EFFECTS OF HYDROCORTISONE AND SODIUM METHOHEXITAL ON GROWTH RATE OF CHRYSONYA CHLOROPYGA WEIDEMANN (DIPTERA: CALLIPHORIDAE): DEVELOPMENTAL AND BEHAVIOURAL INDICATIONS OF PRESENCE OF DRUGS

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Williams, K.A. & Villet, M.H. 2014. Effects of hydrocortisone and sodium methohexital on growth rate of Chrysomya chloropyga weidemann (Diptera: Calliphoridae): developmental and behavioural indications of presence of drugs. Durban Natural Science Museum Novitates 37: 25-29. Larvae of the blowfly Chrysomya chloropyga Weidemann were reared on chicken liver dosed with either a steroid or a barbiturate at concentrations that would be half the median lethal dose (MLD), the MLD and twice the MLD for humans. No significant differences were observed between dosages in terms of durations of any stage of development or total development or mortality for larvae exposed to either drug. These results show a different trend to those obtained using Sarcophaga tibialis Macquart, suggesting that extrapolations from one taxonomic family or species to another are unreliable. Activity levels of larvae exposed to the barbiturate and the steroid were significantly increased from the control on Days 1 and 2 of the experiments, indicating stimulation experienced by the larvae in the presence of the drugs. Uncharacteristic behaviours of maggots may thus indicate the presence of drugs, providing toxicological investigations with a new tool in the form of behavioural bioassays.

KEYWORDS: behaviour, behavioural bioassay, Calliphoridae, Chrysomya chloropyga, development, post mortem intervals.

INTRODUCTION

The use of insects, especially maggots, as toxicological material for the detection of drugs and toxins has received substantial attention (Beyer et al. 1980; Catts & Goff 1992; Wilson et al. 1993; Goff & Lord 1994, 2010; Tracqui et al. 2004), as have the effects of these substances on the development rate of dipteran larvae (Goff & Lord 1994; Lopes de Carvalho 2010; Rivers & Dahlem 2014). Medico-criminal forensic entomologists use larval development to estimate the duration of the postmortem interval (PMI) in criminal investigations (Catts 1992; Rivers & Dahlem 2014). When estimating a PMI, it is assumed that during the first few weeks of decay, insects (especially flies) will develop at predictable rates under the prevailing climatic conditions. This may not always be true if drugs (or their metabolites) are present.
Drug response of Chrysomya chloropyga

This study examined the effects of sodium methohexital (a barbiturate) and hydrocortisone (a steroid) on the developmental rate of the blowfly Chrysomya chloropyga Weidemann. Our primary reason for using these drugs was that sodium methohexital is a metabolic depressant, while hydrocortisone is a metabolic stimulant, and they may therefore have pronounced and opposite effects on development (Musvasva et al. 2001). Furthermore, previous work on the same drugs (Musvasva et al. 2001) used a flesh fly (Sarcophagidae) and it would be useful to establish if different species of fly respond to the same drugs similarly. Barbiturates are potentially dependence-producing drugs and are therefore likely to be abused, and can occur in large quantities in a body (Foye 1976). Steroids are used to treat a variety of common inflammatory conditions, but are not usually abused. Chrysomya chloropyga was chosen because it is a blowfly (Calliphoridae), and one of the first and commonest insects to colonise dead bodies in southern Africa, which gives it forensic significance.

Materials and Methods

A culture of C. chloropyga was bred from wild flies trapped on carrion in Grahamstown, South Africa. The culture was kept in a constant environment room at 25°C with a photoperiod of 12L:12D hours. The flies were fed on sugar, milk powder and water ad libitum and fresh chicken liver was provided prior to the start of the experiment as a protein meal for the flies. The flies were allowed to lay eggs on the liver and when eggs were discovered they were removed with a small portion of liver. When they hatched, the first instar larvae were transferred to the experimental jars.

Three concentrations (3 μl/g, 6 μl/g and 12 μl/g) of hydrocortisone were prepared using 5 ml of 0.9% saline and 50 g of chopped chicken liver, and placed in 150 ml glass bottles. Solutions of sodium methohexital (50 μg/g, 100 μg/g and 200 μg/g) were also made using 5 ml of 0.9% saline and 50 g of chopped chicken liver, and placed in 150 ml glass bottles. These concentrations were half of, equal to, or double the median lethal dose (MLD) for mammals (S. Daya, pers. comm.).
It is probable that breakdown products of the drugs formed during the course of the experiment due to the presence of bacteria, enzymes in the chicken liver, and the metabolism of the larvae (Goff & Lord 1994). Five replicates of each concentration for both drugs were used. Five replicates containing only 5 ml of 0.9% saline in 50 g of chicken liver were made up in 150 ml glass bottles to act as controls.

To preclude the effects of competition and metabolic heating on growth (Goodbrod & Goff 1990; Davies & Ratcliffe 1994), only ten larvae were transferred to each bottle, which was then placed in a 2 l tub containing a layer of sand to allow larvae to burrow and pupariate. The tubs were covered with fine netting secured by an elastic band to prevent larvae from escaping. The larvae were kept in a controlled environment room at 25.9° C (SD = 1.69° C). The sand was sieved daily and when larvae were found, they were placed separately in Petri dishes to complete metamorphosis. The dates of migration, pupariation, and eclosion were recorded for each larva. Deaths at each of these stages were noted.

Eclosing flies were provided with liver for feeding and oviposition. Due to significant heteroscedacity (Levene’s test, p < 0.05) or relatively small numbers of replicates (n = 5) in a nested sample design, all analyses involved Kruskal-Wallis tests performed on means of replicates. Median tests were used to identify the cause of significant ANOVA results.

