Ethchlorvynol in Biological Fluids: Specificity of Assay Methods

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Abstract □ The concentrations of ethchlorvynol in blood, urine, and hemodialysate from a patient with acute ethchlorvynol overdosage were determined by chemical methods and GLC. In comparison, the colorimetric and spectrophotometric methods are shown to be nonspecific, apparently measuring metabolites as well as unchanged drug. GLC provides a specific method for the quantitative estimation of ethchlorvynol in biological fluids. It was used in this patient to show that, while the drug can be effectively removed from the body by hemodialysis, the urinary excretion of unchanged drug is negligible.

Keyphrases □ Ethchlorvynol poisoning—metabolism, chemical and GLC determination in blood, urine, and hemodialysate □ Hemodialysis—removal of ethchlorvynol poisoning, man □ Poisoning, ethchlorvynol—metabolism, determination in biological fluids □ GLC—analysis, ethchlorvynol □ UV spectrophotometry—analysis, ethchlorvynol □ Colorimetry—analysis, ethchlorvynol

Acute overdosage with the hypnotic sedative ethchlorvynol[1] (5-chloro-3-ethylpent-1-en-4-yn-3-ol) leads to respiratory depression and hypotension with prolonged deep coma and its associated complications. Among the 28 clinical cases reported in the literature (1–12), there have been seven fatalities, so the management of these patients presents a significant problem. In addition to intensive supportive therapy (13), the management may include an attempt to shorten the period of coma, which is usually longer than that induced by overdosage of the short-acting barbiturates, glutethimide or methaqualone. It was suggested that forced diuresis (2, 6, 11), peritoneal or hemodialysis (4–7, 9, 10), exchange transfusion (9), and resin hemoperfusion (12) may provide an effective treatment by removing the drug from the body. However, any assessment of such methods of treatment, like studies on the metabolism of the drug, requires a sensitive and specific method of ethchlorvynol assay.

Several methods have been developed. That of Algeri *et al.* (1) depends upon the reaction between the drug and phloroglucinol in acid to give a chromophore, while the colorimetric procedure described by Andryauskis *et al.* (14) and Frings and Cohen (15) depends upon the reaction with diphenylamine in acid solution. The indirect method devised by Wallace *et al.* (16) involves acid hydrolysis to a carbonyl derivative, which is separated by steam distillation and assayed spectrophotometrically as the semicarbazone. Several GLC methods were also described (10, 17–24). Methods based on each of these principles have been used for ethchlorvynol assay in studies of poisoned patients. Ogilvie *et al.* (7), Hedley-Whyte and Laasberg (10), and Teehan *et al.* (2) noted that there is disagreement in the literature regarding the recoveries of ethchlorvynol in urine and dialysate obtained from patients with ethchlorvynol overdosage. This, together with chemical considerations, led the authors to believe that some methods of ethchlorvynol assay are not specific for unchanged drug.

Cummins *et al.* (24) recently described a GLC assay of ethchlorvynol and suggested that the method of Wallace *et al.* (16) might lack specificity and be unable to differentiate between ethchlorvynol and related metabolites. However, Cummins *et al.* (24) did not refer to other conventional chemical methods of ethchlorvynol assay, nor did they provide experimental evidence to support their suggestion. Recent studies on a patient acutely poisoned by ethchlorvynol helped to provide experimental evidence which suggests that the method of Wallace *et al.* (16) and the colorimetric procedures of Algeri *et al.* (1) and Andryauskis *et al.* (14) are indeed nonspecific.

MATERIALS AND METHODS

Case History—A 27-year-old male was admitted in grade III coma (13). After intubation, gastric lavage yielded a dark-red fluid with the characteristic smell of ethchlorvynol. A sample of blood taken on admission was found to contain ethchlorvynol (51 mcg./ml.), cyclobarbitone (20 mcg./ml.), and methaqualone (12 mcg./ml.). During the following 24 hr., the patient's level of unconsciousness deepened to grade IV; he became hypotensive and required assisted ventilation. On each of the following 5 days, he was treated by hemodialysis[2] with four 2-hr. changes of 100 l. of standard dialysis bath solution. His conscious level fluctuated, becoming progressively lighter; on the 6th day, it was possible to discontinue assisted ventilation. On regaining consciousness, the patient was confused and hallucinated but eventually made a complete recovery.

