ROLE OF ACTIVE POTASSIUM TRANSPORT BY INTEGUMENTARY EPITHELIUM IN SECRETION OF LARVAL–PUPAL MOULTING FLUID DURING SILKMOTH DEVELOPMENT

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SUMMARY

1. The exuvial side of the pharate pupal integument is usually positive to the haemolymph-side, both in vivo and in vitro, during the period when the moulting fluid is being secreted.

2. The ratio of potassium flux toward the exuvial space is higher than that toward the haemolymph, under both open-circuit conditions and short-circuit conditions, demonstrating by the Flux Ratio test that potassium is actively transported across the isolated integument during this secretion period.

3. Just prior to ecdysis, while moulting fluid is being reabsorbed, the potassium flux ratios become unity, suggesting that active potassium transport has ceased, but the short-circuit current that remains suggests that some other ion is actively transported at this time.

4. We argue that the potassium salt solution, formed in the exuvial space (as water presumably follows the actively transported potassium), has three functions (1) to accomplish the gel-sol transformation, (2) to activate the gel enzymes and (3) to buffer the enzyme solution at a pH favourable to the activity of the gel enzymes.

INTRODUCTION

Curiously little is known about the moulting process in insects, although the nucleic acid and protein metabolism of moulting and its hormonal control have been studied exhaustively (see reviews by Wigglesworth, 1970; Chen, 1971; Wyatt, 1972, and Doane, 1973). After mitosis of the integumentary epithelial cells and prior to formation of a new epicuticle, the moulting gel is formed from zymogen granules produced by these cells and is secreted into the ecdysial space which will persist between the old and new cuticles. The ultrastructure of gel formation has been studied in a Lepidopterous insect (Locke, 1969; Locke & Krishnan, 1973; Hikida & Jungreis, 1975) and the gel has been shown to contain inactive forms of hydrolytic enzymes (Lensky, Cohen & Schneiderman, 1970; Katzenellenbogen & Kafatos, 1971a, b, c; Bade & Shoukimas, 1974). These latter workers adhered to the classical view of...
Passonneau & Williams (1953) that moulting fluid is simply a sol form of the moulting gel (Katzenellenbogen & Kafatos, 1970; Lensky et al. 1970). However, Jungreis (1973, 1974) has shown that the fluid present in the exuvial space when the old cuticle is being actively hydrolysed and reabsorbed is a potassium salt solution in which the enzymes of the gel are dissolved and presumably activated (see Lensky et al. 1970). An apt analogy is the formation of cocoonase solution by adult silkmoths in which a crystalline pro-enzyme is secreted by the galea and subsequently is activated by a potassium bicarbonate buffer solution which is secreted by the labial glands (Kafatos, 1968). In the case of the moulting fluid, however, the enzyme-containing gel and the potassium salt solution are both secreted by the same tissue, the integumentary epithelium, but at different times. This paper will deal with the mechanism of formation of the potassium salt solution by the pharate pupal integument (PPI) of *Hyalophora cecropia* (L): this solution constitutes the bulk of the fluid volume (moulting fluid) in the exuvial space prior to ecdysis.

We will show that the potassium salt solution is formed by the active transport of potassium across the integument followed by a presumed osmotic flow of water. The potassium pump in the integument resembles that in the labial gland (Kafatos, 1968) and indeed it has many properties in common with that in other insect tissues which secrete or reabsorb fluids in conjunction with active potassium transport (reviews by Maddrell, 1971; Berridge & Oschman, 1972). However, the biophysics of the potassium pump has been difficult to study in these small, fluid-secreting tissues and many of its properties have been analysed in the large, isolated, silkworm midgut (Harvey & Wood, 1972, 1973; review by Harvey & Zerahn, 1972). Keynes (1969) has classified the K-pump in Malpighian tubules, labial gland, and midgut as a Type V pump. Its principal properties are that (1) potassium is secreted from blood-side to apical side of an epithelium, rendering the apical side electrically positive to the blood-side, (2) no counter ion is required, (3) the pump is electrogenic, and (4) it is not inhibited by ouabain. However, unlike the other K-transporting insect tissues, the midgut does not secrete a fluid. The pharate pupal integument has great potential as a model for analyzing the role of the K-pump in fluid secretion by insects.

