

EFFECT OF PRECOCENE II, ECDYSONE AND JUVENILE HORMONE ON THE GLYCOGEN CONCENTRATION IN PUPAE OF *STOMOXYS CALCITRANS* (DIPTERA MUSCIDAE)

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Third instar larvae of Stomoxys calcitrans (L.) were treated with precocene II, ecdysone and juvenile hormone. The larvae were allowed to develop until pupation and when it occurred, determination of glycogen levels was assayed. The administration of those three substances have interfered on the glycogen concentration. The precocene II causing a decrease whereas the ecdysone and juvenile hormone causing an increase. The ecdysone administered together with precocene II reverses the effect of the latter. This does not happen when precocene II is administered together with the juvenile hormone. Ecdysone administered together with juvenile hormone causes reduction of the glycogen concentration.

Key word: ecdysone – juvenile hormone – precocene II – glycogen – *Stomoxys calcitrans*

Glycogen, an important component of the insect tissues reserve, is found in the entire insect body, being accumulated in the larvae and used during metamorphosis (Wyatt, 1967). It was reported (Wright & Rushing, 1973; Wright et al., 1973), that *Stomoxys calcitrans* pupae, treated with juvenile hormone, failed to emerge and had a larger quantity of glycogen than those untreated, and also that there is no difference between pupae treated and untreated with juvenile hormone, in relation to glycogen phosphorylase.

It is known that in some insect orders ecdysteroids administration can delay or prevent ecdysis and a recent work (Z'dàrek & Delinger, 1987) showed that in diptera, more exactly in *Sarcophaga crassipalpis*, exogenous 20-hidroxi-ecdysone has prevented the pupal ecdysis.

Failure of ecdysis was also observed through precocene II administration in ticks (Leahy & Booth, 1980).

The purpose of the present study is to search some clarifications on the action of juvenile hormone, ecdysone and precocene II, substances that cause some disfunctions on ecdysis, on the

glycogen use by third instar larvae including the testing of a possible antagonic effect of these substances, since there are scarce records on *Stomoxys calcitrans*, as well as on diptera in general.

MATERIALS AND METHODS

The colony of *Stomoxys calcitrans* was maintained according to Bailey et al. (1975) methods. Larvae at the beginning of the third instar, after removed from the culture environment, were treated with different substances by proper means. Juvenile hormone and ecdysone (Sigma Chemical Co.) were administered through alimentation (10 µg/g and 25 µg/g of culture medium, respectively). Precocene II (Sigma Chemical Co.) was applied by contact (11 µg and 22 µg/cm²) on filter paper, in a covered Petri dish of 9 cm of diameter, the same dish being use in the other groups.

The alimentary medium was offered in these dishes to treated and untreated groups. The control (untreated) group has not received any of those substances, but the solvents that were ethanol to ecdysone and acetone to juvenile hormone and precocene II.

The combination of precocene II plus ecdysone, ecdysone plus juvenile hormone and precocene II was effected complying with the same concentrations used for the groups that received the isolated substances. The means of

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administration were also the same and had been chosen according to the criteria that they evidenced more remarkable effects than the others tested. The treatments occurred over a period of 24 hours, having the precocene II treated groups received culture medium without the administration of any tested substance, and control group received only the solvents.

Third instar larvae were allowed to develop and immediately after pupation and darkening of the cuticle, were weighed and tested one by one for glycogen concentration, this substance isolated and purified according to Van Handel methods (1965). Five pupae were subjected to each repetition, three repetitions having been accomplished. The pupae that had not been tested for glycogen determination were kept under the same conditions of the original colony so that the emergence could occur, which effectively happened.

RESULTS

In order to determine whether precocene II, edcysone and juvenile hormone influence the glycogen concentration, test were conducted, some parameters such as glycogen concentration, pupae weight and adult emergence analysed, and the results are presented on the Table.

As indicated in the Table, pupae from treatment of third instar larvae with edcysone, and juvenile hormone singly, had the highest glycogen concentration. As to precocene II

treated groups, in both concentrations there has been observed a reduction in the glycogen level, that seems to be dose-dependent.

Adult emergence was affected by the three substances, although the precocene II group that has received a medium dose ($11 \mu\text{g}/\text{cm}^2$) and the juvenile hormone treated group had presented the same weight of pupae of the control group.

When precocene II was administered together with juvenile hormone and edcysone together with juvenile hormone the glycogen concentration, pupae weight and adult emergence were reduced in relation to the control group.

DISCUSSION

In 1973, Wright et al. treated pupae of *Stomoxys calcitrans* immediately after pupation, and while still white, with juvenile hormone and verified that the glycogen concentration was higher in this group than in the control one, findings that can be correlated with the present study in relation to juvenile hormone, since both have demonstrated an increase of glycogen level concentration, even though the time of treatment and quantification had been different.

As to edcysone, there is no recorded work trying to show a relation between this substance and glycogen metabolism, auspiciously there are papers as that of Z'dàrek & Delinger (1987), that show a prevention of pupal ecdysis, when exogenous ecdysteroids are

TABLE

Glycogen concentration, pupae weight and adults emergence from third instar larvae of *Stomoxys calcitrans* treated with precocene II, edcysone, juvenile hormone and combinations of these substances

Treatment	Concentration	Ways of administration	Glycogen ($\mu\text{g}/\text{mg}$ pupae)	Pupae weight (mg)	Adult emergence (%)
Control	—	—	80.5	10.0	97.2
Precocene II	(1) $22 \mu\text{g}/\text{cm}^2$ /a	contact	33.5	8.2	31.3
Precocene II/2	$11 \mu\text{g}/\text{cm}^2$ /a	contact	58.8	10.0	79.2
Ecdysone	(2) $25 \mu\text{g}/\text{g}$ /b	alimentation	114.9	6.2	49.2
Juvenile hormone	(3) $10 \mu\text{g}/\text{g}$ /b	alimentation	145.7	9.5	50.0
Precocene II + Ecdysone	1 + 2	1 + 2	86.9	8.8	80.2
Precocene II + J. hormone	1 + 2	1 + 3	37.1	9.4	35.3
Ecdysone + J. hormone	2 + 3	2 + 3	47.0	5.6	25.4

a = contact on filter paper; b = weight of culture medium.

