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Preliminary Observations of the Effects of Amitriptyline in Decomposing Tissues on the Development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and Implications of This Effect to Estimation of Postmortem Interval

REFERENCE: Goff, M. L., Brown, W. A., Omori, A. I., and LaPointe, D. A., **"Preliminary Observations of the Effects of Amitriptyline in Decomposing Tissues on the Development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and Implications of This Effect to Estimation of Postmortem Interval,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 2, March 1993, pp. 316–322.

ABSTRACT: Larvae of *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae) were reared on tissues from rabbits administered different dosages of amitriptyline to study the effects of this drug on the development of this insect species. The rabbits were given 300, 600, and 1000 mg of amitriptyline via ear vein infusion. No significant differences in rates of larval growth were observed among the colonies. Durations of the larval stage were significantly longer for larvae fed on tissues from rabbits receiving amitriptyline. Larval mortality was observed to be 5.5% for the control colony, but ranged from 40.5 to 57.5% for the test colonies. Durations of the puparial stage were significantly longer for the colonies fed on tissues from the rabbits receiving the 600 and 1000 mg dosages of amitriptyline than for the control and colony fed on tissues from the rabbit receiving the 300 mg dosage. Observed differences in the durations of the larval and pupal stages from the test colonies were sufficient to alter a postmortem interval estimate by up to 77 h, if based on normal developmental patterns for this species at 26°C. Presence of amitriptyline and nortriptyline could be detected in larvae from all colonies fed on tissues from the rabbits receiving amitriptyline using high-performance liquid chromatography (HPLC).

KEYWORDS: toxicology, postmortem interval, entomology, Diptera, amitriptyline, nortriptyline, drugs

The use of maggots as alternate specimens for toxicologic analyses for drugs and toxins has been well documented [*1–4*]. While these studies have dealt with detections of both prescription and illegal drugs and toxins, fewer studies have concerned themselves with the effects of these substances on the developmental patterns of the maggots. Work by Goff et al. [*5–7*] has documented differences in rates of growth for species of flesh fly maggots (Diptera: Sarcophagidae) fed on tissues containing varying amounts of cocaine, heroin, methamphetamine, and their metabolites. These studies have emphasized the

Received for publication 10 June 1992; revised manuscript received 30 July 1992; accepted for publication 31 July 1992.

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significance of these differences in rates of development when estimating a postmortem interval using techniques of forensic entomology. These estimates are generally based on the period of time required for arthropods using decomposing remains as a food source to reach a given stage of development or through interpretations of succession patterns for the species associated with the remains [*8,9*]. An example of adjustment of the postmortem interval estimate due to presence of cocaine has recently been documented by Lord [*10*]. Nolte et al. [*11*] have detailed another case in which detection of cocaine in Calliphoridae larvae contributed to the determination of the circumstances of death.

This study concerns the effects of the tricyclic antidepressant amitriptyline on the rate and pattern of development of the sarcophagid fly *Parasarcophaga ruficornis* (Fabricius). In the Hawaiian Islands, amitriptyline is the drug most frequently encountered in suicide cases (AIO) and *P. ruficornis* is frequently associated with decomposing human remains on the island of Oahu [*12*].

Materials and Methods

Three domestic rabbits (4.0 to 5.0 kg in weight) were given dosages of 300, 600, and 1000 mg of amitriptyline in 10 mL of normal saline via ear vein infusion to produce different concentrations of amitriptyline in tissues. These dosages were calculated to represent sublethal, median lethal and 2.0X median lethal dosages of the drug by body weight. A fourth rabbit (4.5 kg in weight) was used as a control and received only 10 mL of normal saline by ear vein infusion. The rabbits receiving the 600 and 1000 mg dosages of the drug expired almost immediately following administration of the drug. The rabbit receiving the 300 mg dosage became moribund within 1 min following administration of the drug and died within 3 min. The control rabbit was killed in a carbon dioxide chamber.