The first clue that active potassium transport plays a role in the formation of the moulting fluid came from the demonstration that potassium is present at virtually the same high concentration in both moulting fluid (132 m-equiv./l) and integument (135 m-equiv./kg), but at a much lower concentration (31 m-equiv./l) ($P < 0.001$) in the haemolymph of pharate pupae of *H. cecropia* (Jungreis & Tojo, 1973; Jungreis, 1974). In addition, the osmotic pressure of moulting fluid was 38% greater than that of blood collected from the same insects. These observations suggested that potassium is actively transported from blood-side (haemolymph) to exuvial side (moulting fluid) of the developing pupal integument, and that fluid movement between the integumentary epithelial cells is restricted (see Hikida & Jungreis, 1975). Water would follow potassium passively, but not to equilibrium, resulting in the formation of a moulting fluid hyper-osmotic to haemolymph. In the present study this model has been tested both in vivo and in vitro and has been confirmed. Preliminary evidence has also been obtained which suggests that resorption of the moulting fluid is not driven by potassium transport, but that it is driven by the active transport of some other ionic species, possibly bicarbonate.
Potassium transport and moulting fluid

METHODS AND MATERIALS

Larvae of the giant American silkmoth, Hyalophora cecropia (L.) were reared either in the laboratory on a synthetic diet (Riddiford, 1968) at 25 °C and 65 % R.H. or out of doors on wild cherry foliage. Pharate pupae were selected for analysis at stages described elsewhere (Jungreis, 1974; Hikida & Jungreis, 1975). The pharate pupal integument was dissected from the larval cuticle at 24 to 1 h before ecdysis, when it consists of a delicate cuticle developing over a one-cell-thick layer of pupal integumentary epithelial cells. Midgut, fat body, and other adhering tissues were scraped gently from the haemolymph-side of the integument and the larval cuticle was then separated from it by inserting blunt-tipped forceps between the two layers and separating them carefully at the major points of attachment, the spiracular junctions. The isolated integument was transferred as a flat sheet to a glass or Plexiglass chamber, was tied in place with fine cotton twine, and an area of 0.75 cm² was exposed to separate, identical bathing solutions on both sides. The standard solution, designated as 32-K-S-Tris, consisted of 32 mM-KCl, 5 mM-MgCl₂, 5 mM-CaCl₂, 5 mM Tris-HCl and 160 mM sucrose, had a pH of 8.60, and had a total osmotic equivalent of 260 milliosmolal (Harvey & Nedergaard, 1964; Wood, 1972). Other solutions are described in the appropriate table or figure.

The potential difference (PD) across the PPI was measured by calomel electrodes, was read on a Keithley Electrometer (Model 602 A), and was recorded on a Servoscribe 1 S Recorder. The PPI was short-circuited as a flat sheet by Wood's modification (1972, 1975) of the classical technique of Ussing & Zerahn (1951). Wood's principal modification was to introduce a third calomel electrode to record the potential gradient in the solution when current was passed through it and to use this value to correct automatically for the resistance of the bathing solutions. The short-circuiting was accomplished manually using a current supply, designed by Mr Dennis Unwin of Cambridge University, which passed current through the solutions via Ag–AgCl electrodes. The short-circuit current, Iₛₑ, was read on a Keithley Picoammeter (Model 410A). Fluxes of potassium, Jₖ, were measured with either ^48K or ^86Rb by comparing the radioactivity in samples on the cold side to the specific activity of the isotope on the side to which it was added. To confirm that ^48K and ^86Rb could not be distinguished by the epithelial transport system, both were placed simultaneously on the exuvial side of the integument, which was bathed in 32-K-S-Tris, and the uni-directional fluxes were measured over a 2 h period. ^86Rb was transported at 94–110 % (mean = 105 %) of the rate of ^48K, indicating that ^86Rb is virtually indistinguishable from ^48K and can be used as a tracer for potassium fluxes in this solution.

The PD across the integument was measured in vivo in selected body segments at 60–12 h before the larval–pupal ecdysis. For this purpose two tuberculin syringes were fitted with 0.5 in, 27-gauge hypodermic needles and were attached to polyethylene tubing. Each of the two bridges formed in this way was filled with a 3 % agar, 32-K-S-Tris solution and was connected via a calomel electrode to the Keithley electrometer. One needle was pushed through the integument and into the haemolymph, where it served as a reference electrode, whereas the other one was used to probe the exuvial space.
Table 1. *The potential difference measured across the pharate pupal integument in vivo in selected body segments of Cecropia silkmoths at 60–12 h before the larval-pupal ecdysis*

