TECHNICAL NOTE

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Preliminary Observations of the Effect of Methamphetamine in Decomposing Tissues on the Development Rate of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and Implications of this Effect on the Estimations of Postmortem Intervals

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ABSTRACT: Larvae of *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae) were reared on tissues from rabbits administered different dosages of methamphetamine to study the effects of this drug on development of this species. The rabbits were given 37.5, 71.4, and 142.9 mg of methamphetamine via ear vein infusion. From Hours 30 to 60, larvae feeding on tissues from rabbits receiving 71.4 and 142.9 mg of methamphetamine developed more rapidly than larvae from the control colony and those feeding on tissues from the rabbit receiving 37.5 mg of methamphetamine. The time required for pupariation was significantly greater for colonies fed on tissues from methamphetamine-dosed rabbits than for the control. These differences were sufficient to alter postmortem interval estimates based on larval development by up to 18 h and estimates based on puparial development by up to 48 h. The presence of methamphetamine or amphetamine could not be detected in Diptera larvae in this experiment using radioimmunoassay techniques, as there was a nonspecific reaction, resulting in a false positive.

KEYWORDS: toxicology, postmortem interval, entomology, Diptera, methamphetamine, amphetamine, development drugs

Drug-related deaths are, in many instances, not discovered for a period of several days and, due to changes associated with decomposition, estimates of postmortem intervals are made using techniques involving arthropod development and successional patterns

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[*1–3*]. Concentrations of drugs and toxicants have been determined by analyses of Diptera larvae taken from decomposed tissues [*4,5*]. Nuorteva and Nuorteva [*6*] have demonstrated that tissues containing mercury may have an adverse effect on Diptera larvae feeding on them. Recently, Introna et al. [*7*] have demonstrated a correlation between the concentration of opiates in tissues and the concentrations in larvae feeding on those tissues. With the exception of work by Goff et al. [*8,9*], studies have not been published concerning the effects of drugs, such as cocaine, heroin, and methamphetamine, in tissues on the developmental patterns of Diptera larvae feeding on them. As these larvae and their developmental patterns are used extensively in estimations of postmortem intervals in cases involving decomposing remains [*1–3*], data on the effects of these drugs on larval development are important, particularly in cases presented during the early stages of decomposition, where Diptera larvae are the predominant group present [*10*].

The present report deals with the effects of methamphetamine or "ice" in tissues on the development of the sarcophagid fly *Parasarcophaga ruficornis* (Fabricius). This species of fly has been associated with decomposing human remains on the island of Oahu, Hawaii [*11*].

Materials and Methods

Three domestic rabbits (4.2 to 4.6 kg in weight) were given dosages of 37.5, 71.4, and 142.9 mg of methamphetamine in 5 mL of normal saline by ear vein infusion to produce different concentrations of methamphetamine in tissues. The dosages were calculated to represent sublethal, median lethal, and $\times 2$ median lethal dosages of the drug by weight. A fourth rabbit (4.2 kg) was used as a control and received only 5 mL of normal saline by ear infusion. The rabbit receiving the 142.9-mg dosage expired within 2 min following administration of the drug. All other rabbits were sacrificed in a carbon dioxide chamber, 10 min following administration of the drug.

Immediately after death, a 2-mL blood sample was taken from each rabbit and frozen for later analysis of drug content. The livers, spleens, and kidneys were also removed from each rabbit. The livers and spleens had combined weights ranging from 126 to 167 g, with a mean of 145 g, and the kidneys 25.4 to 34.0 g, with a mean of 31.5 g. A sample of tissue from each liver was frozen for later analyses of methamphetamine and amphetamine content. Blood and liver samples were analyzed by the Chemical Toxicology Institute, Foster City, California, using radioimmunoassay process.

The flies used in this study were from a stock colony of *Parasarcophaga ruficornis* (F.) established from specimens collected from a suicide case during 1990 and maintained in the laboratory for five generations. The livers were all exposed to this colony for larviposition for 3 to 5 min. This period of exposure in previous experiments has resulted in deposition of 300 to 500 larvae—a number sufficient for this type of study, although no actual counts of each colony were attempted. Colonies thus established were maintained in the laboratory at 26°C in a Labline Ambi-Hi-Low environmental chamber. From this point on, colonies will be referred to by the dosage of methamphetamine administered (37.5, 71.4, or 142.9, and control). At 6-h intervals, total lengths were recorded for ten larvae from each colony to indicate growth rates. At 24-h intervals, a sample of ten larvae was removed from each colony and frozen for later analyses of drug and metabolite content. After completion of larval development, the pupae were observed at 6-h intervals and adult emergence recorded. Emerging adults were maintained in separate colonies in the laboratory and provided with a standard diet of sugar, protein hydrolysate, and water. Thirteen days following emergence, liver was supplied to each colony for larviposition. The data were analyzed by analysis of variance (ANOVA) and Waller-Duncan multiple range tests [*12*].