Immediately following death, a 2 mL blood sample was taken from each rabbit and frozen for later analysis of drug content. The livers were removed from each rabbit and a sample of tissue removed from each and frozen. Livers weighed from 96 to 126 g, with a mean of 111.5 g. Blood and liver samples were analyzed by the Chemical Toxicology Institute, Foster City, California, using high-performance liquid chromatography (HPLC).

Flies used in this study were from a stock colony of *Parasarcophaga ruficornis* (F.) established from specimens collected off a suicide case during 1990 and maintained in the laboratory for 12 generations. Beef liver was exposed to this colony for a period of 15 min to allow for larviposition. From this larviposition, approximately 210 larvae were placed onto each test liver to establish the test colonies. Colonies thus established were maintained in the laboratory at 26°C in a Labline Ambi-Hi-Low environmental chamber with a 12 h photoperiod. At 6 h intervals, total body lengths were recorded from random samples of 10 larvae from each colony to indicate growth rates. At 48 h, a sample of 10 larvae was removed from each colony and frozen for later analyses of drug content. After completion of larval development, pupae were observed at 6 h intervals and adult emergence recorded. Emerging adults were maintained in separate colonies and provided with a standard diet of water, sugar, and protein hydrolysate. Thirteen days following emergence, liver was supplied to each colony for larviposition. The data were analyzed using analysis of variance (ANOVA) and Waller-Duncan multiple range test [*13*]. The various colonies will be referred to from this point on by the dosage of amitriptyline administered to the rabbits (300 mg, 600 mg, 1000 mg, and control colonies).

Results

The analyzed blood and liver samples showed the presence of amitriptyline and nortriptyline for all rabbits that had received the drug (Table 1). Blood and liver samples

TABLE 1—*Concentrations of amitriptyline and nortriptyline in blood and liver tissues from rabbits administered different dosages of amitriptyline via ear vein infusion.*

Dosage	Control		300 mg		600 mg		1000 mg	
Tissue	blood (mg/mL)	liver (mg/kg)	blood (mg/mL)	liver (mg/kg)	blood (mg/mL)	liver (mg/kg)	blood (mg/mL)	liver (mg/kg)
Amitriptyline	0.3	1.4	38.0	24.0	656.0	154.0	10.0	49.0
Nortriptyline	0	0	0.8	3.3	16.0	7.1	0.1	0.9

TABLE 2—*Durations of larval stage, larval mortality, puparial weights, lengths, durations and mortalities for colonies of* Parasarcophaga ruficornis *reared on rabbit liver tissues containing varying amounts of amitriptyline and nortriptyline at a temperature of 26°C.*

Colony	$\bar{X}$ Duration of[a] larval stage (h)	Larval mortality	$\bar{X}$ Puparial weight (mg)	$\bar{X}$ Puparial length (mm)	$\bar{X}$ Duration of puparial stage (h)	Puparial mortality
Control	159.9 a (N = 189)	5.5%	10.1 a (N = 189)	10.78 a (N = 20)	313.8 a (N = 185)	4.2%
300 mg	173.4 b (N = 85)	57.5%	10.6 a (N = 85)	10.95 ab (N = 20)	311.8 a (N = 82)	3.5%
600 mg	190.1 c (N = 119)	40.5%	11.5 b (N = 119)	11.28 b (N = 20)	316.1 b (N = 111)	5.0%
1000 mg	172.7 b (N = 115)	42.5%	11.4 b (N = 115)	11.35 b (N = 20)	348.2 c (N = 115)	0.0%

[a]Figures in a column followed by the same letter are not significantly different ($P > 0.05$).

from the control rabbit showed traces of amitriptyline, but were negative for nortriptyline (Table 1).