<table>
<thead>
<tr>
<th>Time before ecdysis of an individual (h)</th>
<th>PD of exuvial side (+) with respect to hemolymph-side (−) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Most posterior segment</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>−20</td>
</tr>
<tr>
<td>48*</td>
<td>+7.6</td>
</tr>
<tr>
<td>44</td>
<td>+8.9</td>
</tr>
<tr>
<td>40</td>
<td>+8.8</td>
</tr>
<tr>
<td>36</td>
<td>+10.4</td>
</tr>
<tr>
<td>30 †</td>
<td>+5.6</td>
</tr>
<tr>
<td>24†</td>
<td>+9.6</td>
</tr>
<tr>
<td>24</td>
<td>+6.8</td>
</tr>
<tr>
<td>18–16</td>
<td>+2.1</td>
</tr>
<tr>
<td>12</td>
<td>+3.2</td>
</tr>
</tbody>
</table>

* PD's were also measured in the following abdominal segments: 7th +0.0, 6th −4.40, 4th −4.00, 3rd −4.00, 1st +9.0; prothoracic segments: 3rd +9.0, 1st +2.3; head capsule +3.5.
† Moulting gel rather than moulting fluid was present.
§ Mounting fluid was completely reabsorbed at this stage of development.

Table 2. *Unidirectional potassium fluxes across the integument isolated from the pharate pupa and bathed in oxygenated 32-K-S-Tris (pH 8.60)*

(The influx \( J_{in} \) from haemolymph to exuvial side was measured with \( ^{40} \)K and the efflux \( J_{out} \) was measured with \( ^{85} \)Rb under both open-circuit and short-circuit conditions. \( I_m \) is given for the short-circuit experiments. 'Ant.' and 'Post.' refer to those respective regions of the PPI when isolated from the same insect. Insects fed on foliage are designated by (F) and those fed on synthetic diet by (S). Measurements were made at from 20.5 to 26°C.)

<table>
<thead>
<tr>
<th>Time before ecdysis of an individual (h)</th>
<th>Steady state PD (mV) ((\psi_{ex} - \psi_{haemol}))</th>
<th>Unidirectional fluxes (μEquiv. K⁺/cm²/h)</th>
<th>( I_m ) (μ-equiv./h)</th>
<th>( J_{in}/J_{out} )</th>
<th>Observed</th>
<th>Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 (S)†</td>
<td>+6.0</td>
<td>0.067</td>
<td>0.212</td>
<td>105</td>
<td>4.33</td>
<td>1.00</td>
</tr>
<tr>
<td>24 (F)</td>
<td>+9.6</td>
<td>0.151</td>
<td>0.380</td>
<td>162</td>
<td>1.52</td>
<td>1.36</td>
</tr>
<tr>
<td>24 (F)</td>
<td>+8.4</td>
<td>0.122</td>
<td>0.659</td>
<td>12.5</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>24 (S)†</td>
<td>+3.9</td>
<td>0.021</td>
<td>0.160</td>
<td>49</td>
<td>7.54</td>
<td>1.00</td>
</tr>
<tr>
<td>16 (F)</td>
<td>+14.0</td>
<td>0.062</td>
<td>0.260</td>
<td>41.7</td>
<td>1.73</td>
<td>1.73</td>
</tr>
<tr>
<td>16–12 (F)</td>
<td>+10.0</td>
<td>0.218</td>
<td>0.342</td>
<td>15.7</td>
<td>1.48</td>
<td>1.48</td>
</tr>
<tr>
<td>8–6 (F)</td>
<td>+12.0</td>
<td>0.144</td>
<td>0.073</td>
<td>3.6</td>
<td>6.76</td>
<td>1.60</td>
</tr>
<tr>
<td>8–6 (F)</td>
<td>+5.4</td>
<td>0.144</td>
<td>0.068</td>
<td>0.4</td>
<td>3.6</td>
<td>1.00</td>
</tr>
<tr>
<td>6 (Ant., S)</td>
<td>+12.4</td>
<td>0.148</td>
<td>0.104</td>
<td>83</td>
<td>3.6</td>
<td>1.00</td>
</tr>
<tr>
<td>6 (Post., S)</td>
<td>+11.0</td>
<td>0.061</td>
<td>0.104</td>
<td>41</td>
<td>2.41</td>
<td>1.00</td>
</tr>
<tr>
<td>3 (F)</td>
<td>+6.8</td>
<td>0.155</td>
<td>0.036</td>
<td>75</td>
<td>5.02</td>
<td>1.00</td>
</tr>
<tr>
<td>2–1 (Ant., S)</td>
<td>+7.0</td>
<td>0.093</td>
<td>0.104</td>
<td>75</td>
<td>1.12</td>
<td>1.00</td>
</tr>
<tr>
<td>2–1 (Post., S)</td>
<td>+4.6</td>
<td>0.026</td>
<td>0.025</td>
<td>0.96</td>
<td>1.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* Percentage of the \( I_m \) contributed by net K transport.
† Integument bathed in 32 mm-KCl, 200 mm sucrose, 5 mm glucose and 5 mm Tris-HCl at pH 8.6.
**RESULTS**