**RESULTS**

No significant differences in survivorship through the larval, wandering or puparial phases or in total survivorship for either drug were detected (Table 1) despite trends in the data indicating higher mortalities in the sodium methohexital at higher concentrations. No morphological defects were observed in the adult flies that emerged and they were all fecund and fertile, laying eggs that hatched.

Although trends to faster larval development at higher drug concentrations were found (Fig. 1), no significant effects of hydrocortisone or sodium methohexital (and/or their possible breakdown products (Goff & Lord 1994) were detected in any stage of development (Table 2).

Activity levels, measured as the rate of locomotory contractions,
showed no significant differences in the hydrocortisone treatments ($H_{3,40} = 7.148253, p = 0.0673, \text{Fig. 2a}$), but a significant trend of increasing activity with increasing drug concentrations was detected in the presence of sodium methohexital ($H_{3,59} = 23.83089, p = 0.0000, \text{Fig. 2b}$). However, significantly more immature larvae left the liver and wandered around the jar when levels of hydrocortisone were high ($H_{3,20} = 9.052674, p = 0.0286, \text{Fig. 3a}$). There was also a trend for more larvae to leave the liver as levels of sodium methohexital increased, but it was not significant ($H_{3,20} = 7.548892, p = 0.0563, \text{Fig. 3b}$).

**DISCUSSION**

This study makes two important findings. First, it confirms that the same drugs produce different results in different fly species, and that extrapolations from these results to other families of flies may be unreliable. Second, this study provides an example of the potential of behaviour to provide a bioassay for the presence of drugs or their metabolites.

**Developmental responses to drugs**

The larval and puparial stages in *C. chloropyga* were reported by Prins (1982) as being 162 – 230 and 188 – 204 h, respectively, at a room temperature of 22 – 25°C. In another study the larval stage was approximately 140 hours and the pupal stage 250 hours at 25°C (Richards et al. 2009). These values are comparable with the control results obtained in this experiment, the average temperature of which was slightly higher (25.9°C). Survivorship at the larval and pupal stages did not differ significantly from the control. These two details imply that the experiment was not confounded by anomalous developmental conditions.

Development was unaffected by the presence of either hydrocortisone or sodium methohexital, even though steroids like hydrocortisone are metabolic stimulants (Foye 1976). Contrarily, both drugs significantly delayed larval development by a few hours at lower concentrations in *Sarcophaga tibialis* Macquart, but less so at higher concentrations (Musvasva et al. 2001). On the other hand, barbiturates are metabolic depressants acting on the neural and muscular system in mammals (Foye 1976). Thus the expected effect would be a reduction in the developmental rate. Sodium methohexital’s effects of increasing locomotion rate (Fig. 2) and wandering (Fig. 3) were thus not anticipated. It appears that the effects of the drugs are ameliorated by the changes in behaviour of the larvae, and we hypothesise that they are leaving the liver to avoid the drugs.

No significant effects were detected in the length of the puparial developmental stage for either drug. By comparison, in *S. tibialis* the duration of the puparial stage was significantly shorter only at the lowest concentration of both drugs (Musvasva 2001). The pattern in *C. chloropyga* may be because maggots are able to eliminate, metabolise or sequester a wide variety of drugs (Wilson et al. 1993; Sadler et al. 1995, 1997; Bourel et al. 2001). If the drugs were eliminated or metabolised before pupariation, they may then not have an effect on the developmental rate of pupae. We did not have the opportunity to assay the puparial remains for drugs, and so cannot tell if they were sequestered in the larval cuticle. However, barbiturates are not always recoverable from larval tissue (Sandler et al. 1997), and no predictable pattern in this characteristic has been detected (Sandler et al. 1995, 1997).

The effects of sodium methohexital and hydrocortisone on larval developmental rates of *C. chloropyga* were different to the effects of the same drugs on *S. tibialis* (Musvasva et al. 2001). A similar pattern has been found using heroin and its metabolite morphine: Goff et al. (1991) reported an accelerated growth rate in the larval stage of the fleshfly *S. peregrine* in response to cocaine, while Bourel et al. (1999), reported a retarded growth rate in the blowfly *Lucilia sericata* Meigen in response to morphine. In another study using *L. sericata* and codeine, the development rates were shown to be accelerated and two metabolites of codeine, morphine and norcodeine, were detected in the larvae (Kharbouche et al. 2008). The design of the experiment and the use of live animals or dead animal tissue as the food source for these experiments may also affect the results due to the presence or absence of metabolites. The issues of whether these trends are widespread, and whether they have a phylogenetic component, need to be clarified.

**Behavioural responses to drugs**

Levels of activity of the maggots, as measured by the rate of locomotory contractions, increased significantly with an increase in the concentration of sodium methohexital (Fig. 2b). This agitation parallels the number of immature maggots that were counted wandering outside the food (Fig. 3b). On the second day of the experiment, the number of maggots outside the food was significantly different from the control, but by the third day it was no longer significant. This suggests that either the sodium methohexital had degraded and that its metabolites were no longer a stimulant to the maggots, or that the maggots had acclimated to the levels of the drug (or both). This response was also observed in the highest concentration of hydrocortisone, although it was less marked.

While an effect on behaviour was not persistent in this example,
forensic scientists should be aware that unusual necrophage behaviour may indicate the presence of drugs. The development of behavioural bioassays for drugs would provide forensic science with an additional tool.

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REFERENCES


