Preliminary observations on the effects of hydrocortisone and sodium methohexital on development of *Sarcophaga* (*Curranea*) *tibialis* Macquart (Diptera: Sarcophagidae), and implications for estimating post mortem interval

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Abstract

Larvae of *Sarcophaga* (*Curranea*) *tibialis* (*S. tibialis*) were reared at constant temperature on chicken liver treated with a steroid or a barbiturate at concentrations that would be lethal, half-lethal and twice-lethal doses for humans. Trends to greater mortality at higher drug concentrations were not statistically significant. Larvae exposed to either drug took significantly longer to reach pupation compared to those in the control, while larvae exposed to sodium methohexital passed through pupation significantly faster than those in the control. No systematic relationship was found between drug concentration and development time of larvae or pupae. The total developmental period from hatching to eclosion did not differ between treatments, implying that estimates of post mortem intervals- (PMI) based on the emergence of adult flies will not be affected by the involvement of these drugs in a case. On the other hand, anomalous pupation spans may indicate the presence of barbiturates. These findings are compared with patterns found in another fly fed other contaminants.

Keywords: *Sarcophaga tibialis*; Larvae; Post mortem intervals; Development

1. Introduction

Too often, drug-related deaths are not discovered for days, especially if the victim lives alone, and ensuing decomposition and loss of body fluids can complicate forensic investigations. This has led to new applications of forensic entomology [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15]. In particular, knowledge of the effects of different drugs on the developmental rates of immature carrion-breeding insects could be useful in refining the estimation of post mortem intervals (PMI) [16], which involves deducing the minimum and maximum time interval between death and the discovery of a corpse [17].

For example, cocaine and heroin residues and metabolites accelerate development of larvae of the fleshfly *Boettcherisca peregrina* (*B. peregrina*, Robineau-Desvoidy) [7 and 8], higher concentrations of methamphetamine ("ice") accelerate development of *Parasarcophaga ruficornis* (*P. ruficornis*, F.) [9], and lower (but not high) concentrations of methylenedioxymethamphetamine (MDMA, Ecstacy) delayed larval development of the same species [12]. These effects can potentially cause misestimates of PMI of as much as 70 h [9 and 10]. Conversely, when toxicological analysis of a corpse is complicated by decomposition, unusual developmental patterns, mortality rates or fecundity may be primary evidence of the presence of drugs or their metabolites.

Goff et al. [7, 8 and 10] emphasised the need for studies of the effects of further classes of drugs on the development of more species of necrophagous flies. This study examines whether sodium methohexital, a barbiturate, and hydrocortisone, a steroid, affected the developmental rate of larvae of the carrion-breeding fleshfly *Sarcophaga* (*Curranea*) *tibialis* (*S. tibialis*) Macquart. Barbiturates are potentially dependence-producing drugs, and are, therefore, fairly commonly abused, and can occur in large quantities in a body [18]. Steroids are used to treat a variety of common inflammatory conditions, but are not usually abused. Sodium methohexital is a metabolic depressant, while hydrocortisone is a metabolic stimulant. These contrary actions were the prime reason for selecting these two compounds for this study. *S. tibialis* is found throughout the Afrotropical region, Madagascar and parts of Europe [19 and 20], and causes traumatic dermal myiasis [19]. It also breeds in carrion, giving it significance in forensic investigations.

2. Materials and methods

S. tibialis was cultured through one generation from wild flies trapped on carrion in Grahamstown to obtain large numbers of adults. The culture was maintained in a constant environment room at 25°C under a lighting cycle of 12:12 h light:dark. The flies were fed only sugar, dried skimmed milk and water ad libitum. Larvae were collected by placing 200 g of chicken liver in the holding cage for 24 h for adults to oviposit.