The moulting fluid was, in all regions, invariably positive with respect to the haemolymph (mean = 8.8 mV, n = 20) at 40–24 h before ecdysis, when moulting fluid is being secreted from haemolymph to exuvial space (Table 1). The PD was still positive but smaller in magnitude (mean = +1.2, n = 8) at 18–12 h before ecdysis, when moulting fluid is being resorbed. Significantly, the material in the exuvial space was negative with respect to the haemolymph (approximately -20 mV) at 60 h before ecdysis, when moulting fluid is not yet abundant (Table 1). When the integument was isolated and bathed with the same physiological saline (32-K-S-Tris) on both sides,
the exuvial side was positive to the haemolymph-side in all cases which extended from 30 to 1 h before ecdysis (Table 2). The isolated integument was short-circuited and a typical time-course of the $I_{sc}$ and K-fluxes is shown as Fig. 1. This preparation was bathed in a 32 mM-KCl solution but showed a similar time-course of $I_{sc}$ and K-fluxes as those preparations bathed in 32-K-S-Tris. The average steady-state value of the $I_{sc}$ was 0.182 $\mu$-equiv. cm$^{-2}$ h$^{-1}$ ($n = 5$) during the period from 30 to 3 hours before ecdysis (Table 2). The ratio of the potassium influx ($I_{in}$) toward the exuvial side, to efflux ($I_{out}$) toward the haemolymph-side, ranged from 5.4 to 64 under open-circuit conditions and from 2.4 to 7.5 under short-circuit conditions at 30 to 3 h before ecdysis (Table 2). Despite values of the PD and $I_{sc}$ at 2 to 1 h before ecdysis being similar to those at earlier times, the potassium flux ratios at that time were close to unity. The net flux of potassium toward the exuvial side of the integument had an average value of 0.126 $\mu$-equiv. cm$^{-2}$ h$^{-1}$ ($n = 5$) and accounted for 70% ($n = 5$) of the $I_{sc}$ at 30 to 3 h before ecdysis. However, at 2 to 1 h before ecdysis the net potassium flux accounted for only 9% of the $I_{sc}$ (Table 2). The $I_{sc}$ of the isolated integument was maintained equally well in chloride or bicarbonate solutions, and persisted even in simple solutions consisting of 32 mM potassium chloride or 32 mM potassium bicarbonate and sucrose, glucose, and Tris (Fig. 2).

**DISCUSSION**

**Electrical potential difference across integument.** The exuvial space of the living insect was mostly between 5 and 10 mV positive with respect to the haemolymph during the period from 48 to 24 h before ecdysis, when moulting fluid is being secreted (Table 1). During this same period the potassium concentration was 176 and 132 $\mu$equiv./l in moulting fluid and 53 and 31 $\mu$equiv./l in haemolymph from insects reared on foliage and synthetic diet respectively (Jungreis & Tojo, 1973; Jungreis, 1974). The potassium equilibrium potential, $E_K$, between exuvial fluid and haemolymph was calculated from these concentration values assuming that the activity coefficients for potassium are the same in both fluids, as

$$E_K = -\frac{RT}{zF} \ln \frac{K_{\text{exuvial fluid}}}{K_{\text{haemolymph}}}$$

where $R$ is the gas constant, $T$ is the absolute temperature, $z$ is the valency of potassium, $F$ is Faraday's constant, and $K$ is the potassium concentration measured in exuvial fluid and haemolymph respectively. The sign of $E_K$ is negative, i.e. opposite to that of the measured PD and its magnitude is $-31$ mV and $-36$ mV in insects reared on foliage and synthetic diet respectively. Clearly, potassium is not in equilibrium across the pharate pupal integument. This result suggests either that potassium is being actively transported across the integument from haemolymph to exuvial space during this period or else that its flux in this direction is coupled to that of some actively transported ionic species. However, at 60 h before ecdysis, when little or no moulting fluid is present, the measured potential is $-20$ mV, a value close enough to the potassium equilibrium potential to suggest that potassium movements are passive at this stage.

