated uridine in the ligated larvae is not significantly lower than that in the starved controls. Nearly all the ligated larvae have a higher incorporation after 24 or 36 hours.

Type of operation	Time after operation		
	12 hr	24 hr	36 hr
Control	38 ± 2.2	50 ± 2.4	40 ± 2.0
	35 ± 1.5	52 ± 1	42 ± 1.7
Nerve cord cut	30 ± 1.4	42 ± 2.4	36 ± 1.5
	23 ± 1.8		33 ± 2.1
Head ligated	23 ± 1.4	40 ± 1.8	53 ± 1.9
	32 ± 1.6	36 ± 1.4	
Prothorax ligated	34 ± 2.5	32 ± 1.8	50 ± 2.5
	23 ± 2.1	42 ± 2.9	30 ± 1.1

The control of intermolt activities therefore depends on a complicated meshwork of intrinsic and extrinsic factors. By intrinsic we mean that the cell at that time is independent of external agents (other than substrates) which modify the occurrence and rate of the synthesis. By extrinsic we mean that the cell is being continuously monitored from without with respect to type and rate of synthesis. At some time before molting the epidermis receives a command to synthesize RNA. This is perhaps the last effect of ecdysone stimulation in the previous stadium. As a result of this command, RNA synthesis is at a maximum 30 hours after ecdysis. Twelve or more hours later the intermolt activities of endocuticle deposition and wax secretion have begun.

Similar syntheses taking place at molting are not controlled in this way. A dense bloom of wax appears on the pupa about 12 to 24 hours after ecdysis. This bloom still appears in abdomens surgically isolated within minutes of eclosion from the larval exuvium. Thus, unlike the secretion of wax during the intermolt period, the immediate post-ecdysial wax bloom is an event determined at an earlier stage and is probably the terminal event in a sequence initiated by ecdysone. Similarly, lamellate endocuticle is secreted by the epidermis at molting even after decapitation in the preceding stadium. Studies of the extrinsic controls of intermolt syntheses may give a clue to the mode of action of the intrinsic controls of the events at molting.

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