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QUANTITATIVE ELECTRON PROBE ANALYSIS OF HAEMOLYMPH AND URINE COMPOSITION FROM INSECT CALLIPHORA ERYTHROCEPHALA DURING METAMORPHOSIS

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Résumé - Les teneurs en Na, K, Mg, Ca et P de l'urine distale et de l'hémolymphe de la mouche Calliphora e., ont été mesurées au cours de la métamorphose à l'aide d'un analyseur à sonde électronique.

Abstract - Na, K, Mg, Ca and P concentrations in distal urine and haemolymph of the blowfly Calliphora e., have been measured during metamorphosis. Quantitative analysis of liquid microsamples has been performed with an electron probe analyser.

Microtechniques of analysis were initiated by RAMSAY (1) who applied them to the understanding of the physiology of excretion and osmoregulation of small invertebrates like insects. Nowadays the electron probe analysis allows accurate elemental concentration measurements of insect internal fluids (2) or of insect transporting epithelia (3). Data have been obtained from fluids pooled from several Drosophila hydrid (2) and from samples from individual Heteroptera (4). Results always concerned one animal stage, either the adult (= imago) or the larvae stage. However very little is known about the changes in ion composition and excretion of the insect during metamorphosis. The Malpighian tubules of Diptera are the only organs to keep a permanent structure during metamorphosis, although their intracellular organization and their size are modified (5). In the present work, samples of distal urine and haemolymph from individual Calliphora erythrocephala were analysed at several stages of metamorphosis. The concentrations of the elements contained in both fluids were determined with an electron probe analyser (6).

Material and Methods

The different stages examined were: three day old larvae (L3), 2, 5 and 7 day old pupae (P2, P5, P7) and imago (I). In each stage, eight laboratory-bred animals were used. Haemolymph micropuncture samples were obtained and kept under water-saturated paraffin oil (on account of their very high protein content it was not possible to prepare samples of haemolymph from P5 for probe analysis). Distal urine contains a great number of spherical concretions. The analyses were performed upon both liquid and concretions, the latter being dissolved prior to analysis in 4% TCA and the results corrected for this dilution. The volumes analysed were 0.3 nI. The electron probe analyser was a Cameca MBX adjusted as follows: 15 kV, 300 nA, beam diameter 80 μm.

Results

Haemolymph and distal urine concentrations values (in mmoles/l) are given for each stage studied in table I and Figs. 1, 2 and 3.

In haemolymph, Na, Mg and Ca decreased significantly from 116, 14 and 6 mmol/l respectively (in L3) to 58, 7 and 1 (in P2), then increased again to 140, 4 and 4 (in I). K and P increased significantly from 11 and 18 (in L3) to 39 for both (in P7) and decreased to 23 and 32 (in I).

In distal urine, a large increase in the K, Mg, Ca and P concentrations was observed during the three first days of metamorphosis (from L3 to P2). Mg, Ca, P and K concentrations increased significantly from 560, 210, 370 and 140 mmoles/l (in L3) to 3000, 1500, 4400 and 430 (in P2). Between P2 and P5 no significant change occurred. The values for Ca and P for the final stage (I) were similar to those found in L3, while the values for K and Mg were two to...
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<th>Stages</th>
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Table I: Na, K, Ca, Mg and P distal urine (U) and haemolymph (H) concentrations during metamorphosis. Mean value ± SEM.

Fig. 1: Na, K, Mg, Ca and P haemolymph concentrations during metamorphosis.
Fig. 2: Na and K distal urine concentrations during metamorphosis

Fig. 3: Ca, Mg and P distal urine concentrations during metamorphosis.

three times lower in the stage I than in stage L3. Na decreased significantly from 2800 (in L3) to 500 (in I).

Thus the distal urine concentration of every analysed element was invariably higher than the corresponding haemolymph concentration, irrespective of the stage involved.

Discussion

The general tissue histolysis taking place during the first three days (from L3 to P2) induces the release of the ionic cell components. Among them, Ca, Mg and P are strongly drawn towards Malpighian tubules and accumulated in distal urine. Such an accumulation might explain the increase in size and number of the distal urine concrements in which Ca, Mg and P content is very high (5). Alkaline earth precipitation in concrements could be induced by an acid-phosphatase rich organic phase (2). During the same period (from L3 to P2) haemolymph is depleted in Ca and Mg. The concentrations of these ions, which are low compared to those in the larvae and imago, are probably conducive to the formations of new tissues. On the contrary, it seems that high P and K concentrations in haemolymph during the same period are not prejudicial for organogenesis.

Between stages P2 and P5, the stability of the concentration values observed for all measured elements (except Na) in distal urine is the consequence of two conflicting processes: the end of histolysis which still yields free ions and the start of organogenesis which reuses them.

From P5 to I, a seven or eight day period during which the animals still do not take food, elements supplied to newly organized tissues come from haemolymph and possibly from concretions in the distal urine. However the decrease in number and size of the concretions which is observed during this period may also be due to their partial elimination in the urinary flow which starts at this period.

Sodium storage during metamorphosis is not effected by the distal urine and further investigations are necessary to answer to this question. As the Na contents of the haemolymph are similar for larvae and imago, while the Na contents of their urine differ (that of imago being lower) one concludes that sodium transport by the Malpighian epithelia is different in the two stages. The large Mg and Ca content differences observed between larvae and imago haemolymphs might be related to differences in diet or in metabolic processes.

References