

the right fore-leg and the left hind-leg. A large proportion of the shocks which were given near the end of ventricular systole, as determined by their coincidence with the T-wave of the electrocardiogram, caused ventricular fibrillation and death, while but one shock (0.1 second duration) distinctly outside the T-wave resulted in fibrillation.

It seems probable that the difference in the susceptibility of the heart to fibrillation from shocks in different phases of its cycle is related to the known variation of its excitability. Further investigation is being carried forward.

7356 P

Estimation of Small Traces of Alkaloids.

BURNHAM S. WALKER AND ELISABETH W. WALKER. (Introduced by A. W. Rowe.)

From the Evans Memorial of the Massachusetts Memorial Hospitals, and Boston University School of Medicine.

The need to measure the amount of cocaine penetrating to the aqueous humor of an experimental animal after instillation of a cocaine solution into the conjunctival sac led to the following analytical procedure, which we find applicable to the quantitation of traces of many alkaloids in aqueous solution, and of certain alkaloids, particularly cocaine and strychnine, in body fluids. The method, which is accurate within the limits of colorimetric observations, is strikingly simple, and we believe will be of interest to workers in pharmacology, toxicology and experimental medicine.

A. *Procedure for aqueous solutions.* To a measured amount of solution containing from .5 to .05 mg. of the alkaloid is added 1 cc. of a 1% solution of phosphomolybdic acid in normal sulphuric acid. This is best done in a small test tube of about 5 cc. capacity. After mixing and standing for 15 minutes, the mixture in the test tube is centrifuged and the supernatant fluid decanted. The residue is resuspended in water, again centrifuged and the supernatant discarded. To the washed precipitate is now added 1 cc. of fifth normal sulphuric acid, 1 cc. of 2% aqueous solution of hydroquinone, and after shaking, 1 cc. of the carbonate-sulphite solution used in the Bell and Doisy¹ method for phosphate determination (15% so-

¹ Bell and Doisy, *J. Biol. Chem.*, 1920, **44**, 55.

dium carbonate and 3% sodium sulphite). The color developed is read promptly in the small Duboscq type colorimeter against a standard prepared by treating 1 cc. of a solution of known and approximately equal alkaloid content (using the same alkaloid that is being determined) simultaneously with the same reagents. If necessary, it is a relatively simple matter to prepare several standards (5 of the small test tubes can be spun in an ordinary 50 cc. metal centrifuge cup) and the proper one can be selected by inspection.

B. *For body fluids of low protein content.* To a measured amount, 1 cc. or less, of aqueous humor or cerebrospinal fluid suspected of containing a certain alkaloid, is added 1 cc. of 20% sulphosalicylic acid. The protein is separated by the centrifuge, and the clear supernatant fluid removed by decantation and treated as in the procedure for aqueous solutions.

C. *For fluids of high protein content.* It is advisable to dilute blood serum with 4 volumes of physiological salt solution before deproteinization, in order to minimize the error resulting from adsorption of the alkaloid on the precipitated protein.

Results. With aqueous solutions of brucine, strychnine, cocaine, atropine, homatropine, sparteine, procaine, quinidine, caffeine, physostigmine, morphine, codeine, pilocarpine and apomorphine the analytical error was less than 6% if more than .1 mg. was present. With amounts from .05 to .1 mg., the error increased with decreasing amounts up to 20% at .05 mg., which is about the limit of possible colorimetric reading.

With solutions of cocaine or strychnine salts in aqueous humor or cerebrospinal fluid the analytical error is about the same in the weaker solutions, and up to 9% in the stronger. With blood serum, the error is up to 12%. With morphine there appears to be a marked tendency for the alkaloid to adsorb on the precipitated protein (it is not precipitated from simple aqueous solution by sulphosalicylic acid), hence this method is not applicable to morphine in body fluids.