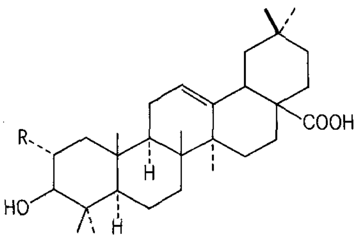
acid (**1**) from various spectral data (UV, IR, EI-MS, $^1$H-NMR and $^{13}$C-NMR)[2, 3]. Another triterpene present in very small amounts [$C_{30}H_{48}O_4$, m.p. 264–266 °C and $[\alpha]_D^{20}$+60.0° (c = 0.01, $CHCl_3$)] was another triterpene, maslinic acid (**2**), was also identified by spectral data (UV, IR, EI-MS, $^1$H-NMR and $^{13}$C-NM)[2, 3]. The total yield of these triterpenes from the leaf surface is at least 3.2% of the total fresh leaf weight, and is comparable to the yield reported by Roncero and Janer[2] for the total leaf.

This means that almost all oleanolic acid present is on the surface of the leaf. *O. europaea* may be protected against fungal attack at least to some extent by the simple mechanism of excreting these triterpenes onto the leaf surface. Although oleanolic acid itself does not exhibit antifungal activity, it acts as a barrier at the leaf surface, a site where the physiological environment is normally favorable for spore germination. The degree of surface wettability of the leaf is important, since spores cannot germinate and grow unless sufficient moisture is available. If a leaf is heavily coated with the non-water soluble oleanolic acid, moisture will not remain on the leaf surface. We consider this simple mechanism of excreting triterpenes onto the leaf surface to be part of a 'multichemical' defense against fungal attack[5].



**1** R = H, oleanolic acid
**2** R = OH, maslinic acid

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# Age-dependent tolerance to *Baculovirus* in last larval instars of the codling moth, *Cydia pomonella* L., induced either for pupation or for diapause

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*Summary.* $LD_{50}$ values as well as time-dependent parameters of granulosis virus infections were determined at different times during the last larval instar ($L_5$) of the codling moth, *Cydia pomonella* L., induced either for pupation or for diapause. A significant increase of tolerance to virus was found in 48-h-old $L_5$ induced for pupation, and 24 h later in $L_5$ induced for diapause.

*Key words.* *Baculovirus;* granulosis virus; codling moth; tolerance, age-dependent; pupation; diapause; *Cydia pomponella.*

Although the larvae of Lepidoptera are usually very susceptible to infections with baculoviruses, several authors reported an increased tolerance to virus among different larval instars[1]. Multiplication of baculoviruses in pupae and adult insects is not common; only few cases have been reported[2]. In order to estimate the influence of the pupation on the susceptibility to *Baculovirus* infection, experiments were performed with $L_5$ of the codling moth induced for either uninterrupted development to pupation or for diapause as fully grown $L_5$[3]. The larvae were reared individually according to our standard method[4], either under continuous light in order to induce pupation or under a short day-regime with a light: dark cycle of 10:14 h, which induced diapause.

Experimental groups of 30–40 individuals were formed with synchronized $L_5$. Computing of age started as larvae moulted to the last instar. The age of larvae within a group varied by ± 2–4 h. Infections with subgroup B *Baculovirus*[5] (granulosis virus) were scheduled for 3-, 24-, 48- and 72-h-old $L_5$. Granulosis virus was purified by conventional methods[6]. A stock suspension containing $3 \times 10^{11}$ granulosis capsules per ml was prepared with phosphate buffer ($\frac{1}{15}$ M; pH 7) and stored at 2 °C. Dilutions were made with destilled water. 1 µl virus suspension per larva was orally administered by means of microinjection[7]. Larvae not surviving the first 2 days after injection had probably been wounded when handled for infection and were discarded. The average time till death was recorded and the $LD_{50}$ values were determined by probit analysis with a computer program[8].

$LD_{50}$ values calculated from the dose-mortality responses for larvae infected at different times after the last larval molt are presented in table 1. The doses necessary to kill 50% of 3- and 24-h-old $L_5$ induced for pupation were similar. However, in the 48- and 72-h-old larvae about 2500 and 25000 times higher doses were necessary respectively. On the other hand, whereas

Table 1. $LD_{50}$ values of the granulosis virus of *Cydia pomonella*. Effect of the age of last instar larvae ($L_5$) induced for either pupation (IP) or diapause (ID) on mortality and time parameters

| Age of $L_5$ at infection | $LD_{50}$ | 95% confidence level |  | Slope |
|---|---|---|---|---|
|  |  | Lower | Upper |  |
| IP 3 h | $9.2 \times 10^1$ | $7.2 \times 10^1$ | $1.2 \times 10^2$ | 2.033 |
| 24 h | $7.6 \times 10^1$ | $4.1 \times 10^1$ | $1.1 \times 10^2$ | 1.332 |
| 48 h | $2.1 \times 10^5$ | $1.4 \times 10^5$ | $3.1 \times 10^5$ | 1.169 |
| 72 h | $2.1 \times 10^6$ | $1.1 \times 10^6$ | $3.6 \times 10^6$ | 1.380 |
| ID 3 h | $1.2 \times 10^2$ | $8.0 \times 10^1$ | $1.8 \times 10^2$ | 1.262 |
| 24 h | $1.1 \times 10^2$ | $5.7 \times 10^1$ | $1.9 \times 10^2$ | 1.238 |
| 48 h | $7.7 \times 10^2$ | $3.4 \times 10^2$ | $1.2 \times 10^3$ | 1.344 |
| 72 h | $2.0 \times 10^5$ | $8.8 \times 10^4$ | $7.3 \times 10^5$ | 0.758 |

