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The Fluorometric Detection of Salicylate in Bloodstains

The prospect of using drugs or their metabolites as discriminating markers in bloodstains was first discussed by Curry [*1*]. This approach could prove particularly useful in cases where blood protein has been denatured or where other evidence suggests that a particular drug has been consumed. The collection of body fluids as stains could also be of interest to the toxicologist. Filter paper techniques offer advantages over conventional methods in terms of sample stability and ease of storage and shipment. A recent publication described the radioimmunoassay of morphine recovered from blood and urine stains [*2*].

Aspirin and paracetamol (acetaminophen) are widely used and abused. A recent survey in the United Kingdom has shown that 11% of the population will consume aspirin in one week [*3*]. In a more restricted survey, Murray [*4*] reported that on any given day 3% of the population of Glasgow will take aspirin.

This report describes a method for the identification of traces of salicylate, the major metabolite of aspirin, in bloodstains.

Procedure

A volunteer ingested 600 mg aspirin (British Pharmacopeia) (two tablets) at 8:40 a.m. Immediately afterwards a square (15 by 15 mm) of Whatman No. 1 filter paper was saturated with a sample of blood obtained by finger-prick. (A previous trial had shown that this size paper absorbed 25 μl blood.) This procedure was repeated at hourly intervals throughout the day. After drying at room temperature, the bloodstains were each triturated with 1 ml absolute ethanol for 2 h. The fluorescence of the ethanol extracts was measured at room temperature by using 0.5-ml micro cells in a Perkin-Elmer MPF-2A spectrophotofluorometer. The excitation and emission monochromators were set at 300 and 400 nm, respectively. The corresponding half-bandwidths were 6 and 4 nm. Calibration was achieved by using a standard solution of salicylic acid in ethanol (158 ng/ml).

Results and Discussion

In a control experiment, it was found that salicylate could be recovered with an efficiency of 60% from a bloodstain prepared from whole blood freshly spiked with salicylic acid. Allowance for this recovery has been made in expressing the following results. The blood level of salicylate as a function of time is shown in Fig. 1. The peak concentration of salicylate in blood was reached 4 h after dosing; the half-life was close

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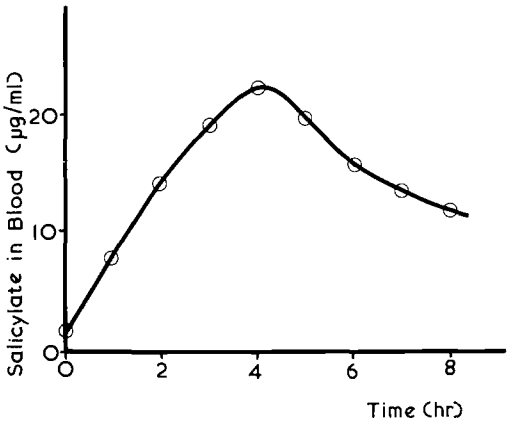

FIG. 1—*Blood level of salicylate following ingestion of 600 mg aspirin.*

to 4 h, in agreement with previous findings [5]. The fluorescence of the ethanol extract of the bloodstain taken at zero-time was equivalent to 1.3 µg/ml salicylate in whole blood. Extrapolation of these results suggests that blood salicylate would still be detectable 16 h after a therapeutic dose of aspirin was taken. If necessary, the identity of salicylate could be confirmed by fluorometric titration in aqueous solution (pKa = 3.2 [6]).

The detection of other widely used drugs such as barbiturates and benzodiazepines could provide useful information about the donor of a bloodstain. The discrimination of smokers from nonsmokers might be achieved, in theory, by the determination of the nicotine metabolite cotinine. (Nicotine itself would be unsuitable because of its high vapor pressure and short biological half-life.) A radioimmunoassay for cotinine has been described and appears to be sufficiently sensitive for very small quantities of blood [7].

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