TECHNICAL NOTE

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Detection of Digoxin in Bloodstains

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ABSTRACT: Picogram quantities of digoxin were detected in 100-μL samples of dried bloodstains by using radioimmunoassay. The extractability of digoxin by various surfactants and the problems encountered with aged stains were investigated.

KEYWORDS: toxicology, digoxin, blood

In two previous accounts [*1,2*] we reported the persistence of phenytoin and morphine in dried bloodstains. These data were obtained with the rapid, sensitive, specific, and relatively inexpensive radioimmunoassay (RIA) technique.

In this study, picogram quantities of digoxin were detected in 0.1-mL dried bloodstains. The age of the stain versus detectability of digoxin and the extractability of digoxin by various surfactants were investigated.

Experimental Procedure

Standard Curve

A standard concentration curve was prepared by assaying known amounts of unlabeled digoxin equal to 50, 100, 200, 400, and 800 pg in duplicate by the RIA method as described by New England Nuclear [*3*] and depicted in Fig. 1.

The "logit" method [*4*] shown in Fig. 2 represents a mathematical manipulation of the same numbers where the logit of the efficiency-corrected counts per minute is plotted versus the log of the dose. Results quoted in this report were extrapolated from the second (logit) standard curve. Experimental error for the digoxin RIA is ±12% as described by the manufacturer. All duplicates agreed within this error rate.

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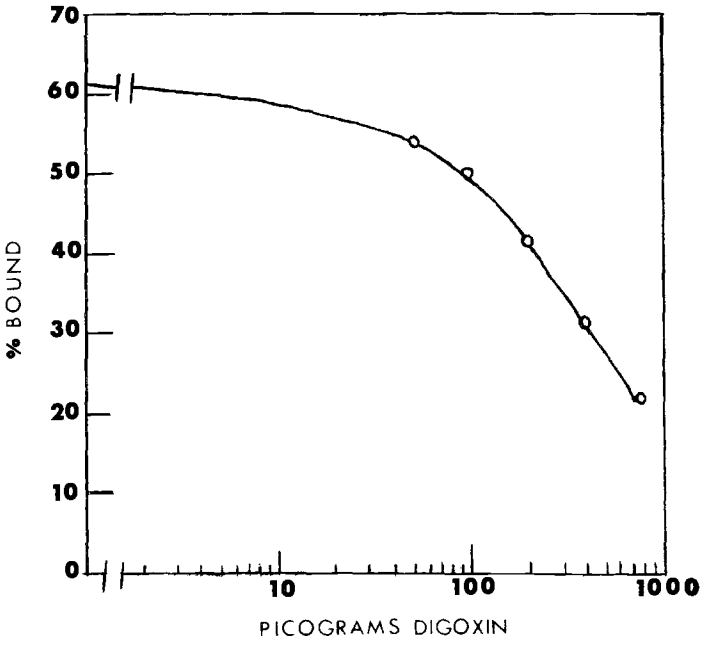