the $LD_{50}$ values for 3- and 24-h-old $L_5$ induced for diapause were but slightly higher than in $L_5$ induced for pupation, the $LD_{50}$ values in the former increased only by the factors 6.4 and 2000 in the 48- and 72-h-old larvae respectively. These results indicate that the tolerance to the virus increases in diapause-induced $L_5$ about 24 h later than in $L_5$ induced for pupation. The decrease of the slopes of the dose-mortality regression-lines with larval age indicate that the populations become less homogeneous with respect to granulosis infection.

Time-mortality data were recorded for the standard dose $3 \times 10^3$ capsules per larva (table 2). This dose is close to the minimal dose necessary to cause 100% mortality in a population of freshly molted L5. The results reveal that an increased tolerance to the *Baculovirus* occurs already in 24-h-old $L_5$, since the larvae survive longer after infection at 24 h than at 3 h. In older experimental groups average time till death as well as the minimal time requested for the appearance of mortality increased generally and mortality decreased. Whereas the standard dose caused still 39% mortality in 72-h-old $L_5$ induced for diapause, no mortality was caused by this dose in 72-h-old $L_5$ induced for pupation. Only at the age of 96 h the diapause-induced $L_5$ were also fully resistant to the standard dose.

Table 2. Effect of age of last instar larvae – induced for pupation (IP) or diapause (ID) – on mortality, and time-parameters after infection with a single dose of $3 \times 10^3$ capsules/larva. Larval weight is given as $\bar{x} \pm SD$

| Age of $L_5$ at infection | | Larval weight (mg) | Mortality % | First observed mortality at Day | % | Average time till death (days) |
|---|---|---|---|---|---|---|
| IP | 3 h | 39.1 ± 6.2 | 100 | 5 | 15 | 6.0 |
| | 24 h | 68.5 ± 9.8 | 89 | 6 | 4 | 8.8 |
| | 48 h | 88.5 ± 12.0 | 18 | 10 | 9 | 10.8 |
| | 72 h | 85.0 ± 14.6 | 0 | – | – | – |
| | 96 h | 73.0 ± 11.7 | 0 | – | – | – |
| ID | 3 h | 45.5 ± 7.9 | 97 | 6 | 7 | 7.5 |
| | 24 h | 75.5 ± 10.5 | 97 | 7 | 23 | 9.8 |
| | 48 h | 95.0 ± 14.0 | 67 | 8 | 4 | 10.6 |
| | 72 h | 105.5 ± 16.7 | 39 | 10 | 18 | 12.2 |
| | 96 h | 93.5 ± 15.8 | 0 | – | – | – |

The weight gain of the larvae cannot explain the rapid increase of tolerance to the virus. Tolerance is not correlated with weight (table 2) and is still increasing when larval weight decreases after 72 h.

Our data suggest that the physiological changes in the last instar larva connected with the change of the cellular commitment to pupal differentiation are responsible for the development and rapid increase of virus tolerance. The delay by 24 h of this increase in larvae induced for diapause fits well with this interpretation. The physiological changes that prepare the insect for pupation take also place in diapause-induced larvae, but 24 h later than in $L_5$ programmed for immediate pupation[9, 10].

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# In vivo effects of epinephrine in a freshwater fish

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*Summary.* In vivo effects of epinephrine were investigated in a freshwater teleost, *Barbus conchonius* Hamilton. Fish given 2 mg/kg epinephrine in a single i.m. dose showed significant hypocholesterolemia and elevated liver and kidney cholesterol levels 1–8 h postinjection. Plasma amino nitrogen evinced a transient yet significant fall at 2 h followed by a significant increase after 24 h. A marked reduction occurred in the plasma FFA and organic $PO_4$ levels after 1–8 h. The results offer little evidence for a lipolytic effect of epinephrine in this species, and the changes in metabolite levels are attributable, in part, to the catecholamine-induced modification of insulin secretion.

*Key words.* Freshwater fish; *Barbus conchonius;* epinephrine, hypocholesterolemia, insulin secretion; lipolytic effect.

The adipokinetic effects of catecholamines in mammals are well known and elevated levels of plasma and tissue free fatty acids (FFA) have been demonstrated following epinephrine administration[2]. Evidence for a possible lipolytic effect of catecholamines in fishes is still fragmentary and inconsistent. Norepinephrine in the bream, *Abramis brama* and epinephrine in the pike perch, *Lucioperca lucius,* did not stimulate lipolysis[3]. By contrast, epinephrine treatment raised the FFA content[4] in the blood of the eel, *Anguilla anguilla.* A significant norepinephrine-induced decrease occurred in the total uptake of $^{14}C$-acetate in the liver lipids of the nurse shark, *Ginglymostoma cirratum* indicating stimulation of lipolysis[5]. The object of the present investigation was to evaluate epinephrine-induced changes in blood cholesterol, FFA, organic $PO_4$ and amino nitrogen, and liver and kidney cholesterol in *Barbus conchonius* Hamilton.

*Material and methods.* Adults of *Barbus conchonius* Ham., weighing 4.5–5 g, were procured from the local lake and accli-