When the integument was isolated during the period from 30 to 1 h before ecdysis and bathed in 32 mM potassium on both sides the exuvial side was always positive with
Fig. 2. Time-course of short-circuit current in solutions in which potassium is the only cation added. The integument was isolated at 16 to 14 h before ecdysis and bathed in 32 mM-KHCO₃, 200 mM sucrose, 5 mM glucose and 5 mM Tris-HCl at pH 8.6 (open circles). After 2 h the solution was changed to one in which the 32 mM-KHCO₃ was replaced by 32 mM-KCl, with no effect on the time-course of the $I_{sc}$ (closed circles).

respect to the haemolymph-side, after an occasional temporary negative phase (Figs. 1, 2; Table 2), suggesting that the active potassium transport postulated above to take place in the living insect still continues in the isolated preparation. (The rapid initial decrease in $I_{sc}$ occurred in all preparations although the $I_{sc}$ did not always become negative. The phase usually lasted no more than $\frac{1}{2}$ h in solutions free of calcium and magnesium. It might be caused by a reversal of the K-pump, by an activation of an anion pump oriented toward the exuvial side of the PPI, or by other mechanisms such as a loss of tissue K towards the haemolymph-side. It may be induced by the removal of the tissue from a hormonal influence in the haemolymph as discussed on page 364 below. However, we are concerned here only with the steady state $I_{sc}$ and with the fluxes responsible for it.)

**Short-circuit current and K-fluxes across integument.** When the integument, isolated during the period from 30 to 3 h before ecdysis, was short-circuited (thereby eliminating a net flux of any ionic species moving passively), a net flux of potassium toward the exuvial side was always observed. The ratio of flux toward the exuvial side (influx) to flux toward the haemolymph-side (efflux) was calculated under both open-circuit and short-circuit conditions and compared to the values predicted by the flux ratio equation (see Ussing, 1960).

$$\frac{J_{K_{\text{in}}}}{J_{K_{\text{out}}}} = \frac{[K]_{\text{in}}}{[K]_{\text{out}}} \exp \left[ (P_D) zF/RT \right],$$

(2)
where $J_{K_{in}}$ is the unidirectional potassium flux toward the exuvial side, $J_{K_{out}}$ is the unidirectional potassium flux toward the haemolymph-side, and PD is the electrical potential difference. The measured flux ratios exceeded the calculated ratios by factors ranging from 42 to 2.4 during this period (Table 2). Clearly potassium is being actively transported from haemolymph-side to exuvial side of the isolated integument. The net potassium flux accounted for a large fraction of the short-circuit current (Fig. 1, Table 2). At 2 to 1 h before ecdisis, the potassium flux ratios were close to predicted values and we conclude that active potassium transport has ceased. However, a substantial $I_{sc}$ remains and must be accounted for by the active transport of some other ionic species at this stage: the net potassium flux now accounts for only 9% of the $I_{sc}$ (Table 2).

**Properties of integumentary K-pump.** Consistent with our demonstration of active potassium transport is the requirement for oxygen in maintaining the PD across the isolated PPI (Jungreis, 1973). The potential difference cannot be maintained when potassium is replaced either by sodium or by choline (Jungreis, 1973), suggesting that the transport mechanism has a strong preference for potassium, at least in solutions containing calcium. Sodium is not present in the bathing solution and, indeed, the potential and short-circuit current are readily demonstrated in a simple solution consisting of 32 mM-KCl or 32 mM-KHCO3, sucrose, glucose and Tris buffer (Fig. 2). This lack of counter-ion involvement suggests that the K-transport is electrogenic. Finally, both the PD across the PPI, and the $I_{sc}$, are unaffected by ouabain (Jungreis, 1973). In these respects the K-pump in the isolated pharate pupal integument resembles that of the larval midgut (Harvey & Nedergaard, 1964) and can be classified as a Type V Pump (Keynes, 1969).

