TECHNICAL NOTE

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Detection of Phenobarbital in Bloodstains, Semen, Seminal Stains, Saliva, Saliva Stains, Perspiration Stains, and Hair

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ABSTRACT: Low nanogram quantities of phenobarbital were detected in a 10-μL dried blood stain and in other physiological fluids by using radioimmunoassay. The age of the stain versus detectability of phenobarbital and the cross-reactivity of vaginal secretions were investigated.

KEYWORDS: toxicology, phenobarbital, radioimmunoassay

In earlier accounts [*1-3*] we reported that phenytoin, morphine, and digoxin were detectable in dried bloodstains using the radioimmunoassay (RIA) technique. Salicylate in bloodstains was detected fluorometrically by King [*4*]. The application of RIA to barbiturates was presented by Spector and Flynn [*5,6*] and Cleeland et al [*7*]. In our present study physiological fluids, their stains, and a single head hair were tested for phenobarbital.

Experimental Procedure

Standard Curve

Standard curves for phenobarbital were prepared with each assay by testing, in duplicate, the designated quantities plotted in Fig. 1. The assay was completed according to the manufacturer's instructions [*8*]. All duplicates agreed within the manufacturer's ±12% experimental error.[3]

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[3]R. Cleeland, personal communication, 1978.

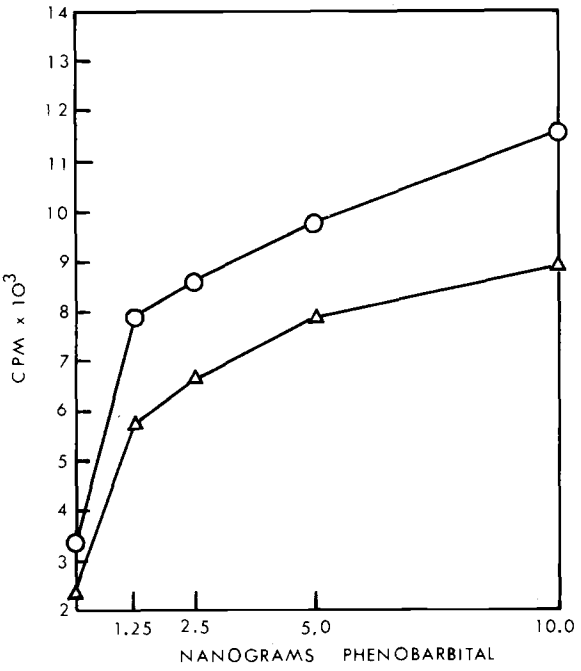

FIG. 1—*Phenobarbital standard curve.*

Sample Preparation

Dried Bloodstains—Blood was obtained from an individual on long-term phenobarbital therapy (30 mg sodium phenobarbital/four times daily), placed in 10-μL aliquots on a white unbleached cotton sheet to dry, and eluted with 2.0 mL of 0.1% sodium dodecyl sulfate (SDS) in physiological saline as previously described [*1*]. For phenobarbital determination by RIA, 0.1-mL aliquots of eluate from a 10-μL bloodstain were sampled and tested. This dilution produced an equivalent sample of 0.5 μL from the original 10-μL bloodstain.

Saliva and Semen—Samples were collected and stored as previously described [*1*] and diluted for analysis as follows: 0.1 mL of neat sample was added to 2.0 mL of deionized, distilled water and mixed thoroughly on a vortex-type mixer. Aliquots of 0.1 mL of this dilution were tested by the RIA procedure. That amount was the equivalent of 4.8 μL of the neat sample.

Saliva Stains—A filter-type cigarette was used to obtain phenobarbital-positive and negative-control saliva stains as previously described for phenytoin [*1*].

Seminal Stains—Semen samples obtained as described above were measured into 10-μL aliquots and dried by the same manner as bloodstains. Elution took place in 0.2 mL of eluant, and 0.1 mL was tested.

Whole Blood and Plasma—The phenobarbital concentration was determined by diluting 0.1 mL whole blood or plasma in 20.0 mL physiological saline solution and assaying 0.1 mL by the RIA method.

Red Blood Cells—Red blood cells were prepared as described in Ref *1*.

Perspiration Stains—A circular patch of approximately 7 cm^2 was cut from the underarm area of a 50% polyester, 50% cotton tank top man's T-shirt from an individual on phenobarbital therapy. The patch was cut into 4- by 4-mm squares and eluted with 4.0 mL 0.1%

SDS/saline for 1 h, after which 0.1 mL of the eluate was assayed in duplicate by the RIA method. Another 7-cm^2 section was cut from this T-shirt at the waist band and subjected to the above treatment. A new T-shirt of the same style without barbiturate present served as a negative control on which the above procedure was performed.

