Determination of Tetracaine in Blood

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This paper describes a spectrophotometric procedure that is more specific for tetracaine and offers greater sensitivity than the method of Woods *et al.* Recovery of more than 90 per cent of the anesthetic can be realized with good precision, and the method is sensitive to less than 2 mcg. of tetracaine per ml. of blood.

THE FEASIBILITY of determining blood levels of tetracaine after tracheal instillation of 1 mg./Kg. of the local anesthetic to humans was explored. Rapid absorption and measurable blood levels of tetracaine in dogs was reported by Adriani (1) and Adriani and Campbell (2, 3). They found 30 mcg./ml. of blood 4 to 6 min. after tracheal instillation of 6 mg. of tetracaine per Kg. in dogs. Human levels were not reported by these workers. At one-sixth the dose per Kg. of body weight, a blood level of about 5 mcg./ml. would be expected in man under comparable conditions.

The halogenated sulfonphthalein dye complex method of Woods *et al.* (4), used by Adriani and Campbell, was applied to the *in vitro* addition of tetracaine to oxalated human blood. The sensitivity of this procedure was found to be borderline for detection of the small quantity of anesthetic expected.

Other methods for the determination of alkaloids in biological fluids were explored. The colorimetric procedure for the estimation of cinchona alkaloids and other organic bases in plasma, urine, and feces, based on complex formation with methyl orange dye, was introduced by Brodie and Udenfriend (5). Gettler and Sunshine (6) modified this method for application to the quantitative determination of alkaloids in human organs. Both methods were applied with inadequate results. Recovery was low, precision was poor, and some interference from blood components was encountered.

METHOD

Ten milliliters of blood is withdrawn from the cubital vein and oxalated in a test tube in the usual manner. Within 60 sec., 2-ml. samples of this blood are placed in 35-ml. glass-stoppered centrifuge tubes containing 10.0 ml. of distilled water, 1.0 ml. of 1 *N* NaOH, and 10.0 ml. of chloroform. After mechanically agitating this mixture for 10 min., it is centrifuged 20 min. to promote separation of phases. As much as possible the aqueous phase is removed by aspiration, and the organic phase is centrifuged again. The organic phase is carefully removed and dried over anhydrous sodium sulfate. A complete ultraviolet absorption spectrum from 240–400 mμ is obtained on a Cary model 14 recording spectrophotometer, using chloroform as the reference solvent. A characteristic spectrum for tetracaine is obtained, and shown in Fig. 1. For quantitative determination, the intensity of absorbance at 303 mμ is measured and related to a standard.

RESULTS AND DISCUSSION

Solutions of tetracaine added to water were quantitatively recovered when determined by the spectrophotometric procedure described in this paper. These results are shown in Table I.

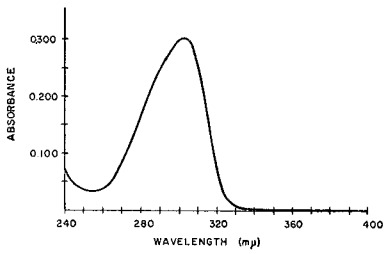

Fig. 1.—Ultraviolet absorption spectrum of solution containing 3.0 mcg. tetracaine per ml. in chloroform.

The recovery of tetracaine added to oxalated human blood gave results that appear to be concentration related up to 10 mcg./ml. These results are shown in Table II.

The differences in recoveries from water and blood can be attributed to hydrolysis of tetracaine by esterase enzymes present in blood (7). Hydrolysis

TABLE I.—RECOVERY OF TETRACAINE FROM A WATER SOLUTION OF THE HYDROCHLORIDE SALT

mcg. of Added Tetracaine/ml. of Soln.	% Recovered
2.5	102
	100
5.0	102
	101
15.0	102
	103

TABLE II.—RECOVERY OF TETRACAINE ADDED TO 2.0 ml. OF HUMAN BLOOD

mcg. of Added Tetracaine/ml. of Soln.	% Recovered	Mean
2.5	85	
	70	
	74	
	85	
	85	
	83	80.3
5.0	84	
	89	
	91	
	88	
	92	
	90	89.0
10.0	93	
	93	
	92	92.7
15.0	89	
	91	
	95	91.7

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of the ester linkage alters its lipophilic character, preventing extraction with chloroform and would be expected to give lower values. This was confirmed by determining that recovery of tetracaine from oxalated human blood was dependent upon the length of time in contact with blood prior to extraction. After 1 hr., complete degradation of tetracaine occurred, and none could be recovered.

Since no mention of any precaution regarding delays in extraction of tetracaine in dog experiments was made by other investigators, an *in vitro* comparison of the rates of hydrolysis of tetracaine in human and dog bloods was made to determine whether they might be different. The results shown in Table III indicate that the rate is much more rapid in human than dog blood.

These data are comparable to results reported for procaine esterases by Kisch and his co-workers (8). They found that procaine was 78% hydrolyzed in 75 min. at 37° in man's blood, whereas under identical conditions, only 9.7% was hydrolyzed in dog's blood.

The use of mercuric chloride and sodium fluoride enzyme inhibitors proved unsatisfactory. It has been shown (9) that 15 to 60 min. preliminary contact with blood after withdrawal is required for effective inhibition of esterase activity by these agents. In that time, significant degradation occurs. The effect of enzyme activity is minimized in the method described here by the rapid extraction of tetracaine from blood immediately after withdrawal. Cotty *et al.* (10) also found this to be an effective method to reduce esterase activity in blood analysis.

TABLE III.—DECOMPOSITION OF TETRACAINE IN HUMAN AND DOG BLOOD AT 37°C.

	Time Elapsed Since Addition of Tetracaine to Blood, min.	% Recovery
Human blood	15	39
	60	Nil
Dog blood	15	87
	60	79

TABLE IV.—RESULTS

Subject	Dose of i.v. Tetracaine, mg./Kg.	Tetracaine Found in Blood, mcg./ml. 1 min.	3, 5, and 10 min.
A	1.0	10.0	0
B	1.5	6.3	0
C	1.5	3.6	0
D	2.0	14.2	0

In a series of experiments conducted by Dr. Robert Bauer, UCLA Medical Center, 1.0 to 2.5 mg./Kg. tetracaine was administered intravenously or transtracheally to patients. Blood samples were withdrawn from the cubital vein 1, 3, 5, and 10 min. after administering the drug and analyzed for tetracaine immediately after withdrawal by the spectrophotometric method described in this paper. The results are shown in Table IV.

In seven subjects to whom 1.0 to 2.0 mg./Kg. tetracaine was administered transtracheally, no drug was found in the blood at 1, 3, 5, or 10 min. after instillation. In one subject given 2.5 mg./Kg., 2.9 mcg./ml. was found 1 min. after instillation, 2.3 mcg. after 3 min., 1.3 mcg. after 5 min., and none 10 min. after transtracheal instillation. The rapid degradation of tetracaine in man accounts for the wide variation in blood levels found.

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