THE ISOLATION AND DETECTION OF ERGOMETRINE IN TOXICOLOGICAL ANALYSIS

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The isolation of ergometrine from viscera is accomplished by extraction with ether from an ammoniacal aqueous solution saturated with ammonium sulphate. Purification is achieved by paper chromatography and detection of 0·1 μg. by ultra-violet fluorescence and the *p*-dimethylaminobenzaldehyde reaction. The detection of ergometrine and procaine in urine samples after administration of therapeutic doses is described.

Ergot is one of the classical abortifacients and has been a drug of toxicological interest for many years. Most workers, however, in discussing its detection in viscera refer to sclererythrin and usually avoid specific reference to isolation and detection of the alkaloids.

In recent years ergometrine has replaced the crude drug in obstetric practice and, although this particular alkaloid has little oxytocic effect on the pregnant uterus before term, it has been used in an effort to procure abortion.

Koppe and Dille[1] added ergometrine to muscle, blood, and liver and using continuous extraction with chloroform of the sodium sulphate dried tissue were able to recover a substantial proportion. The same workers injected guinea pigs in doses of 25 mg./kg. and were just able to detect the alkaloid in the viscera 5 hours after. Notwithstanding these encouraging results no other work has been published. Recently, in a case of insulin poisoning, it was necessary to investigate an allegation that a woman had received 0·5 mg. injection of ergometrine maleate into her buttock some hours before her death[2] and because in the circumstances the method of Koppe and Dille was unsuitable the problem was re-investigated.

Experimental

Extraction Method

Before considering the process which could be used to extract the alkaloid from buttock tissue it was necessary to know the stability of the alkaloid in acid and alkali, and also its partition coefficients between an aqueous phase and organic solvents. The rate of decomposition of the alkaloid was followed by observations on the ultra-violet absorption spectra and by examination of paper chromatograms.

The *E*(1 per cent, 1 cm.) values for ergometrine maleate were as follows:—

Solvent	λ max (mμ)	λ min (mμ)	*E*(1 per cent, 1 cm.) (λ max)
0·1N NaOH ..	310	269	225
0·1N H_2SO_4 ..	312	269	221

Although at room temperature the alkaloid was stable in 0·1N sodium hydroxide or 0·1N sulphuric acid for several hours elevation of temperature or increase in acidity or alkalinity resulted in rapid loss of the alkaloid. In 5 per cent acetic acid the alkaloid survived heating in a boiling water bath for 30 minutes. If ether was used as the solvent for extraction the proportion of alkaloid extracted from ammoniacal aqueous solution could be significantly increased if the aqueous phase was saturated with ammonium sulphate. In this way 80 per cent of the alkaloid was recovered by extracting the ammoniacal solution twice with equal volumes of ether, whereas three extractions in the absence of the ammonium sulphate recovered only 36 per cent. These results led to experiments on the use of the Daubney and Nickolls method[3] for the extraction of the alkaloid from tissue and urine. The method of Stas and Otto was not used because of the high proportion of fat in the buttock tissue. The method used to extract the alkaloid was as follows. The sample of tissue, 60–200 g., was macerated with 350 ml. of 5 per cent v/v aqueous acetic acid and sufficient ammonium sulphate was added to make a saturated solution. After heating in a boiling water bath for 30 minutes the macerated tissue was filtered through a paper pulp pad on a sintered glass Buchner funnel. The tissue on the pad was washed twice with 250 ml. aliquots of hot 5 per cent acetic acid. It is important not to reduce the pressure in the filtration to such a degree that the molten fat is sucked through the pad. After cooling, the combined aqueous filtrate and washings were extracted with an equal volume of ether which was examined in this particular case for the phenolic preservatives which are added to commercial insulin preparations. The aqueous phase was then made alkaline with ammonia and resaturated with ammonium sulphate. Two extractions with equal volumes of ether followed by evaporation of the ether gave the crude alkaloid extract. Experiment showed that this method of extraction gave a recovery of approximately 40 per cent when 1, 10 or 100 μg. were added to 200 g. samples of buttock tissue. Urine was extracted in the same way after the addition of ammonium sulphate.

Purification

Paper chromatography was used as the method of purification. It was found possible to load up to about 60 per cent of the total alkaloid extract from 200 g. of tissue on to one spot. Because the limit of detection was approximately 0·1 μg. and the recovery 40 per cent, this implies that quantities above 0·5 μg. of ergometrine maleate in 200 g. quantities of tissue should be detectable. Experiment showed that 1 μg. added to tissue could be detected and further that storage of the tissue at 0° for several weeks did not lead to a loss of the alkaloid. There is a large volume of literature on the separation of the ergot alkaloids but none of it refers to the separation of ergometrine from body constituents. In this laboratory the *n*-butanol: citric acid solvent system of Curry and Powell[4] using Whatman No. 1 paper buffered with 5 per cent sodium dihydrogen citrate has been extensively used for the separation of alkaloids. Using this system it was found that ergometrine ran at R_F 0·23, a position unoccupied

by any of the constituents found in a normal buttock. Very good spots were obtained. Detection was by observation of the ultra-violet fluorescence and by the blue-violet colour obtained when the paper was gently heated after spraying with 0·5 per cent solution of *p*-dimethylaminobenzaldehyde in ethanol containing 10 per cent of concentrated sulphuric acid. 0·1 μg. of ergometrine could be detected using either method. In buttock tissue faint fluorescence from fat slightly reduced the sensitivity of this method but no such difficulty was experienced in urine samples. Most of this work was done using an ultra-violet light fitted with a Wood's glass filter. It was subsequently found that when a lamp fitted with a Chance OX7 filter was used the sensitivity of the fluorescence method was substantially increased. This series of experiments investigated only the isolation of the alkaloid from buttock tissue or urine but Dr. E. G. C. Clarke tells me that the method works equally well in the extraction from liver tissue.

The Urinary Excretion of Ergometrine and Procaine

The urinary excretion was followed using the methods described above. Urine was collected from women who had been given a single 0·5 mg. injection of ergometrine maleate during childbirth. 50 ml. samples were extracted once with ether from acid solution and then twice with ether from ammoniacal solution saturated with ammonium sulphate. 1 and 10 per cent aliquots of this alkaloid extract were examined by paper chromatography. It was found that the alkaloid could be detected using the *p*-dimethylaminobenzaldehyde reaction up to 7½ hours after the injection. In one case where the time interval was 8½ hours positive fluorescence spots were obtained using the Chance OX7 filtered light, although no chemical reaction could be obtained. The maximum concentration of ergometrine in the urine generally occurred 2–3 hours after the injection.

Procaine was the only common base that interfered with the reaction. This local anaesthetic was present in several urines usually in much higher concentration than the ergometrine after injections of procaine penicillin. It runs slightly faster than ergometrine ($R_F = 0{\cdot}25$) and gives a yellow colour immediately on spraying with the *p*-dimethylaminobenzaldehyde reagent.

Procaine is known to be hydrolysed in tissue by procaine esterase and doubts have been expressed about the chance of its successful detection by forensic toxicologists[5]. No difficulty was experienced in its detection in this series of experiments even after the urines had been stored for several weeks.

Discussion

In toxicological analysis emphasis is usually placed on the detection of toxic quantities of poisonous compounds. Occasionally, however, it is necessary to analyse viscera for non-toxic drugs alleged to have been given in therapeutic doses. Such an occasion arose recently when a murderer who used injections of insulin as the means to kill alleged that the hypodermic marks were caused by injections of ergometrine maleate. Despite

the extremely small doseage of this drug it was found possible to demonstrate that had such an injection been given its detection would have been ensured.

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