

## THE DISAPPEARANCE OF MOULTING FLUID IN THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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(Received 23 May 1983–Accepted 27 June 1983)

In *Manduca sexta*, and other insects, moulting fluid (MF) accumulates after apolysis in the exuvial space which underlies the old cuticle, and then most of it disappears shortly before ecdysis (see reviews by Zacharuk, 1976; Jungreis, 1979). Moulting fluid digests and weakens the old endocuticle so that the animal may free itself at ecdysis, and the digested components of the old endocuticle are reabsorbed. In *M. sexta*, Jungreis (1979) found that MF is produced between 30 and 12 h before the larval–pupal ecdysis (LPE) and it begins to disappear about 9 h before the LPE (LPE – 9 h). By LPE – 3 h, most, but not all, of the MF has disappeared. We have found that MF represents a significant fraction of the total body water, 14.7 % body weight (bw) [ $0.579 \pm 0.042$  (5) ml, mean  $\pm$  s.e. (N)] at LPE – 6 h.

It has been reported that the integument reabsorbs MF (nymph–adult ecdysis, *Rhodnius prolixus*, Wigglesworth, 1933; pupal–adult ecdysis, *Hyalophora cecropia*, Passonneau & Williams, 1953; Lensky, Cohen & Schneiderman, 1970) and that animals drink MF (fourth–fifth larval ecdysis, *Bombyx mori*, Watcher, 1930; elaterid larvae, Zacharuk, 1973). In previous studies of *M. sexta* at the LPE it was assumed that MF was reabsorbed by the integument (Jungreis, 1979); but, the properties of the isolated integument do not lend much support to this assumption (Cornell & Jungreis, 1981). Our studies of the LPE suggest that most of the MF is transferred directly to the gut.

At LPE – 12 h, the midgut contains a small volume [ $4.96$  % bw,  $0.205 \pm 0.029$  (8) g] of a brown viscous fluid; however, at LPE – 3 h, the midgut is full [ $19.3$  % bw,  $0.813 \pm 0.011$  (9) g] of a brown watery fluid (Fig. 1). There is good agreement between the time when MF disappears and the time when new fluid appears in the midgut. Furthermore, the volume increase in the midgut between LPE – 12 h and LPE – 3 h (14.3 % bw) is equivalent to the volume of MF. After the moult, the midgut volume declines to 5.42 % bw [ $0.209 \pm 0.025$  (8) g at LPE + 24 h].

Ligation experiments suggest that MF is transferred from the exuvial space to the midgut *via* two direct routes. When five specimens of *M. sexta* were anaesthetized with CO<sub>2</sub> and ligated between the head and prothorax and between the eighth and ninth abdominal segments (A<sub>8</sub> and A<sub>9</sub>) at LPE – 12 h, MF did not disappear. At LPE – 3 h, when MF has normally disappeared, 15.2 % bw of MF [ $0.622 \pm 0.030$  (5) ml] was collected and the midgut contained 4.67 % bw [ $0.179 \pm 0.040$  (5) g] of a dark brown viscous fluid. A single ligation between A<sub>8</sub> and A<sub>9</sub> at LPE – 12 h resulted in

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Key words: Ecdysis, integument, moulting fluid.

the disappearance of MF in five experiments. At LPE -3 h, the midguts of these animals were full [ $15.4\%$  bw,  $0.608 \pm 0.048$  (5) g]. In addition, a single ligation between the head and the prothorax at LPE -12 h produced results which were similar to those described above. Finally, in two animals a ligation was placed in the middle of the animal (between  $A_3$  and  $A_4$ ). In both cases MF disappeared from the exuvial space and the midgut contained a watery fluid on both sides of the ligation, although the ligation had separated the midgut into two sections. These results indicate that MF can be confined between two ligations but that it cannot be confined to either end of an animal by a single ligation. Thus, there are at least two routes for the disappearance of MF, an anterior route and a posterior route.

One difficulty is that the ligations would be expected to prevent the normal circulation of haemolymph and hormonal factors. To avoid this difficulty, five animals were anaesthetized with  $\text{CO}_2$  at LPE -12 h and a ring of old cuticle was removed from segments  $A_1$  and  $A_7$ . The two exposed rings of integument were dried with compressed air, and cyanoacrylate glue was used to seal the old cuticle to the integument. At LPE -12 h this may be carried out since MF does not readily leak past the inter-segmental connections between the integument and the old cuticle. These animals failed to reabsorb the MF trapped between  $A_1$  and  $A_7$  and their midguts contained little fluid [ $5.83\%$  bw,  $0.239 \pm 0.043$  (5) g]. Thus, the failure of the integument to reabsorb MF is not the result of disrupting the circulation.