Results

The analyzed blood and liver samples showed the presence of methamphetamine and amphetamine in all the rabbits which had received the drug. Samples from the control were negative by radioimmunoassay (RIA) for both substances. The blood sample from the rabbit receiving the 37.5-mg dosage showed methamphetamine at 5380 ng/mL and amphetamine at 140 ng/mL, while liver tissue had methamphetamine at 3026 ng/mL and amphetamine at 2157 ng/mL. Blood from the rabbit receiving 71.4 mg of methamphetamine showed methamphetamine at 12 240 ng/mL and amphetamine at 510 ng/mL, while the liver tissues had methamphetamine at 7594 ng/mL and amphetamine at 5249 ng/mL. Blood from the rabbit receiving 142.9 mg of methamphetamine had methamphetamine present at 43 440 ng/mL and amphetamine at 1600 ng/mL, while liver tissues had methamphetamine at 55 698 ng/mL and amphetamine at 7607 ng/mL.

Analyses were made of ten larvae from each colony at 24- and 48-h sample periods using RIA. A cutoff value of 50 ng/mL was established for a positive finding. Larvae from all colonies reared on tissues from rabbits receiving methamphetamine and the control colony showed a weak positive reaction (>50 ng/mL) at both sample intervals.

All tissue samples were highly attractive to the adult *P. ruficornis*, and larviposition began immediately upon exposure of the livers to flies in a darkened environmental chamber. Because of the large numbers of larvae produced, no actual counts were attempted for each colony. Previous published [*8,9*] and unpublished work in the Forensic Entomology Laboratory, University of Hawaii at Manoa, has indicated that exposure of liver to a colony of *P. ruficornis* for a period of 3 to 5 min will result in deposition of 300 to 500 larvae. Colonies in this experiment appeared to be consistent with that range.

Rates of development, as indicated by total body length of larvae, were not significantly different among samples from Hours 0 to 24 (Fig. 1); mean lengths ranged from 2.0 mm for all larvae at Hour 0 to 4.7 to 5.4 at Hour 24. This period corresponded roughly to the first stadium. Beginning at Hour 30, significant differences ($P < 0.05$) were observed

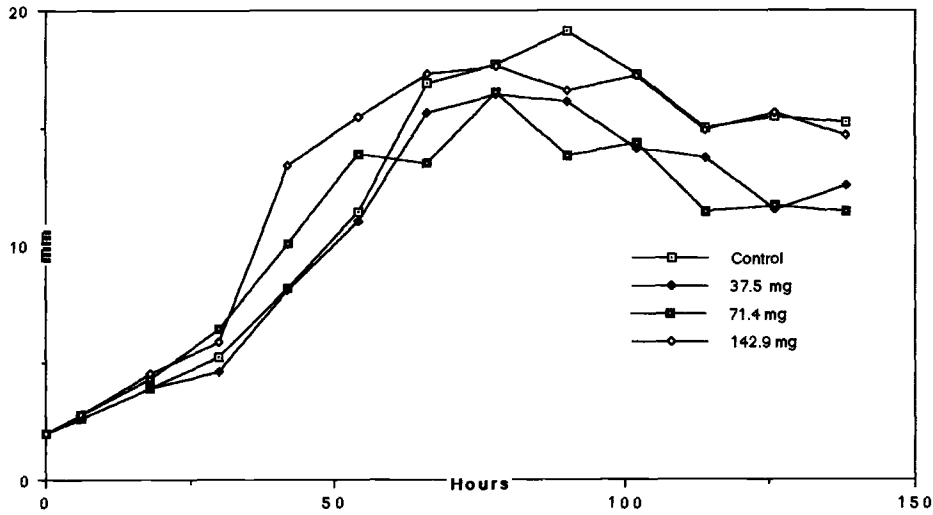

FIG. 1—*Rates of development, as indicated by total body length, of larvae of* Parasarcophaga ruficornis *reared on liver tissues from rabbits dosed with 37.5, 71.4, and 142.9 mg of methamphetamine by ear vein infusion.*

in rates of development for the 71.4 and 142.9 mg colonies when compared with the 37.5-mg and control colonies. This difference continued until Hour 60, at which point the rate of growth for the 71.4-mg colony slowed and only the 142.9-mg colony was significantly different in rate of development. Maximum size was attained by larvae in the 142.9-mg colony (20 mm) and the 71.4-mg colony (17 mm) at Hour 78; growth continued longer in the 37.5-mg and control colonies, with maximum lengths of 18 and 20 mm, respectively, attained at Hour 90.