Sodium methohexital (Eli Lilly, Indianapolis) and hydrocortisone (as hydrocortisone sodium succinate) (Upjohn, Isando) were used. A lethal dose of sodium methohexital is about 100 mg/kg (Daya, personal communication), and that of hydrocortisone is about 6 ml/kg for mammals (Daya, personal communication). Each drug was made up in lethal, half-lethal (50 mg/kg or 3 ml/kg) and double-lethal (200 mg/kg or 12 ml/kg) doses in 5 ml of 0.9% saline carrier. Three replicates of each dose were mixed with 50 g of chicken liver in 100 ml jars. Three replicates of two control treatments of chicken liver were made up in 100 ml jars, one with 5 ml of 0.9% saline only, and the other without saline or drugs. Ten similar sized, but randomly selected, larvae were transferred to each of the 24 jars. This density is well below the level that might cause competition for food or retention of metabolic heat [21 and 22]. Each jar was placed on a layer of sand in a 2l plastic container covered with netting, and housed in a controlled environment room at 24.5°C (S.D.=0.39°C). The sand was sieved daily, and when larvae were found, they were placed individually in petri dishes to complete metamorphosis. The dates when the larva migrated and when the adult eclosed were recorded for each specimen, and note

was made of deaths before these developmental landmarks. Two weeks after eclosion, liver was supplied to the culture for oviposition.

Mortalities were initially analysed with Kruskal–Wallis-tests because of the relatively small sample sizes and because of minor departures from normality. Developmental times were analysed with nested ANOVA (because larvae were nested within jars), and survivorships were analysed with one-way ANOVA. In all cases, the same pattern of significant results (and very similar probabilities) was obtained from ANOVA as from more robust but less sensitive non-parametric-test. Post hoc pairwise comparisons were performed with Tukey's HSD-test for unequal sample sizes to identify the cause of significant ANOVA results.

3. Results

3.1. Acute effects

Although examination of the data indicated weak trends of greater mortality at higher pharmaceutical doses, especially of sodium methohexital (Fig. 1), no statistically significant differences between any treatments were detected in survivorship of either larvae (F=1.933, P=0.1303) or pupae (F=0.726, P=0.6943), or in survivorship to adulthood (F=1.839, P=0.1482).

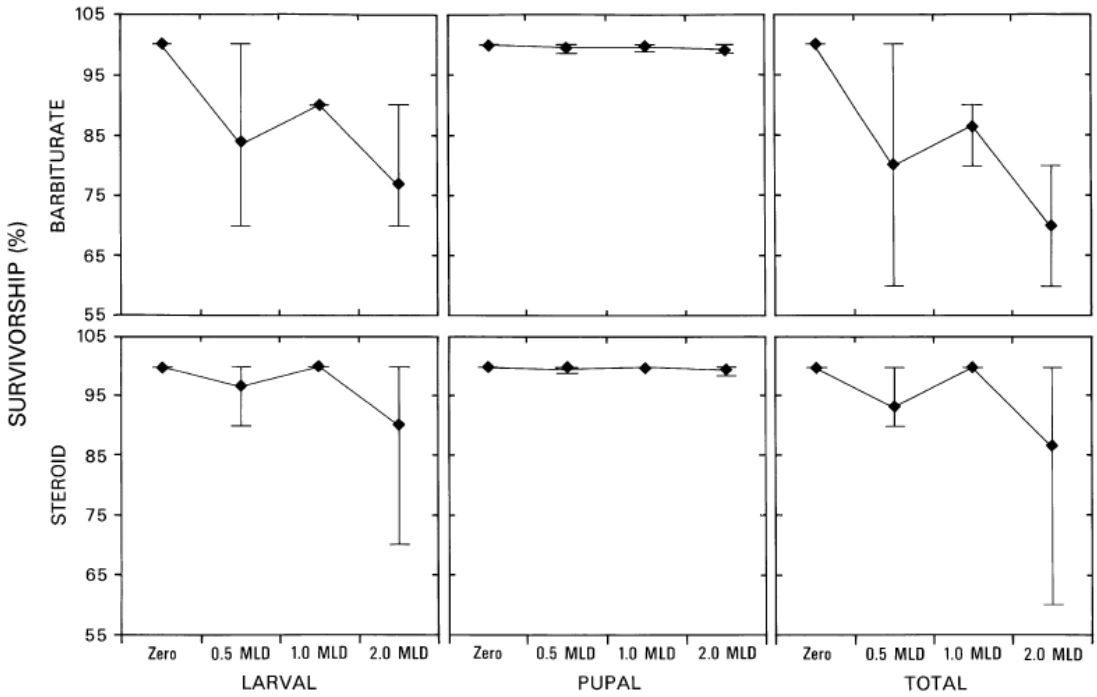

Fig. 1. Mean survival rate (with ranges) for larval, pupal and total post-hatching development of *Sarcophaga tibialis* exposed to various concentrations of sodium methohexital and hydrocortisone. No statistically significant differences were found between any treatments. MLD = median lethal dose.