Vaginal Secretions—Negative control samples of vaginal secretions were donated by laboratory personnel with no known use of barbiturates. Two sections of approximately 3 cm^2 were cut from the upper end of a woman's sanitary tampon that had been worn for 24 h. They were cut into four pieces, placed in separate disposable round-bottom culture tubes, and eluted with 1.0 mL eluant as described for the other stained samples. Duplicate aliquots of 0.1 mL of each extract were assayed by the RIA method.

Hairs—A single head hair weighing 0.4 mg from an individual on long-term phenobarbital therapy was washed in 20 mL of distilled deionized water ten times, dried, cut into approximately 2-mm lengths, and added to 0.2 mL 0.1% SDS/saline in round-bottom glass culture tubes. At the 24-h time point, 0.1 mL was sampled and assayed by the RIA technique. Equivalent single hairs from a drug-free individual served as negative controls and were treated as above.

Materials

Abuscreen® radioimmunoassay for barbiturates (^{125}I) was obtained from Roche Diagnostics, Nutley, N.J.

Results and Discussion

The RIA method used has the capability of responding to as little as 1.25 ng phenobarbital (Fig. 1). Because of the rapid radioactive decay of ^{125}I (half-life equal to 60 days), two standard curves are plotted. The first experimental results, from studies with plasma, whole blood, washed red blood cells, fresh bloodstains, and aged bloodstains, were extrapolated from the higher standard curve. Later studies, on seminal fluid, seminal stains, saliva, hair, perspiration, cigarette filter paper, cigarette filters, vaginal secretions, and neat saliva, were extrapolated from the lower standard curve generated at the time these experiments were conducted.

The results of the preliminary investigation of the phenobarbital levels in blood samples are shown in Table 1. Although the plasma has a larger concentration of barbiturate than does the whole blood, 5.46 versus 4.44 μg/mL, the washed red blood cell particulates do contain a measurable quantity of drug, 0.32 μg/mL. The whole blood samples were tested to give levels that would be expected from bloodstains made from that same volume of blood. The earlier precedent set by the successful detergent extraction of 100% of the drug phenytoin from an 18-month-old bloodstain [*1*] was applied to this problem. Essentially 100% of the drug was recovered from 18-month-old stains when 0.1% SDS in saline was used as the eluant. Thus, this solvent may be used to extract measurable amounts of the drug from aged bloodstains.

Table 1 also shows the amount of phenobarbital found in saliva and seminal fluid: 1.19 μg/mL saliva and greater than 2.10 μg/mL semen. This concentration is sufficient to detect the drug in less than 1 μL of neat fluid.

Tests on saliva stains prepared on a cigarette show that although no barbiturate was detected in the filter itself, 15.2 ng was determined from the filter paper.

Seminal stains contained 14.9 ng/10 μL stain. Therefore, phenobarbital could be detected in a seminal stain of less than 2 μL. This illustrates both the sensitivity of RIA and the ubiquity of this drug in all body fluids tested.

Since the findings cited regarding the drug in semen could be useful in processing evi-

TABLE 1—*Results of the preliminary investigation.*

Sample	Phenobarbital Detected, ng	Calculated, μg/mL Sample, or ng Total
Serum	2.68	5.36
Whole blood	2.22	4.44
Washed red blood cells	1.54	0.32
Bloodstain aged 18 months	2.11	4.22
Seminal stain	7.45	1.49
Neat semen	10.00	2.10
Neat saliva	5.66	1.19
Saliva stains		
Cigarette filter	none detected	...
Cigarette paper	3.79	15.2
Perspiration stains on shirt		
Underarm area	100	400
Bottom hem area	100	400
One hair, 0.39 mg in weight	4.42	8.84
Vaginal secretions on tampon	none detected	...

dence from sexual assault cases, the possibility of endogenous cross-reactivity from vaginal secretions was investigated. No cross-reactivity was observed.

Perspiration stains from the phenobarbital-positive individual were tested as described and yielded the following results: the underarm area of the shirt contained so much drug that it exceeded the upper limit of the standard curve (greater than 10.0 ng). The bottom area of the shirt tested also produced off-scale readings. The negative (drug-free) control T-shirt tested showed no signs of cross-reactivity.

A single human head hair from a man on continuous phenobarbital therapy was tested by the method described. The amount of 8.84 ng was observed, as shown in Table 1. This result provides additional discriminating information that can be used on hair collected as trace evidence from crime scenes, discarded clothing, and the bodies of victims of violent crime.

Acknowledgments

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