FIG. 1—*Standard concentration curve for digoxin.*

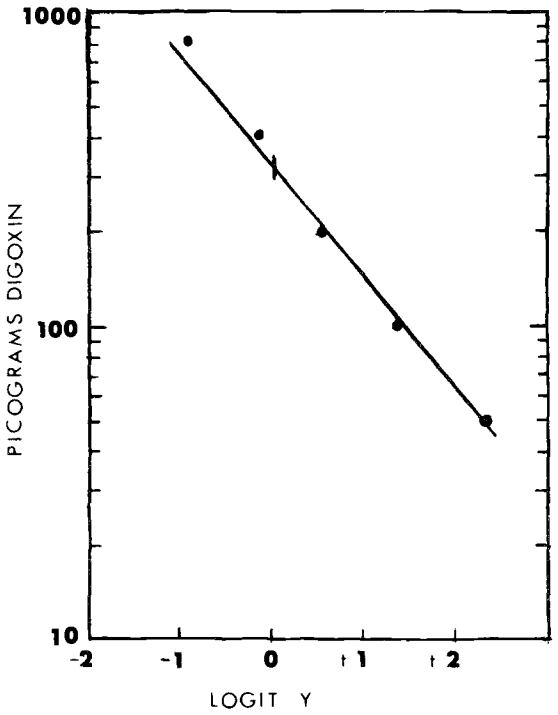

FIG. 2—*"Logit" plot of standard concentration curve for digoxin.*

Sample Preparation

Dried Bloodstains—Whole blood drawn by venipuncture into ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes was measured by using a 100-μL pipetting device and placed on white, unbleached cotton sheet cloth to dry. Storage was at room temperature in an open space for the designated aging times. Immediately prior to assay the dried bloodstains were cut with scissors into approximately 1-mm squares, placed in 13- by 100-mm round-bottom culture tubes, and eluted with the described solvents for the specified time intervals.

Bloodstains containing digoxin were created from two basic sources. First, the blood of patients on digoxin therapy was used to create 0.1-mL bloodstains. Second, blood tested to be digoxin-free was spiked to a concentration of 1.9 ng/0.1 mL and dried into 0.1-mL bloodstains. The purity of the digoxin used in spiking was checked by silica gel thin-layer chromatography (TLC). The mobile phase of acetone/glacial acetic acid/*c*-hexane, 49:2:49, resulted in a single spot with an R_f value of 0.32 that was visualized by iodoplatinate spray [5].

Whole Blood—EDTA-anticoagulated whole blood (0.1 mL) was analyzed without dilution for the presence of digoxin by the RIA method. Necessary column chromatographic purification was performed before the RIA analysis to remove interfering blood chromophores that had caused quenching when the ^{3}H-digoxin test kit was used [3]. This problem can be avoided by using the ^{125}I-digoxin derivative with a gamma counter.

Plasma—Whole blood was centrifuged for 3 min at 1000*g* to separate plasma from the red blood cells. Digoxin concentrations were determined directly from 0.1 mL of plasma by the RIA method.

Column Chromatography—Bloodstain eluates and whole blood were purified by quantitative transfer to #1001 Jet® tube columns. The eluting solvent was 90:10, methylene chloride/isopropanol. Three successive 4-mL washes were spaced by 2- to 5-min equilibration periods. The drug was recovered from Jet tube columns at 89% efficiency as described by the manufacturer and confirmed by radioactive tracer recovery. This was taken into account in calculating these results.

An ionic detergent, sodium dodecyl sulfate, $CH_3(CH_2)_{11}OSO_3^-Na^+$ (SDS), and a nonionic detergent, Triton X-100, $CH_3C(CH_3)_2CH_2C(CH_3)_2C_6H_4O(CH_2CH_2O)_nH$ (n = 10), were examined for their efficiency at extracting digoxin from dried bloodstains. Detergent solutions (0.1 and 1.0%) of each were prepared in physiological saline.

Materials

The RIA for digoxin was purchased from New England Nuclear, Boston, Mass. Digoxin-positive blood and negative-control blood were obtained from the Central Blood Bank of Pittsburgh. Digoxin was purchased from Sigma Chemical Co., St. Louis, Mo. Jet tubes were obtained from Har-Len Associates, Pittsburgh, Pa. Uniplate silica gel TLC plates were purchased from Quantum Industries, Fairfield, N.J.

Results and Discussion

The standard curves (Figs. 1 and 2) illustrate the extreme sensitivity of the RIA procedure for digoxin determinations. The therapeutic range of four patients on digoxin therapy was 0.9 to 5.6 ng digoxin/mL plasma. The concentrations of digoxin in whole blood, considering the dilution factor of the red blood cells (hematocrit value), were from 54 to 340 pg/0.1 mL.

However, the analyses of 0.1-mL bloodstains made from these samples and aged several weeks yielded negative results. Digoxin-positive bloodstains could not be distinguished

from negative-control bloodstains of equivalent age and volume, and both were of the same value as the zero standard. This shows that endogenous cross-reacting substances were not detected and therefore did not interfere with this assay. Also, the question was raised, Under what circumstances, if any, could digoxin be detected in bloodstains?