Samples—Venous blood samples were taken at intervals into heparinized tubes, all urine was collected in 24-hr. periods, and aliquots of each change of bath fluid were removed from the dialyzer bath after use. All samples were stored at 4° until assayed.

Chemical Assays of Ethchlorvynol—The method of Wallace *et al.* (16) was first used to measure ethchlorvynol in 2 ml. of whole blood, 20 ml. of dialysis fluid, and 1-ml. samples of urine. The methods of Algeri *et al.* (1) and Andryauskis *et al.* (14) were used to estimate ethchlorvynol in the urine collected from the patient between 70 and 94 hr. after admission.

GLC Assay of Ethchlorvynol—A GLC method was developed using a gas chromatograph with a flame-ionization detector[3], 1-mv. recorder[4], and integrator[5]. The column used for the assays was 2.5% XE-60 on AW-DMCS Chromosorb G, 80–100 mesh[6]. A second column, 3% SE-30 on Gas Chrom Z, 100–120 mesh[7], was also used to determine the retention times of ethchlorvynol and the internal standard. Both columns were of glass, 1.5 m. × 4 mm. i.d. The column temperature was 100°, the detector temperature was 200°, and the injection port temperature was 120°. The carrier gas (argon) flow was 70 ml./min. 1,1,1-Trichloro-2-methylpropan-2-ol (chlorbutol) was used as the internal standard.

One-milliliter samples of whole blood lysed by the addition of 2 ml. water, 20-ml. samples of hemodialysate, and 2-ml. samples of

[1] Placidyl, Abbott Laboratories.

[2] Using a Kolff-Travenol UF145 coil.
[3] Pye series 104–24.
[4] Honeywell.
[5] Hewlett Packard 3370A.
[6] Perkin Elmer.
[7] Pye.

Table I—Comparison of Results of Ethchlorvynol Assays by the Method of Wallace *et al.* (16) (A) and by GLC (B) in Blood, Hemodialysate, and Urine from a Poisoned Patient

Hours after Admission	Blood Level, mcg./ml.			Dialysis Recovery, g.			Urinary Excretion		
	A	B	Ratio (A/B)	A	B	Ratio (A/B)	Volume, ml.	Total Recovery, g. A	Total Recovery, g. B
0	51	50	1.02				530	0.02	0.01
28	125	127	0.98						
				1.7	1.2	1.42	440	1.1	0.02
36	117	111	1.05						
47	117	120	0.98						
				2.6	1.9	1.37	430	1.0	0.02
55	102	91	1.12						
70	93	90	1.03						
				2.7	1.4	1.93	290	0.8	0.01
78	94	64	1.47						
94	110	54	2.04						
				2.5	0.7	3.57	480	1.1	0.01
102	69	40	1.73						
121	83	30	2.77						
				2.1	0.3	7.0	370	0.7	0.005
129	41	20	2.05						
143	50	10	5.00				1520	3.8	0.10
167	45	2	22.5						
191	27	0							
Total recoveries, g.				11.6	5.5			8.4	0.16

urine were extracted by shaking with 1 ml. analytical reagent grade chloroform followed by centrifugation; 4-μl. samples of the chloroform extract were applied to the column using a microsyringe.

Ten standard solutions, covering the range of 20–200 mcg. ethchlorvynol/ml. in chloroform containing chlorbutol, were prepared and applied to the column in the same way. The ratios of the integrated areas of the ethchlorvynol and internal standard peaks were plotted against concentration to provide a calibration curve. Recoveries of ethchlorvynol from water, urine, and lysed whole blood were determined by adding ethchlorvynol to these fluids to concentrations covering the range of 40–200 mcg./ml. and extracting them as test samples.

Analysis of Steam Distillates—Steam distillates of aqueous solutions of ethchlorvynol and of urine samples, after treatment with sulfuric acid according to the method of Wallace *et al.* (16), were extracted with a tenth of their volume of chloroform and analyzed by the GLC method previously described. Similar distillates were extracted with analytical reagent grade carbon tetrachloride, and the IR spectra of the extracts were measured using a spectrophotometer[8], with carbon tetrachloride in a variable pathlength cell in the reference beam.