Five pupae were subjected to each repetition; three repetitions had been accomplished.

injected into pupae of *Sarcophaga crassipalpis*. The present authors also observed partial prevention of pupae ecdysis when third instar larvae of *Stomoxys calcitrans* were treated with ecdysone. In this case, the results were not so drastic as that of Z'dàrek & Delinger, probably because the ecdysone was administered in the beginning of the last larva instar and the administration happened through alimentation, while in the first mentioned work it was injected into the pupae.

As to precocene II treated group, on both concentrations, it has been observed a decrease on the glycogen level, that at first was considered as possible anti-juvenile hormone effect, since these substances promoted an increase of that carbohydrate and based also in the anti-juvenile hormone effects of prococene II, demonstrated by the reports of Landers & Happ (1980) and Wilson et al. (1983), on the reproduction of *Drosophila melanogaster*. Thus, in order to amplify the understanding of the precocene II action on diptera and verify whether these substances would really cause an anti-juvenile hormone effect, third instar larvae were subjected to treatment with precocene II plus juvenile hormone and it has been verified that the glycogen concentration in this group was quite similar to that presented by the group treated with precocene II solely. Thus, it has not been evidenced an anti-juvenile hormone action of precocene II under these experimental conditions. These results can not be considered in opposition to those obtained in *Drosophila melanogaster* as observed by the two mentioned researchers, who worked with adult diptera, and others such as Bowers et al. (1976), Pratt et al. (1980), Tarrant et al. (1982), Bitsch & Bitsch (1984) and others who have noticed them in other insect orders.

The simultaneous treatment with precocene II plus ecdysone, performed due to the fact that it had been suggested by Bowers et al. (1976) and by Cupp et al. (1977) the hypothesis of anti-ecdysone action, showed a return of glycogen concentration and adult emergence to a level near that of the control group in the newly formed pupae, thus strengthening the hypothesis brought by such researchers, although only the last, have worked on diptera.

The treatment of third instar larvae with ecdysone plus juvenile hormone caused a decrease in the glycogen level in the pupae,

showing the inexistence of antagonic effect between these substances in the third instar larvae of *S. calcitrans*, under the considered parameters, since ecdysone and juvenile hormone when separately administered cause a glycogen increase.

As the glycogen levels of pupae from the third larval instar of *Stomoxys calcitrans* treated with juvenile hormone and ecdysone were higher and with precocene II smaller than the untreated larvae it may be suspected that there is a critical level of glycogen, above or below which the emergence is prevented and this effect could be directly promoted by precocene II, ecdysone and juvenile hormone or in, an indirect way, by other disfunctions of the general metabolism.

A diminution of adult emergence has occurred in the ecdysone and the precocene II treated groups, that can not be attributed only to the small weight of the pupae, since the juvenile hormone treated group showed a weight roughly closer to the control group.

The pupae low weight observed when the larvae are subjected to treatment with ecdysone is in accordance with Z'dàrek & Slàma (1972), who observed the effect of different concentrations of ecdysone on *Calliphora* larvae, verifying the formation of reduced weight pupae.

The pupae from the precocene II group, treated with both concentrations, exhibited a reduction of adult emergence, though the treatment with half of the dose has caused no reduction in the pupae weight, showing that this was not the cause of that effect.

The results of the present work indicate that precocene II, ecdysone and juvenile hormone interfere on the glycogen use by pupae originated from treatment of *Stomoxys calcitrans* third instar larvae, and the emergence of adults from these larvae was likewise altered, and it has been observed that increase as well as decrease in glycogen concentration have been verified in groups that presented reduction of adult percentage.

Concerning the adults that emerged from the eight groups formed, it has been observed that they were fertile. It has not been possible to compare each other because of the great variation among the groups and, according to

Benettova & Fraenkel (1981), the size of the diptera female determines the number of ovarioles in its ovary, directly reflecting on the number of laid eggs.

RESUMO

Efeito de precoceno II, ecdisona e hormônio juvenil sobre a concentração de glicogênio em pupas de *Stomoxys calcitrans* (Diptera: Muscidae) – Larvas de terceiro instar de *Stomoxys calcitrans* (L.) foram tratadas com precoceno II, ecdisona e hormônio juvenil. O desenvolvimento ocorreu até acontecer a pupação, quando os níveis de glicogênio foram determinados. A administração daquelas três substâncias interferiu na concentração de glicogênio. O precoceno II causou uma diminuição enquanto a ecdisona e o hormônio juvenil um aumento. A ecdisona administrada junto com o precoceno II reverteu o efeito do último, o que não aconteceu quando o precoceno foi administrado junto com o hormônio juvenil. A ecdisona, administrada junto com o hormônio juvenil, causou redução na concentração do glicogênio.

Palavras-chave: ecdisona – hormônio juvenil – precoceno – glicogênio – *Stomoxys calcitrans*

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