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Carrier-Mediated Uptake of Pyrrolizidine Alkaloids in Larvae of the Aposematic and Alkaloid-exploiting Moth *Creatonotos*

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Many warning-colored (aposematic) insects store defensive plant substances for their own protection [1]. Since such secondary metabolites of plants are often polar and charged molecules which cannot readily pass biomembranes by simple diffusion, the question arises as to the mechanisms of the resorption of such compounds by the respective herbivorous insects.

Larvae of the East Asian arctiid *Creatonotos transiens* are polyphagous. When feeding on plants which contain pyrrolizidine alkaloids (PA's) these substances are taken up and preferentially stored in the integument, whereas other alkaloids (e.g., quinolizidines, indoles, caffeine) are eliminated with the feces [2]. PA resorption indicates therefore a selective – in vivo – process. This PA uptake has, in addition to the protection of the insects, a further function in a number of Lepidoptera, where these alkaloids serve as essential precursors for the biosynthesis of male dihydropyrrolizine pheromones [3], and in *Creatonotos* even act as a morphogen for the development of the male scent organ gland, the corema [4, 5].

Pyrrolizidine alkaloids are mainly present in plants as hydrophilic N-oxides and should not be able to pass plant membranes by simple diffusion but rather by means of carrier-mediated transport [6]. Specific carrier mechanisms have been demonstrated for the transport of other alkaloids (e.g., isoquinolines and quinolizidines) across plant biomembranes such as the tonoplast [7]. In order to explain the selective uptake of PA's by larvae of *C.transiens*, we have analyzed in vitro whether simple diffusion or carrier-mediated transport are the processes involved.

Larvae of *C.transiens* were reared in the laboratory on a semi-synthetic diet [8]. Midguts were isolated, cut into pieces of ca. 2 mm in length and washed thoroughly. Midgut segments were incubated with a PA, ^{14}C-senecionine-N-oxide, and the uptake of the radioactive label was measured by liquid scintillation counting (Fig. 1). PA uptake is time-dependent and proceeds against a concentration gradient which can be deduced from a parallel experiment with tritiated water. Midguts of larvae of *Syntomis mogadorensis*, a polyphagous ctenuchid moth, neither resorb PA's in vivo [2] nor take up the senecionine-N-oxide label (Fig. 1). This shows that the capacity to resorb PA is not a general phenomenon, but specific to *Creatonotos*.

Following the above observation that alkaloids other than PA's are not taken up in vivo by *C.transiens*, we now incubated the gut segments with several radio-labeled substances (nicotine, lupanine, 13-hydroxylupanine, and colchicine) but found no uptake against the concentration gradient as expressed for hydroxylupanine in Fig. 1 (HL).

Specifications of the uptake process are: at pH 5, the optimum for the PA resorption is reached (Fig. 2 left); the temperature dependency (Fig. 2 right) allows the calculation of an activation energy of 55 and 81 kJ mol^{-1}, respectively (in two separate experiments); the PA resorption can be competitively inhibited by the addition of an unlabeled PA (heliotrine, Fig. 2 middle), which is known to be accumulated by the larvae in vivo [3, 4]. The addition of the indole alkaloid strychnine had no effect. As compared to untreated controls, PA uptake was inhibited to 60 – 99% by 1 mmol KCN per l, indicating the energy dependency of the process.

The in vivo data [2 – 4] and our in vitro experiments (Figs. 1 and 2), convince us that the resorption of senecionine-N-oxide (and probably of other PA's) in the *C.transiens* midgut is a specific, pH-, temperature-, and concentration-dependent process. Because of these criteria and its activation energy of above 20 kJ mol^{-1}, such resorption cannot be a simple diffusion process.