Analyses were made of 10 larvae from each colony collected at the 48 h sample period using HPLC. Low levels of amitriptyline (1.0 mg/kg) and nortriptyline (1.2 mg/kg) were detected in the sample from the control colony. The larvae from the 300 mg colony had amitriptyline at 4.7 mg/kg and nortriptyline at 3.6 mg/kg. Larvae from the 600 mg colony had amitriptyline at 27 mg/kg and nortriptyline at 3.7 mg/kg. The larvae from the 1000 mg colony had amitriptyline at 2.0 mg/kg and nortriptyline at 0.6 mg/kg.

Rates of development were determined by increases in total body length over time for larvae until maximum size was attained. These rates were not significantly different among the colonies and there were no significant differences in the maximum lengths attained (Fig. 1). The greatest lengths were observed for larvae in the 1000 mg colony, with a mean length of 21.3 mm observed at hour 78 (Fig. 1). Pupation was first observed in the control colony at 132 h, followed by the 300 mg colony at 138 h, and both the 600 and 1000 mg colonies at 144 h. There were significant differences among colonies in the duration of the larval stage (total period of time required for development from 1st instar larva to pupariation), with a mean duration of 159.9 h for the control colony (Table 2), 173.4 h for the 300 mg colony, 190.1 h for the 600 mg colony, and 172.7 h for the 1000 mg colony. Significant differences were also observed in the larval mortality rates (Table 2), with the control colony having a rate of 5.5% and colonies reared on tissues from rabbits receiving the drug ranging from 40.5 to 57.5%. There were also significant differences among colonies in the weights of the pupae, with heavier pupae produced in the 600 and 1000 mg colonies. Pupae from colonies fed on tissues from rabbits receiving the drug were also longer than pupae from the control colony (Table 2). There were significant differences ($P < 0.05$) observed in the duration of the pupal stage (defined as the period of time within the puparium until adult emergence) (Table 2). Larvae from the 600 and 1000 mg colonies required longer to complete the process than those from the 300 mg and control colonies. Significant differences were not observed among colonies in pupal mortality (Table 2).

When supplied with liver for larviposition 13 days following adult emergence, normal larvae were produced by the control and 300 mg colonies. Adults from the 1000 mg

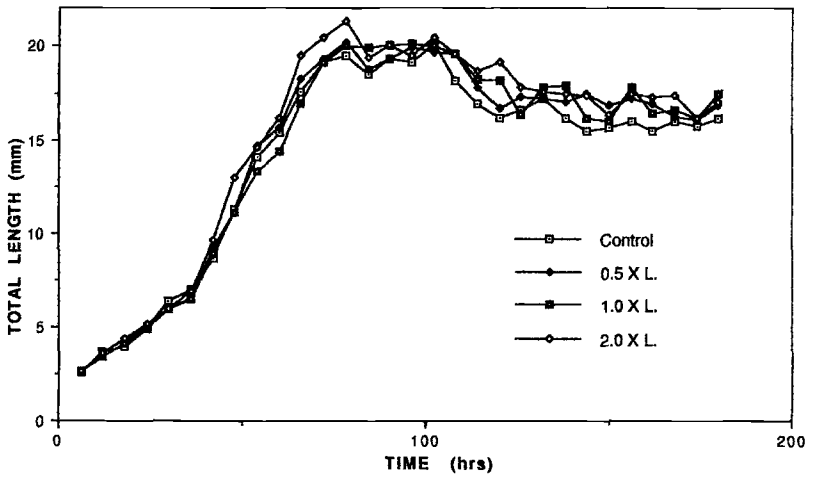

FIG. 1—*Rates of development, as indicated by total body length, of larvae of* Parasarcophaga ruficornis *reared on rabbit liver tissues dosed with different amounts of amitriptyline by ear vein infusion. Dosage of 300 mg = 0.5XL; 600 mg = 1.0XL; and 1000 = 2.0XL.*

colony produced larvae, however, only approximately 50% of these were viable. The adults from the 600 mg colony failed to produce larvae.

