Francesco Introna, Jr.,[1] *M.D.; Charles Lo Dico,*[2] *M.S.;*
Yale H. Caplan,[2] *Ph.D.; and John E. Smialek,*[2] *M.D.*

Opiate Analysis in Cadaveric Blowfly Larvae as an Indicator of Narcotic Intoxication

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ABSTRACT: Specimens of liver were collected from 40 cases in which the cause of death had been determined to be opiate intoxication. Rearings of Calliphora vicina larvae were then promoted on the decomposing liver. A control group of 10 decomposed liver specimens from non-opiate deaths was treated similarly. Analysis of larvae and liver for opiates (morphine) was conducted by radioimmunoassay. Good qualitative and quantitative correlation was observed in both the positive and negative groups. Regression analysis comparing the concentrations of opiates found in the larvae with those found in the liver in the positive group resulted in a correlation of $r = 0.790$.

KEYWORDS: pathology and biology, larvae, radioimmunoassay, opiates, liver (decomposed)

Blowfly larvae can be useful in estimating the minimum time since death. However, they have seldom been used to obtain information on chemical contaminants and toxicological substances present in severely decomposed bodies where no other tissues were available for toxicological analysis. Nuorteva [*1*] described an analysis for mercury on blowflies collected from the decayed corpse of a young woman discovered in a pit. The mercury content was 0.12 to 0.15 ppm, which indicated that the blowflies developed in nonpolluted biological material. Blowflies are capable of accumulating mercury to highly elevated concentrations surpassing the natural level (threshold 0.2 ppm). Consequently, a conclusion was drawn that the murdered girl had not lived in a mercury polluted area. This may have been the first such use of a mercury analysis.

Only two cases have been reported in which the cadaveric fly larvae were used as toxicological specimens to define the cause of death [*2,3*]. In 1980 Beyer et al. [*2*] reported a case in which toxicological analysis performed on larvae of Cochliomyia macellaria, collected from a decomposed body found in a wooded area, revealed the presence of phenobarbital, indicative of a suicidal overdose. In 1988, Gunatilake and Goff [*3*] reported a case in which toxicological analysis, performed on larvae of Chrysomya megacephala

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[1]Fellow in forensic pathology, University of Maryland, Baltimore, MD and researcher, Institute of Forensic Medicine, University of Bari, Policlinico, Bari, Italy.

[2]Assistant toxicologist, toxicologist, and chief medical examiner, respectively, Office of the Chief Medical Examiner, Baltimore, MD.

and Chrysomya rufifacies recovered from decomposing remains, revealed the presence of malathion, thus confirming the diagnoses of organophosphate intoxication.

Previously, no systematic toxicological studies have considered the possibility of using blowfly larvae as a specimen for toxicological analysis. Furthermore, no studies that support any relationship between the values obtained from the larvae and those present in the organs on which the larvae were grown were evident in the literature. A study of the usefulness of blowfly larvae in fatal drug intoxication investigations as well as the relationship between larvae and substratum concentrations is presented. In this first report, the analyte was limited to opiates (morphine). Blowfly eggs were deposited on liver samples from cases previously determined to be positive for opiates. At the third instar of development, the larvae were removed and both the liver and larvae were analyzed for opiates. Similarly treated was a control group of liver specimens in which opiates were absent.

Materials and Methods

Chemicals, Reagents, and Standard Solutions

Abuscreen® radioimmunoassay (RIA) for morphine was supplied by Roche Diagnostic Systems, Inc. (Nutley, New Jersey). Sodium chloride, potassium phosphate monobasic, and ammonium sulfate were obtained from Mallinckrodt, Inc. (Paris, Kentucky). Sodium hydroxide was supplied by Fisher Scientific (Fair Lawn, New Jersey). Morphine alkaloid was supplied by Merck and Co. (West Point, Pennsylvania). Methanol was gas chromatography (GC) grade.