Additional evidence comes from experiments in which a methylene blue solution was injected into the MF at either the anterior ( $N=9$ ) or posterior ( $N=9$ ) end of animals at 6-2 h before LPE. In every case, within 15-30 min of the injection, the dye was found in the midgut and either the foregut or the hindgut, depending upon the injection site (Fig. 2). One complication is that methylene blue crosses the integument and is taken up by the Malpighian tubules. (We have also found that  $^{14}\text{C}$ -inulin, or the detached  $^{14}\text{C}$  label, crosses the integument, but we do not know if it is taken up by the Malpighian tubules.) Thus, one might argue that the dye reached the midgut by this route; however, this would not explain its presence in the foregut and the midgut, and its absence from the hindgut, when dye was injected into the anterior exuvial space. Furthermore, when methylene blue was injected into the MF between two ligations (anterior to  $A_1$  and  $A_9$ ), only small traces of the dye crossed the integument during a 30-min period and none could be found in the foregut, the midgut or the hindgut. Most importantly, methylene blue did not cross the integument at a sufficient rate to account for its presence in the midgut at the concentrations which we observed in the 15- to 30-min test periods. Thus, any route which depends upon the passage of methylene blue across the integument cannot explain the presence of the dye in the midgut in our experiments. In dissected animals we have seen methylene blue being pumped from the hindgut, past the sphincter muscle, and into the midgut by peristaltic contractions which originated in the hindgut. Thus, before the LPE, MF is transferred to the midgut *via* the space between the old cuticular lining of the foregut and the foregut epithelium, and *via* the space between the old cuticular lining of the hindgut and the hindgut epithelium. These spaces provide two direct and continuous routes to the gut.

We cannot eliminate the possibility that MF is also reabsorbed by the integument; however, this possibility seems unlikely at the LPE. If all MF were reabsorbed, the

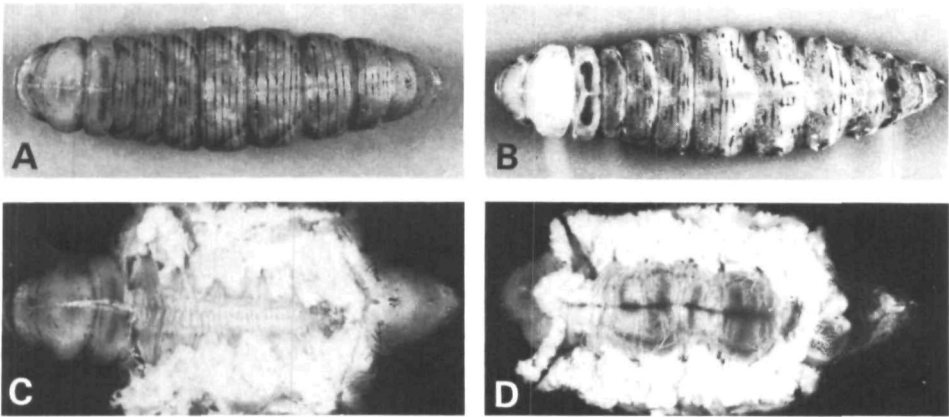


Fig. 1. The increase in the volume of the midgut between 12 and 3 h before the larval-pupal ecdysis (LPE). (A) External view at LPE - 12 h. (B) External view at LPE - 3 h. (C) Dissection of animal in (A) at LPE - 12 h. (D) Dissection of animal in (B) at LPE - 3 h.