The prepupal stage, marked by a decrease in total body length and emigration away from the food source, was first observed in the 71.4- and 142.9-mg colonies at Hour 84, followed by the 37.5-mg colony at Hour 90, and the control colony at Hour 96. Pupation was first observed in the 71.4-mg colony at Hour 114, followed by the 37.5-mg colony at Hour 132, and both the 142.9-mg and control colonies at Hour 138 (Table 1). Total lengths of pupae ranged from 7.5 to 10.0 mm, and no significant differences were detected among colonies ($P > 0.05$). Significant differences were observed between colonies ($P < 0.05$) in pupal weights, with the heaviest pupae in the control colony ($\bar{X}$ = 9.2 mg, N = 50), followed by the 142.9-mg colony ($\bar{X}$ = 7.2 mg, N = 50), the 37.5-mg colony ($\bar{X}$ = 4.7 mg, N = 50), and the 71.4-mg colony ($\bar{X}$ = 4.2, N = 50).

Durations of the pupal stage and pupal mortalities are given in Table 1. The minimum duration of the pupal period was significantly shorter for all colonies fed on tissues from rabbits receiving the methamphetamine. While pupal mortality rates were approximately equal for the control and 142.9-mg colonies, there was an inverse relationship between pupal mortality and dosage of methamphetamine administered, with the greatest mortality recorded for the 37.5-mg colony (60.6%). Minimum time required for development from larva to adult was greater for larvae from the control colony (476 h) than for larvae fed on tissues from rabbits receiving the dosages of methamphetamine (37.5 mg—426 h; 71.5 mg—409 h; 142.9 mg—428 h). Adults from the 37.5-mg, 142.9-mg, and control colonies produced viable larvae when supplied with liver 13 days following adult eclosion. Adults from the 71.5-mg colony did not produce viable larvae, but only dead larvae and eggs which did not hatch. While both the 37.5-mg and control colonies continued, the 142.9-mg colony failed to produce viable larvae during the 2nd generation.

Discussion

Unlike the situation observed for previous studies involving cocaine [*8*] and heroin [*9*] in decomposing tissues, there was a weak positive result for RIA analyses of larvae from both the control and treated colonies of *P. ruficornis* (>50 ng/mL). This uniform weak

TABLE 1—*Maximum larval lengths, minimum durations of developmental stages and puparial mortality for* Parasarcophaga ruficornis *reared at 26°C on rabbit liver tissues containing varying amounts of methamphetamine.*[a]

Colony	Maximum Length of Larva, mm / Time Maximum Observed, h	Minimum Duration of Larval Stage, h	Minimum Duration of Puparial Stage, h	% Puparial Mortality
Control	20/90	138	338 *a*	4.4 *a*
37.5 mg	18/90	132	294 *b*	60.6 *b*
71.4 mg	17/78	114	295 *b*	21.4 *c*
142.9 mg	18/78	138	290 *b*	5.5 *a*

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

reaction may be the result of a nonspecific reaction to some substance other than the actual presence of methamphetamine or amphetamine (R. C. Baselt, 1991, personal communication). The presence of such a reaction would preclude use of Diptera larvae in RIA analyses as alternate toxicologic specimens in cases involving methamphetamine as a result of the possibility of a false positive. This is supported by the uniformity of the reaction in all samples and the wide range of concentrations detected by RIA analyses of the tissues sampled (ranging from negative for the control to 55 698 ng/mL for the ×2 median lethal dosage). It is possible that other, more sensitive analytical procedures may prove to be of value in detecting methamphetamine in Diptera larvae. Regrettably, because of breakage of vials during shipment, we were unable to attempt other techniques during this experiment.