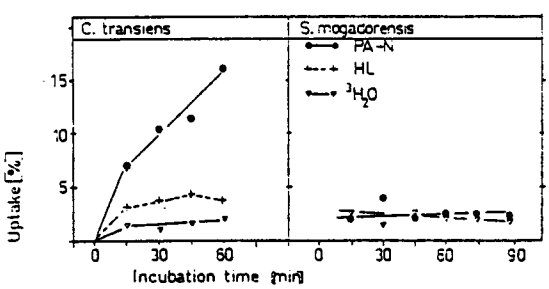

Fig. 1. Time course of senecionine-N-oxide uptake by midgut tissue of *Creatonotos transiens* and *Syntomis mogadorensis*. Midguts of 4 – 8 late instar larvae were dissected in a Ringer solution (8 g NaCl, 0.2 g KCl, 0.1 g glucose per l, 50 m*M* MES, pH 5) and slit open. The peritrophic membrane and gut contents were discarded. Midgut pieces (2 pieces per vial) of ca. 2 mm length were incubated in an Eppendorf vial in 200 µl Ringer solution at 20 °C. The reaction was started by adding either 600 pmol senecionine-N-oxide (37 Bq) (*PA-N*), or 10 kBq tritiated water, or 5 kBq tritiated 13-hydroxylupanine (*HL*). At the time intervals given, midgut pieces were collected, washed in the Ringer solution and homogenized in 0.5 *M* HCl. The radioactivity of the homogenate and of the remaining Ringer solution were determined by liquid scintillation counting. All values are means of two to three experiments and are expressed as the percentage of total radioactivity that was taken up by one piece of midgut tissue. We prepared ^{14}C-senecionine-N-oxide (0.6 mmol, 37 MBq) as described in [9] but used roots of flowering *Senecio vulgaris* plants instead of root organ cultures

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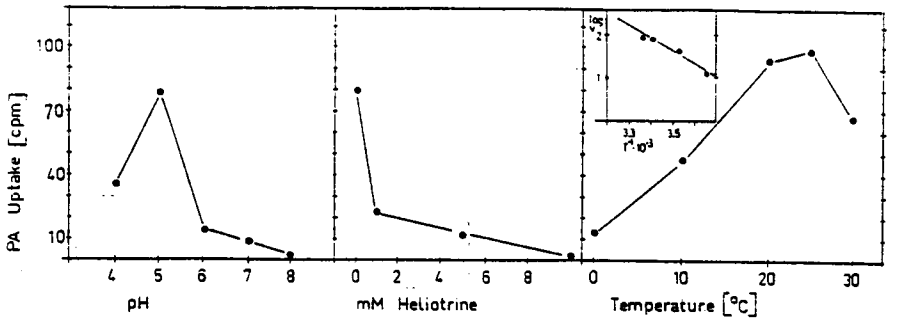

Fig. 2. Uptake of senecionine-N-oxide by midgut tissue of *Creatonotos transiens* in relation to pH value of the buffer, incubation temperature, and competition with "cold" PA (heliotrine). Experimental conditions as in Fig. 1. Values are expressed as the absolute radioactivity (cpm minus background) found in the tissue after incubation at 20 °C for 60 min. *Inset* illustrates the Arrhenius plot used to calculate the activation energy

We postulate therefore, that PA resorption is catalyzed by specific carrier molecules (presumably proteins) such as those known for the transport of the primary metabolites (sugars, amino acids, etc.).

C. transiens is only one of a number of Lepidoptera which not only use the PA's as protective chemicals but rely on the heterocyclic moiety of the PA ester as an essential pheromone precursor (as in some Arctiidae, most Danainae [3, 12], and possibly in some Ithomiinae [10]). Many more insect species are also attracted by the odor of PA's (or rather their metabolites [11]) and avidly ingest even pure PA's [12].

It is tempting to speculate whether such specific carrier systems are also present and active in the other PA feeders and in analogous systems of other insect species which were reported to be unpalatable to some of their predators because they sequester such plant compounds as quinolizidine alkaloids, cardenolides, glucosinolates, or terpenes.

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