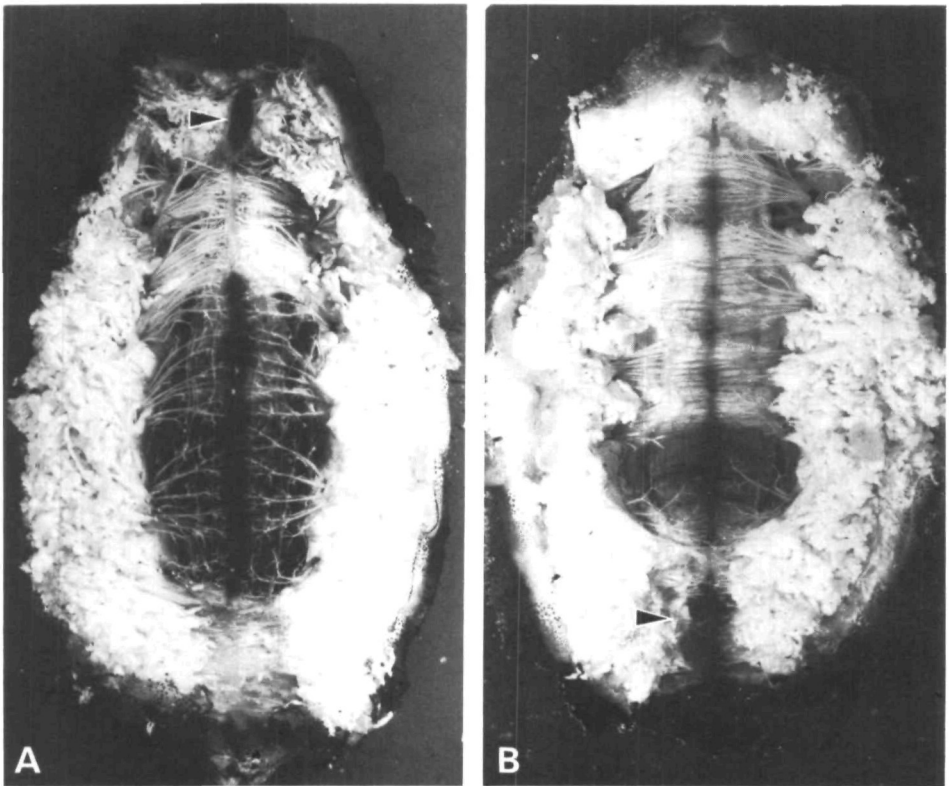


Fig. 2. The arrows indicate the uptake of methylene blue by the foregut (A) and the hindgut (B) 20 min after the injection of the dye into the anterior (A) or the posterior (B) regions of the exuvial space. In this particular case, a greater amount of the dye was present in the midgut of the animal in (A) at the end of the test period. This accounts for its darker appearance. No traces of the dye were found in the hindgut of the animal in (A) or the foregut of the animal in (B). The posterior portion of the foregut intima of the animal in (B) was stained dark brown, possibly from melanized moulting fluid.

integument would be expected to transport fluid at about  $10 \mu\text{l cm}^{-2} \text{h}^{-1}$ ; however, the properties of the isolated integument (Cooper & Jungreis, 1980; Cornell & Jungreis, 1981, 1983) do not favour this event and they differ greatly from those found in preparations known to transport fluid at this rate, e.g. the rectum of *Schistocerca gregaria* (Spring & Phillips, 1980; Phillips, Meredith, Spring & Chamberlin, 1982). In addition, the osmotic gradient *in vivo* opposes MF reabsorption. At LPE -6 h, the MF [ $445.7 \pm 29$  (10) mosmol  $\text{l}^{-1}$ : vapour pressure osmometer, Wescor] is hyperosmotic to the haemolymph [ $393 \pm 24$  mosmol  $\text{l}^{-1}$  (10)] by 53 mosmol  $\text{l}^{-1}$  ( $P < 0.001$ , paired *t*-test).

There is evidence that *M. sexta* takes MF directly into the gut at other developmental stages. About 20 min before the fourth-fifth larval ecdysis, MF appears in the head capsule. Immediately before this time the head capsule is empty since the head slips out of the head capsule many hours before the moult. If methylene blue is injected into the anterior portion of the exuvial space about 20 min before the moult, it enters the head capsule and some of it may be found in the midgut after the moult, suggesting that MF may be taken in *via* the foregut. Our attempts to demonstrate that MF is taken in through the hindgut at this stage have yielded no support for this idea. Watcher (1930) reported that *Bombyx mori* drinks MF at the fourth-fifth larval moult. Our observations are similar to those reported by Watcher, one difference being that *M. sexta* does not swallow air after swallowing MF, as does *B. mori*.

In *Rhodnius prolixus*, Wigglesworth (1933), found that MF disappeared when a ligation was placed behind the head, and that dyes (e.g. neutral red) crossed the integument when injected into the MF of animals ligated behind the head. These experiments were not designed to eliminate the possibility that MF is taken in *via* the hindgut. Others have also found that dyes cross the integument, e.g. Lensky *et al.* (1970) have shown that buffalo black is absorbed in the intersegmental regions of *H. cecropia* and we have found that neutral red and methylene blue are absorbed by the integument of *M. sexta*. It is clear that the integument is able to absorb a number of dyes, but this should be regarded as independent of its ability to absorb water.

Passonneau & Williams (1953) observed the disappearance of MF before the pupal-adult ecdysis (PAE) in *H. cecropia* through windows cemented to the cuticle. They found that MF disappeared in a normal fashion, either in isolated anterior or posterior ends of metamorphosing pupae, and suggested that MF is reabsorbed by the integument. We have found that both *H. cecropia* and *M. sexta* are able to take solutions into the gut *via* the foregut before the PAE, but there is no evidence that MF is taken in *via* the hindgut at this stage (Pan & Cornell, 1982). Thus, one might explain the disappearance of MF in isolated anterior ends of pharate adults, observed by Passonneau & Williams, by the intake of MF *via* the foregut, but the intake of MF *via* the hindgut does not seem to be a likely cause for the disappearance of MF in isolated posterior ends of pharate adults. The disappearance of MF before the PAE occurs over a period of several days compared to 3 h at the LPE. It is possible that during this extended time-period a significant fraction of the MF is reabsorbed by the integument.

In *M. sexta* MF is taken into the midgut *via* the foregut and the hindgut at the LPE. At the fourth-fifth larval ecdysis and at the PAE our results suggest that MF is taken *via* the foregut, but not *via* the hindgut. In no case can we eliminate the possibility

that MF is reabsorbed by the integument; however, it is unlikely that this occurs to a great extent at the LPE. The transfer of MF to the gut could occur in all arthropods. In particular, this mechanism for the recovery of MF may prove to be widely used among the insects. Its occurrence in elaterid larvae (Zacharuk, 1973) lends support to this idea. Finally, this mechanism provides a means for transferring the MF to a location from which it may be readily absorbed.

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