3.2. Chronic effects

No linear dose-dependent relationship was found between drug dose and development time of larvae or pupae (Fig. 2). Many of the dose-duration graphs show a curved form, with effects more pronounced at lower concentrations than at higher ones. This is evidence of hormesis [23].

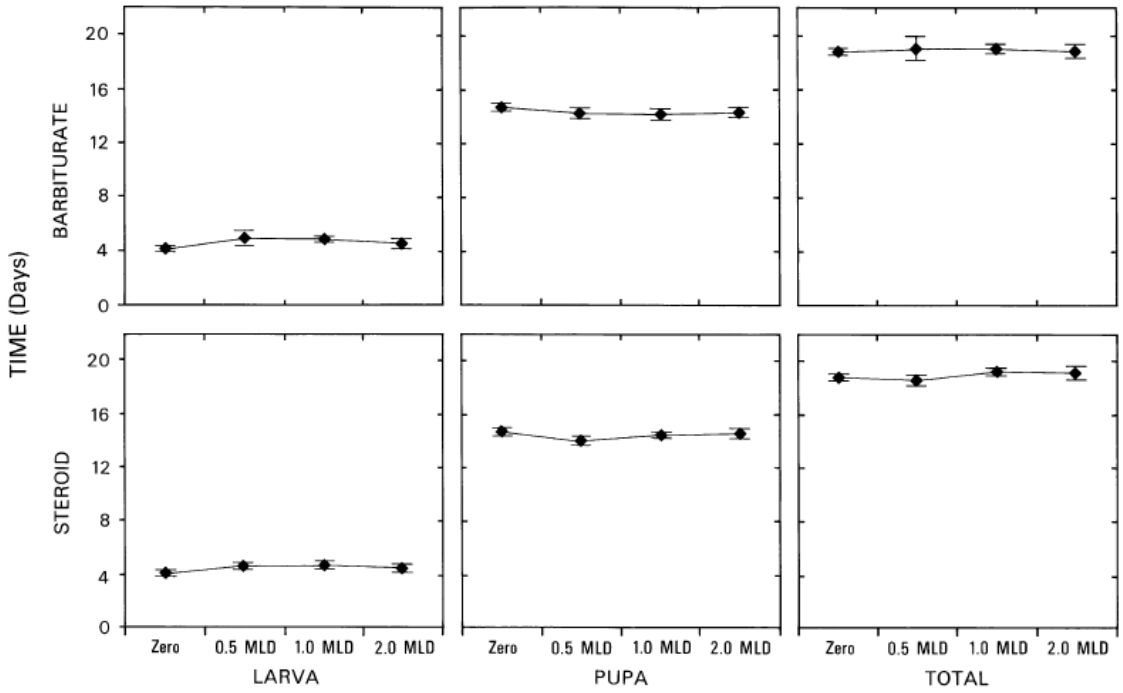

Fig. 2. Mean durations (with 95% confidence interval) for larval, pupal and total post-hatching developmental of *Sarcophaga tibialis* exposed to various concentrations of sodium methohexital and hydrocortisone. See text regarding significance of differences. MLD = median lethal dose.

The presence of sodium methohexital and/or its break-down products, significantly (F=6.327, P=0.0006) retarded development of *S. tibialis* larvae, especially at lower doses (Fig. 2). Pupae also showed a significant (F=6.985, P=0.0003) but opposite trend (Fig. 2), and total development time, therefore, showed no significant differences (F=1.325, P=0.2713) between barbiturate concentrations (Fig. 2).

Larval development was significantly delayed (F=5.788, P=0.0011) by the presence of hydrocortisone and/or its break-down products (Fig. 2). Pupae showed a significant result (F=6.289, P=0.0006) and, although, the overall trend seemed opposite to that in larvae, multiple-range-tests showed only development at the half-lethal concentration was significantly different from the other treatments (Fig. 2). Total developmental time reflected a compromise between these trends (Fig. 2), with significant differences (F=3.336, P=0.0224) being entirely due to slower development at the lethal dose.

Following eclosion, adults fed, mated and eventually produced another generation of flies. No gross morphological maldevelopment was noted in the adults.

