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The occurrence of cyclohexylamine in urines studied for drug use by thin-layer chromatography

In the course of a routine survey of the urines of psychiatric patients for drugs by thin-layer chromatographic procedures as outlined by the authors in a previous communication[1], a recurrent spot was noted. This reaction resembled that of the amphetamines, but did not conform with either the expected R_F value of commonly used amphetamines, nor with the clinical expectation of such a finding. Extensive checks of this material were carried out with the conclusion that this spot represented cyclohexylamine, a breakdown product of cyclamate in the diet. In view of the current interest in the breakdown of cyclamate to potentially toxic cyclohexylamine[2], and the possibility that cyclohexylamine may be confused with amphetamines or ephedrine in routine studies on drug use, this pattern has been investigated.

The breakdown of cyclamate to cyclohexylamine has been described by KOJIMA AND ICHIBAGASE who have developed methods for the analysis of cyclohexylamine by both thin-layer chromatography[3] and gas–liquid chromatography[4]. Other methods for analysis of cyclohexylamine in urine and stool have been reported by SONDERS[5], and by FELTKAMP AND KOCH[6]. LEAHY *et al.*[7] found cyclohexylamine in the urine of only 4 of 35 volunteers 24 h after ingestion of 1 g of sodium cyclamate. Only 1 of 5 volunteers who took 1 g of cyclamate daily over a period of 17 days showed cyclohexylamine in his urine.

Method

The pH 9.3 extract from SA-2 ion-exchange paper soaked in urine and taken to dryness is dissolved in methyl alcohol, spotted on a Silica Gel G plate and developed in ethyl acetate–methyl alcohol–58 % ammonia (85:10:5). In this system, the cyclohexylamine spot shows an average R_F value of 0.40, stains blue with Bromocresol Green, disappears with the application of potassium iodoplatinate and on drying, reappears chalky pink.

R_F values in this area and similar color reactions have been observed with ephedrine, hydroxyamphetamine and phenylpropanolamine. However, when the following solvent systems are used, the R_F values of each of these compounds are readily differentiated: ethanol–pyridine–1,4-dioxane–water (50:20:25:5), ethanol–25 % ammonia (4:1), and butanol–acetic acid–water (4:1:5).

By the use of appropriate standards and confirmation in all systems used, it has been concluded that the material found in the urine was cyclohexylamine since it had an identical R_F value and staining characteristics with standards of cyclohexylamine added to urine.

Incidence in psychiatric patients

A survey of 139 psychiatric patients in whom repeated weekly urine studies over a period of 8 months were available revealed the amine to be present in 39. Of these 31 on careful questioning were aware that they had ingested cyclamates in the form of soft drinks, 8 denied any use of cyclamates to their knowledge. A survey of 100 patients who had no cyclohexylamine in their urine revealed that at least 52 used cyclamates

in their diet. Thus, of 83 known cyclamate using psychiatric patients, 31 showed the presence of cyclohexylamine in their urine.

Incidence in normal volunteers

A group of employees at Hillside Hospital who were apparently in good health and not taking any significant medication were given 0.6 g of cyclamate in a soft drink on two occasions. All pre-ingestion urine samples were negative. On the first occasion, 37 were given the cyclamate at 2 p.m. and the first morning urine specimens were collected the following day. In this group, cyclohexylamine was found in 3. In a second group of 27, urines were collected serially over a period of 8 h. Cyclohexylamine was found in 2 of these people who were also positive in the first series. In one case where the cyclohexylamine was found in the first study, it was not found in the second. In all positive cases, cyclohexylamine was found in the 3 h specimen as well as in the overnight specimen.

Conclusions

(1) Cyclohexylamine, a breakdown product of cyclamate, can be detected in the urine and may be confused with ephedrine or one of the amphetamines. With this in mind, it is suggested that in the use of TLC procedures, cyclohexylamine standards be employed in the interpretation of any findings.

(2) The incidence of appreciable excretion of cyclohexylamine in the urine of cyclamate users may be as high as 28 % and as low as 7 %, depending upon the population studied and the method of urine collection. There is an indication that psychiatric patients may have a higher incidence of cyclohexylamine excretion than the normal population. Conclusions may not be drawn from these results since our observations on psychiatric patients involved repeated urine collections and indeterminate dosages of cyclamates. Following cyclamate ingestion, the presence of cyclohexylamine in the urine, as determined by this method, may be variable.

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