An apparent discrepancy arises regarding the time before ecdisis at which K transport was demonstrated in vitro compared to the time of secretion and reabsorption of the moulting fluid in vivo. Moulting fluid has been fully resorbed in vivo by 16–12 h before ecdisis (Jungreis, 1974), yet isolated integuments actively transported potassium from haemolymph to exuvial sides until 6 h before ecdisis. Furthermore, it was the time at which the insects were dissected, rather than the time when transport was studied (normally 8 to 6 h after dissection), which determined the transport properties of the preparation. These observations suggest that some factor (perhaps hormonal), present in vivo, turns off the pump approximately 18 h before ecdisis. In the absence of this factor in vitro, the K-pump is not turned off until 2 to 1 h before ecdisis. However, within 15 min after ecdisis has occurred in vivo, the isolated integument has lost its ability to generate a PD in vitro.

**Secretion of potassium salt solution by integument.** We propose that in response to a favourable osmotic gradient, maintained dynamically by the active transport of K, larval–pupal moulting fluid is formed as a bulk flow of water from haemolymph to exuvial sides of the pharate pupal integument. Furthermore, we propose that this potassium salt solution, which comprises the bulk of the moulting fluid, acts as a buffer at an alkaline pH. Moulting fluid is reported to have an alkaline pH (7-8) similar to the alkaline pH optima of the hydrolytic enzymes present in moulting fluid (Lensky et al. 1970; Katzenellenbogen & Kafatos, 1971a, b, c; Bade & Shoukimas, 1974), unlike haemolymph, which has an acidic pH (Passonneau & Williams, 1953; Katzenellenbogen & Kafatos, 1970). Transport of K and water is accompanied by morphological
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changes in the integumentary epithelium which occur at approximately 48 h before ecdysis, when the secretion of moulting fluid is at its maximum (Hikida & Jungreis, 1975).

Anion in potassium salt solution. Electroneutrality is maintained in vivo by an as yet unidentified anion: chloride present either in blood or moulting fluid can neutralize only a small fraction of the potassium present (Jungreis, Jatlow & Wyatt, 1973; Jungreis, 1974). A possible anion is bicarbonate, which has been qualitatively measured in silkmoth moulting fluid (Jungreis, unpublished data), and which would account for the alkalinity of moulting fluid. Recall the analogy with the labial gland of adult silkmoths which secretes potassium bicarbonate (Kafatos, 1968). However, bicarbonate would have to originate intracellularly from carbonic acid produced by the integumentary cells because it is not added to the bathing solutions. In vitro the short-circuit current is maintained equally well in bicarbonate or chloride solution (Fig. 2). This hypothesis assumes a transport route through the cells and predicts (1) that when K is actively transported from the cells of the integument to its exuvial side, bicarbonate neutralizes it and (2) that there is a passive exchange of K⁺ in the haemolymph for H⁺ in the transporting cells. Although no studies of bicarbonate transport are reported here, the attractive hypothesis that resorption of moulting fluid could result from bicarbonate transport, from exuvial side to haemolymph-side of the PPI, is supported by the large 1 in the absence of net K transport to 1 h before ecdysis (Table 2).

Active potassium transport and moulting fluid secretion. The clear result of the present study is that potassium is actively transported from haemolymph-side to exuvial side of the integument when it is isolated from the pharate pupa, rendering the exuvial side electrically positive with respect to the haemolymph-side. An active potassium transport from haemolymph to exuvial space also takes place in the living pupa, because the space is invariably positive to the haemolymph during the period when moulting fluid is being secreted, and because the potassium concentration is elevated in the space which is hyperosmotic to the haemolymph during secretion (Jungreis & Tojo, 1973; Jungreis, 1974). Presumably water flows osmotically from haemolymph to exuvial space, accounting for the increase in volume of the moulting fluid during secretion. These observations, together with the demonstration by Jungreis (1973) that the bulk of the moulting fluid is not only formed much later than the time of secretion of the moulting gel but also overlaps the time of gel-sol transformation, forces us to revise current hypotheses regarding a simple gel → sol transformation of moulting gel to moulting fluid. We propose that the formation of the moulting fluid has three steps instead of two: (1) the classical secretion of proteinaceous moulting gel, (2) its transformation to a moulting sol in the presence of a potassium salt solution, and (3) the continued addition to the sol of a potassium salt solution which accounts for the bulk of the moulting fluid volume. We speculate that the potassium salt solution has three functions: (1) to accomplish the gel–sol transformation, (2) to activate the gel enzymes, and (3) to buffer the enzyme solution at a pH favourable to the activity of gel enzymes.

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REFERENCES