The presence of methamphetamine and amphetamine in tissues resulted in increased rates of growth for larvae of *P. ruficornis* feeding on tissues from rabbits receiving the median lethal and ×2 median lethal dosages of the methamphetamine. No significant effect was observed for larvae fed on tissues from the rabbit receiving the sublethal dosage. Unlike larvae fed on tissues containing cocaine and heroin, larvae from all colonies fed on tissues from rabbits receiving methamphetamine were smaller at maximum length than those from the control colony. Additionally, the maximum length was attained earlier in colonies fed on tissues containing methamphetamine and amphetamine than for the control. As was observed for heroin, but not for cocaine, there was a relationship between the concentration of methamphetamine and amphetamine in tissues and the duration of the pupal stage. Unlike the result observed for cocaine [*8*] and heroin [*9*], significant differences were found in the pupal mortality recorded for colonies. While the rates for the control and ×2 median lethal dosages were approximately equal, there was an inverse relationship between the dosage and pupal mortality observed among treated colonies. That there are adverse effects on subsequent generations caused by exposure to methamphetamine was indicated by the failure of the median lethal dosage colony to produce viable larvae during the first generation and the ×2 median lethal dosage colony during the second.

While the presence or absence of methamphetamine and amphetamine in tissues could not be determined using RIA techniques in this experiment, significant differences were observed in rates of development for larvae fed on tissues from rabbits receiving methamphetamine. An estimate based on normal rates of development at 26°C could be in error by up to 18 h, if based on the larval development. Should the estimate be based on pupal development, the error could be up to 48 h, depending on the concentration of the drug in the tissues. Although by the very limited nature of this experiment these data are of only a preliminary nature and only for one species of Diptera, it is reasonable to assume that similar variations may exist for other species when fed on tissues containing these substances. Further studies of analytical techniques to detect methamphetamine and amphetamine in Diptera larvae, and the effects of these drugs on arthropod development, are clearly indicated. Until appropriate baseline data are available, care must be taken in interpretations of arthropod developmental data and succession patterns in cases where methamphetamine or amphetamine may be a factor.

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References

[1] Goff, M. L. and Odom, C. B., "Forensic Entomology in the Hawaiian Islands: Three Case Studies," *American Journal of Forensic Medicine and Pathology*, Vol. 8, No. 1, 1987, pp. 45–50.

[2] Goff, M. L., Omori, A. I., and Gunatilake, K., "Estimation of Postmortem Interval by Arthropod Succession: Three Case Studies from the Hawaiian Islands," *American Journal of Forensic Medicine and Pathology*, Vol. 9, No. 3, 1988, pp. 220–225.

[3] Goff, M. L. and Flynn, M. M., "Determination of Postmortem Interval by Arthropod Succession: A Case Study from the Hawaiian Islands," *Journal of Forensic Sciences*, Vol. 36, No. 2, March 1991, pp. 607–614.

[4] Beyer, J. C., Enos, W. F., and Stajic, M., "Drug Identification Through Analyses of Maggots," *Journal of Forensic Sciences*, Vol. 25, No. 2, April 1980, pp. 411–412.

[5] Gunatilake, K. and Goff, M. L., "Detection of Organophosphate Poisoning in a Putrefying Body by Analyzing Arthropod Larvae," *Journal of Forensic Sciences*, Vol. 34, No. 3, May 1989, pp. 714–716.

[6] Nuorteva, P. and Nuorteva, S. L., "The Fate of Mercury in Sarcosaprophagous Flies and in Insects Eating Them," *Ambio*, Vol. 11, 1982, pp. 34–37.

[7] Introna, F., Jr., Lo Dico, C., Caplan, Y. H., and Smialek, J. E., "Opiate Analysis in Cadaveric Blowfly Larvae as an Indicator of Narcotic Intoxication," *Journal of Forensic Sciences*, Vol. 35, No. 1, Jan. 1990, pp. 118–122.

[8] Goff, M. L., Omori, A. I., and Goodbrod, J. R., "Effect of Cocaine in Tissues on the Development Rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae)," *Journal of Medical Entomology*, Vol. 26, No. 2, 1989, pp. 91–93.

[9] Goff, M. L., Brown, W. A., Hewadikaram, K. A., and Omori, A. I., "Effect of Heroin in Decomposing Tissues on the Development Rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae) and Implications of This Effect on Estimation of Postmortem Intervals Using Arthropod Development Patterns," *Journal of Forensic Sciences*, Vol. 36, No. 2, March 1991, pp. 537–542.

[10] Early, M. and Goff, M. L., "Arthropod Succession Patterns in Exposed Carrion on the Island of O'ahu, Hawaiian Islands, USA," *Journal of Medical Entomology*, Vol. 23, No. 5, 1986, pp. 520–536.

[11] Goff, M. L., Early, M., Odom, C. B., and Tullis, K., "A Preliminary Checklist of Arthropods Associated with Exposed Carrion in the Hawaiian Islands," *Proceedings of the Hawaiian Entomological Society*, Vol. 26, 1986, pp. 53–57.

[12] "SAS User's Guide: Statistics," SAS Institute, Cary, NC, 1982, pp. 217–221.

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