Phosphate buffer solution was prepared by making 0.1*M* potassium phosphate monobasic, and adjusting to pH 7 with 0.1*M* sodium hydroxide. Radioimmunoassay (RIA) saline solution contained 9.0 g of sodium chloride and 10 mL of pH 7 phosphate buffer with distilled water added to 1000 mL. Ammonium sulfate was made saturated with distilled water.

Morphine stock solution was prepared by dissolving 5 mg in 50 mL of methanol; morphine working standard was prepared by transferring 0.1 mL of morphine stock solution and then diluting to 10 mL with distilled water. Morphine standard solutions of 0, 100, 250, 500, 750, and 1000 μg/L were prepared as follows:

(1) blank—add 0.1 mL of blank blood to 2.4 mL of saline solution,

(2) 1000 μg/L standard—add 0.1 mL of blank blood and 0.1 mL of 1000–μg/L working morphine standard to 2.3 mL of saline solution,

(3) 500-μg/L standard— add 0.1 mL of blank blood and 0.05 mL of 1000-μg/L working morphine standard to 2.35 mL of saline solution,

(4) 100-μg/L standard—0.4 mL of blank standard plus 0.1 mL of 500-μg/L standard,

(5) 750-μg/L standard—0.2 mL of 500-μg/L standard plus 0.2 mL of 1000-μg/L standard, and

(6) 250-μg/L standard—0.2 mL of 500-μg/L standard plus 0.2 mL of blank.

Instrumentation

A LKB Automatic Gamma Counter Model 1272 interfaced to an AT&T 6300 personal computer was used.

Preparation of the Sample

Eggs from Calliphora vicina were deposited and promoted on specimens of liver collected from bodies which were previously found to be positive for presence of opiates.

Controls consisted of ten rearings of the same blowfly larvae grown on liver tissue obtained from cases in which opiates were absent. In all cases the larvae used were obtained from deposits in laboratory rearings of adult flies. At the third instar larval stage of development, the larvae were removed from the liver, collected, and washed with distilled water. Liver and larvae were separated and frozen overnight. For each case, 2 mL of distilled water were added to 2 g of larvae or 2 g of liver and each separately homogenized.

Procedure

Remove 0.1 mL of each homogenized sample into a disposable 16- by 100-mm silica tube. Add 2.4 mL of RIA saline solution and vortex thoroughly. Transfer 0.1 mL of the mixture into an appropriately labeled polypropylene test tube. To each tube add 0.2 mL of I-125 morphine (yellow) and 0.2 mL of morphine antiserum (blue). Incubate at room temperature for a minimum of 30 min. After incubation add 0.5 mL of the second antibody reagent (green) and 0.2 mL of ammonium sulfate; centrifuge the tubes for 30 min. Remove 0.5 mL of the supernatant and place in a labeled test tube. Count each tube in a gamma scintillation counter. Concentration of each sample is determined by comparison with standard curve analyzed with each batch.

Statistical Analyses

Linear regression analysis was performed on the data obtained from the liver and larvae opiate analyses. Pearson's test and the student *t*-test were determined for the pairs of values. The correlation coefficient, slope, intercept, and standard error were calculated using the EPISTAT (statistical package for IBM personal computer) version 3.0, 1984.

Results

Larvae were reared on 40 different samples of liver collected from bodies in which the initial testing produced a positive opiate result in blood. The concentration of opiates for all cases was found to range from 8 to 1208 μg/kg for the larvae and 26 to 1769 μg/kg for the liver specimens. All 10 control specimens were found to be negative in both larvae and liver specimens. Five of the 40 positive cases had blood opiate concentrations that were less than 180 μg/L; the paired larvae and liver opiate concentrations in these cases were negative. In the remaining 35 cases in which the blood opiate concentration was greater than 180 μg/L, the paired larvae and liver opiate concentrations were positive. All analyses were conducted using RIA, and a representative sample was confirmed for morphine by gas chromatography/mass spectrometry (GC/MS).