Therefore, the effect of age on the detection of digoxin from dried bloodstains was investigated. Whole blood spiked with digoxin and the corresponding 0.1-mL dried stains were eluted daily. The results (Fig. 3) show that after 24 h only 34% of the drug was recovered. For stains aged one week the recovery slipped to 27%. At two weeks just 6.0% was recovered, and after three weeks none of the drug could be detected. The initially observed drop may be due to the action of enzymes released by the inadequate osmotic protection of drying blood [6]. Any change in the chemical structure of digoxin could drastically affect the antisera's ability to recognize the new compound. Since 20 to 25% of the digoxin in the blood is "plasma bound" [7], the remaining 75 to 80% of the digoxin is "free" in the blood to presumably be broken down by enzymatic action. This correlates well with recovery rates after one day in dried bloodstains. The definitive answer to this question might be explained by analysis of digoxin and its degradation products using gas chromatography/mass spectrometry.

The extractability of digoxin from bloodstains with the described detergents was investigated. Triton X-100 extracted 348 and 444 pg of digoxin at concentrations of 0.1 and 1.0%. This corresponds to 268 and 339% of the amount extracted by saline alone. The anionic detergent SDS extracted 278 and 265 pg of digoxin (or 214 and 204% of the saline value) for 0.1 and 1.0% detergent solutions. Since 1.0% Triton X-100 was the most effective, it was the detergent solution used in the studies described above. It may be significant that the nonionic detergent was more effective than the ionic detergent in the elution of this nonionic drug.

The immediate result of this study is that digoxin may be detectable only in dried bloodstains that are relatively fresh. On another level, this research poses two questions. First, what biochemical forces are at work as blood dries? Second, what happens in a presumably stable dried bloodstain to result in drastic digoxin recovery changes between the first

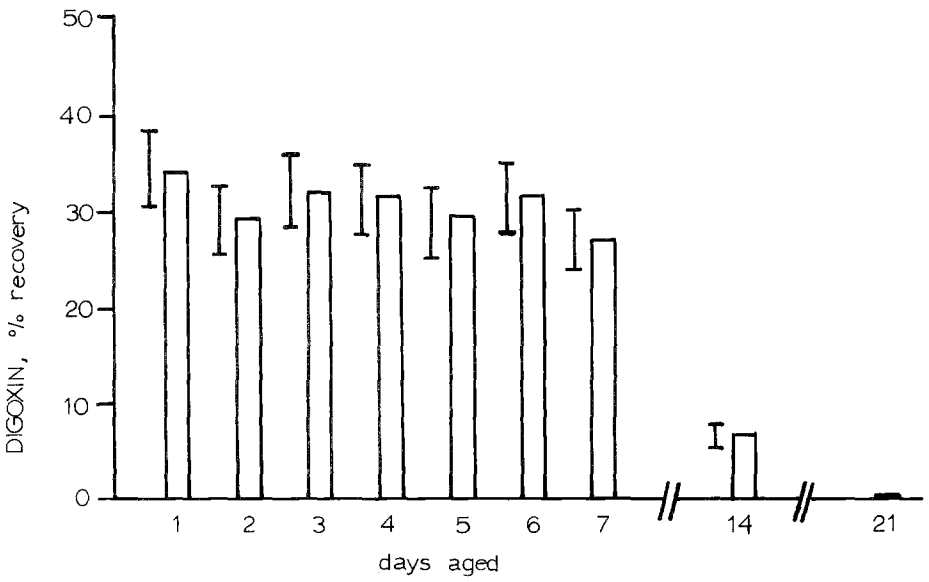

FIG. 3—*Digoxin recovery rates from aged bloodstains.*

and third week of aging? The answers to these questions could help to unravel the unexplained processes involved in bloodstain aging.

Acknowledgments

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