4. Discussion

Aspoas [24] found that *S. tibialis* had a larval stage lasting about 6 days and a pupal stage lasting about 14 days at 25°C, figures that are comparable with the results of this study. Development rates of *S. tibialis* were affected by both drugs, and/or their break-down products, but the effect depended on the life stage and drug dose being considered. In both cases, the opposing trends found in larvae and pupae tended to result in total developmental times that were generally not significantly different between experimental treatments.

Barbiturates act as depressants to neural and muscular activity, marked by a decrease in oxidative metabolic processes and activities in the brain [25]. This may account for the prolonged developmental period of larvae fed on this drug (Fig. 2). However, steroids are metabolic stimulants, which does not explain the response pattern seen in that experiment. Cocaine, a stimulant, [7] and heroin, a narcotic, [8] have both been found to accelerate certain stages of larval development of the fleshfly, *B. peregrina*, while methamphetamine and methylenedioxymethamphetamine have different effects on the rate of development of *P. ruficornis* [9 and 12]. Clearly, the effects of drugs on larval development are apparently not predictable from their structural class or from their effects on vertebrates. Similarly, there was no predictable pattern in the ability of larvae of the blowfly *Calliphora vicina* Robineau-Desvoidy to metabolise related drugs [15].

As with the effect of hydrocortisone on larval development (Fig. 2), non-linear dose-dependent effects were found for cocaine [7] and heroin [8] in *B. peregrina*, and MDMA [12] in *P. ruficornis*. In the cases of hydrocortisone and MDMA, the results may indicate hormesis, which requires a more detailed sampling protocol than used in these studies to be identified unambiguously [23].

With more intensive sampling, the weak rise in mortality with increasing drug exposure (Fig. 1) may provide further evidence of sub-lethal effects of sodium methohexital or hydrocortisone, and/or their break-down products, on *S. tibialis* larvae. As in this study, survival of pupae in *B. peregrina* fed on cocaine- or heroin-contaminated tissue was about 90% and not affected by drug presence or dose [7 and 8]. MDMA affected mortality in *P. ruficornis* only at lower concentrations [12], while amitriptyline killed up to half of the larvae [10]. Maggots are able to eliminate a variety of drugs of different classes [2, 6, 14 and 15], and if they achieve this before pupation, that stage may be unaffected by larval diet.

What are the general, practical implications for forensic entomology of this preliminary study? Acute toxic effects are apparently of no practical forensic significance because dead larvae and pupae are not easily discovered amongst masses of live ones. Chronic effects pose a more likely source of evidence. Because investigators will not generally know when larvae hatched, they will not be able to measure the time span of larval

development directly and, hence, cannot use anomalous developmental durations to refine a PMI estimate. On the other hand, some drugs affect the post-feeding time span [10 and 11] or pupation time span, and these often can be measured. Anomalous post-feeding and pupation time spans, verified with a sufficiently large sample, may usefully indicate the (qualitative) presence of amitriptyline [10], phencyclidine [11], barbiturates (Fig. 2) or heroin [8]. However, it remains to be confirmed that different fly species react in the same way to these drugs. Furthermore, cocaine and MDMA produced no such effect in *B. peregrina* and *P. ruficornis*, respectively [7 and 12], so that the lack of anomalies is not evidence of an absence of contaminants.

Goff et al. [7, 8, 9 and 12] found that standard indirect methods of estimating PMI, based on regression against larval mass or length, may need to take the effects of contaminants into account. Sometimes it is enough to know that a particular drug was present because that drug lacks dose-dependent effects on developmental rate. A simple correction to the PMI is then possible. Where dose-dependent effects are known, one must determine the concentrations of contaminants to which larvae were exposed. Direct measurement of residues in decomposed tissue is probably best because there is not always a useful correlation between the concentration of a drug in tissue and the amount present in larvae that have fed on it [15]. This is probably because maggots can eliminate a variety of drugs with varying success [2, 6, 13, 14, 15 and 26]. However, careful selection of the developmental event on which the PMI is based can sometimes circumvent the need for these corrections. For instance, since total immature lifespan of *S. tibialis* is largely unaffected by the presence of sodium methohexital, hydrocortisone and/or their break-down products (Fig. 2), PMI estimates based on the time of eclosion of adults of *S. tibialis* will not need to be corrected for their presence.

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