The correlation analyses by Pearson's test performed between the two series of values (liver and larvae opiate [RIA morphine] concentrations) indicated a correlation coefficient of 0.790. The paired student *t*-test yielded a significant difference between the opiate liver and larvae concentrations [$T = 7.96$, df $= 39$, $p < 10\ (-6)$]. In the regression analyses, the liver opiate concentrations (dependent variable Y) were correlated with the corresponding direct blowfly larvae opiate concentrations (predictor variable X). The linear regression equation obtained was (liver) = 1.41 (larvae) + 280 with a standard error of 64.8 μg/kg and a determination coefficient (R^2) of 0.625.

Figure 1 compares the liver opiate concentrations with the related larvae opiate concentrations.

Discussion

Blowfly larvae have seldom been used as a toxicological specimen for determining the cause of death in extensively decomposed, preskeletonized bodies. Although in two cases

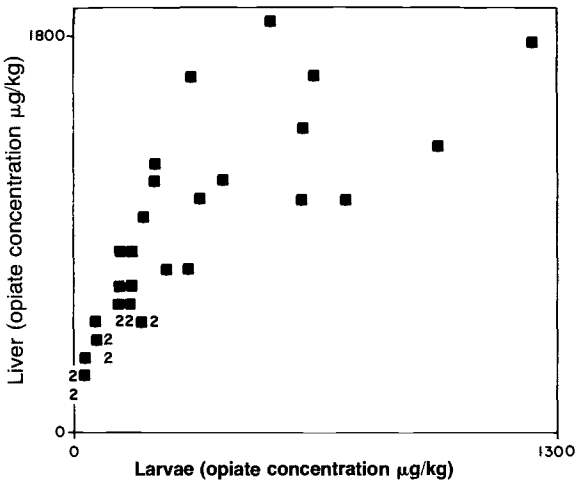

FIG. 1—*Comparison of opiate concentrations in liver with paired concentrations in larvae as measured by RIA. Regression formula is (liver) = 1.41 (larvae) + 280. Correlation was 0.790, with a constant error of 64.8, a deviation of 299, and a standard deviation of 266.*

larvae were used as evidence assisting in the determination of the cause of death, no systematic study had previously considered the possibility of using larvae as toxicological specimens. Furthermore, no previous studies have ever supported a relationship between the toxicological findings in the larvae and the concentration in the biological tissues from which the larvae had been raised.

In this study we developed 40 rearings of Calliphora vicina on samples of liver collected from bodies that were positive for the presence of opiates. The larvae collected at the third instar and the corresponding samples of liver were tested for opiates with RIA. Statistical analysis performed on the paired results show good correlation (r = 0.790). The student t-test (7.96 with a p value $<10[-6]$) revealed a significant difference between the two series of data. The results indicate that the opiate concentrations found in the blowfly larvae correlated well with the opiate concentration of the biological substratum on which the larvae were reared, in our case, liver.

The equation of the best-fit line obtained from linear regression between the two series of data indicates that the larvae opiate concentrations typically underestimate the opiate liver concentration. The constant standard error of 64.8 µg/kg could be considered an acceptable value in relation to the particular situation in which this toxicological analysis was performed. Blowfly larvae can indeed be used as toxicological specimens where other specimens are unavailable, such as in extensively decomposed, mummified, or preskeletonized bodies. In these cases, blowfly larvae as the test material, notwithstanding a relatively high standard error, can be useful.

Conclusions

1. The opiate concentrations in the larvae of blowflies grown on liver of decomposed bodies can effectively be determined using RIA.

2. Blowfly larvae can be used as a toxicological specimen for opiate analysis in cases of narcotic intoxication when other specimens are unavailable.

3. A significant correlation exists between the opiate concentrations observed in the liver samples and those observed in the paired larvae. Therefore, toxicologic analysis of blowfly larvae can be used in the qualitative assessment of potential narcotic related

deaths where limited specimens are available in extensively decomposed or preskeletonized bodies. A quantitative relationship is suggested.

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Address requests for reprints or additional information to
Yale H. Caplan
Office of the Chief Medical Examiner
111 Penn St.
Baltimore, MD 21201