Discussion

Unlike the studies previously conducted dealing with cocaine, heroin, and methamphetamine [5–7], the concentrations of amitriptyline or nortriptyline, or both, detected in tissues were not directly proportional to the dosages of the drug administered. Low levels of amitriptyline were also detected in the control tissues. These low levels represent normal background counts rather than an actual presence of these substances in the control.[3] The concentrations of amitriptyline and nortriptyline detected in tissues from the rabbit receiving the 1000 mg dosage were lower than those detected in tissues from the rabbits receiving the 600 mg dosage. This difference may be attributed to the rapid onset of death in the case of the 1000 mg dosage. This dosage, which corresponded to a 2X median lethal dosage for the rabbit, resulted in almost immediate death. By contrast, while onset of death was also rapid, the 600 mg dosage allowed the rabbit to survive for a slightly longer period of time and the drug was more widely distributed through the tissues.

As was the case for the tissues from the control rabbit, there were low levels of both amitriptyline and nortriptyline detected in larvae from the control colony. Again, these represented normal background values rather than the actual presence of these substances in the larvae.[4] In routine analyses, these values would be subtracted from the values obtained from questioned samples to give the actual concentrations of the drug present. In all cases where amitriptyline was administered, both amitriptyline and nortriptyline were detected in larvae at levels above those for the control colony. As was observed for tissue samples, values for the 1000 mg dosage were lower than those obtained for the 600 mg dosage. Given these results, it appears that larvae may serve as alternate samples for detection of amitriptyline, or nortriptyline, or both, in cases where adequate tissue samples may not be available for analysis.

Unlike the situation observed in previous studies dealing with cocaine, heroin, and methamphetamine [5–7], there were no significant differences observed in the rate of growth among colonies during the larval stage. While the greatest sizes were attained in the 1000 mg colony, differences among colonies in size were not significant. There were significant differences in the total duration of the larval stage (Table 2). The larval stage was longer for larvae fed on tissues containing greater amounts of the drugs. This difference was sufficient to result in an error of up to 30 h, if an estimate of the postmortem interval is based on the total duration of the larval stage under normal conditions at 26°C. While the lowest larval mortality was observed in the control colony, larval mortality in the treated colonies varied inversely with the concentration of the drugs in the liver tissues and all were significantly greater than for the control colony. There were no comparable data recorded from earlier studies on cocaine, heroin or methamphetamine, thus comparisons are not possible here.

Durations of the pupal period were observed to vary among colonies, with 600 and 1000 mg colonies requiring significantly longer to complete the process. This is similar to the pattern observed in earlier work on heroin and the reverse of what was observed for methamphetamine [6,7]. In earlier work dealing with cocaine [5], no significant differences were observed among colonies in duration of the pupal period that could be related to the concentration of cocaine or its metabolite benzoylecognine in tissues. Results of this study indicate that an estimate of postmortem interval based on the duration of the pupal stage could be in error by up to 47 h. When this is combined with

[3]R. C. Baselt, personal communication, 1992.
[4]R. C. Baselt, personal communication, 1992.

the error possibly resulting from the increased duration of the larval stage, the total error could be up to 77 h. There were no significant differences in pupal mortality during this study.

Although these data are of a preliminary nature and only for a single species of Diptera, it is not unreasonable to assume that similar variations may exist for other species when fed on tissues containing amitriptyline. More detailed studies of the effects of this and other drugs in tissues on arthropod development are clearly indicated. Until appropriate baseline data are available, care must be taken in interpretations of arthropod developmental rates for estimations of postmortem intervals where drugs may be a factor. While it may not be feasable in all cases, whenever possible a toxicological analysis of tissues or larvae, or both, should be completed prior to the entomologist's final estimation of the postmortem interval.

Acknowledgments

Thanks are extended to Dr. R. C. Baselt, Chemical Toxicology Institute, Foster City, California, for his assistance in interpretations of results of analyses of tissues and larvae. This study was supported by a grant from the Pathology/Biology Research Committee of the Forensic Sciences Foundation, Inc. This is Journal Series No. 3720 of the Hawaii Institute of Tropical Agriculture and Human Resources.

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