RESULTS

The procedure of Wallace *et al.* (16) gave a linear calibration curve for the concentration range up to 10 mcg./ml. in the final distillate. The results of analyses of the patient's urine, blood, and hemodialysate samples are shown in the columns headed "A" in Table I.

By the GLC method, the relative retention times for ethchlorvynol and chlorbutol were, respectively, 13.5 and 9.5 min. on the XE-60 column and 12 and 7 min. on the SE-30 column. The calibration curve was linear for the range studied (0–200 mcg./ml.), while the recoveries from water, urine, and lysed whole blood ranged from 99% at 40 mcg./ml. to 91% at 200 mcg./ml. The results of analyses of the patient samples are shown in the columns headed "B" in Table I.

The dialysis clearances of ethchlorvynol were 29–71 ml./min. (mean 55) as determined by the Wallace *et al.* (16) method, and 20–35 ml./min. (mean 28) as determined by GLC. The creatinine clearance between 14 and 38 hr. after admission was 79 ml./min., but it later fell and remained between 15 and 28 ml./min. for the period between 62 and 143 hr. after admission.

When a sample of urine was assayed in parallel, using the colorimetric methods (1, 14) as well as the procedure of Wallace *et al.* (16) and the GLC method, the results obtained were: Wallace method, 2.64 mg./ml.; GLC method, 0.032 mg./ml.; phloroglucinol method, 1.0 mg./ml.; and diphenylamine method, 3.2 mg./ml.

Extracts of the steam distillates after acid treatment of pure ethchlorvynol and of the patient's urine had similar GLC chromatograms, with two main and five minor peaks in addition to a small peak corresponding to unchanged ethchlorvynol. The IR spectra of the extracts of the steam distillates were identical, showing the loss of hydroxyl group absorption at 3600 $cm.^{-1}$, the formation of carbonyl group absorption at 1690 $cm.^{-1}$, and the retention of absorption bands attributed to the alkene and alkyne bonds.

DISCUSSION

The appearance on GLC of a single peak in the chloroform extracts of the samples from the patient, with identical retention times to that of ethchlorvynol on XE-60 or SE-30 columns, suggests that GLC provides a specific method for the estimation of unchanged drug. In published methods for the GLC assay of ethchlorvynol (10, 17–24), a variety of solvents such as benzene, chloroform, ethyl acetate, and carbon disulfide was shown to give adequate recoveries of the drug from aqueous solutions; stationary phases such as SE-30, XE-60, Carbowax 20M, neopentyl glycol succinate, and diethylene glycol succinate appear to give satisfactory retention times. In only one published method (22) was an internal standard, 1,3-dichloropropan-2-ol, used; in this study, a similar compound, 1,1,1-trichloro-2-methylpropan-2-ol, was found to be a suitable internal standard. The single chlorine atom of the ethchlorvynol molecule is apparently sufficient to facilitate the detection of 0.25 mcg./ml. of the compound with an electron-capture detector (24). Although the method described here was designed to measure relatively high concentrations of the drug, the use of greater amplification and a higher sample-to-solvent ratio would allow the flame-ionization detector to achieve a comparable sensitivity. The advantage of the flame-ionization detector is that it gives a linear response over a wider range of concentrations than is possible with the electron-capture detector.

It can be seen from Table I that there is a disparity between the results obtained by the two methods. The consistently large differences seen in the urine levels, as well as the progression of differences between the results for both blood and hemodialysate with time, is strong evidence that the method of Wallace *et al.* (16) measures metabolites of ethchlorvynol. The results of the colorimetric assays on urine suggest that this is also true of the procedures of Algeri *et al.* (1) and Andryauskis *et al.* (14).

The GLC and IR studies support this suggestion, because they strongly indicate that steam distillates of acidified urine contain compounds similar to those obtained by steam distillation of ethchlorvynol. It is reasonably certain, therefore, that the additional compounds detected by the colorimetric and spectrophotometric

[8] Unicam SP200.

procedures are derived from ethchlorvynol. The precise mechanisms of these chemical methods of ethchlorvynol assay are not known. However, it is apparent from the disparity between the results obtained for the ethchlorvynol concentration of the patient's urine sample that these three methods differ in their ability to detect metabolites of the drug.

Little appears to be known about the metabolism of ethchlorvynol. P'an *et al.* (25), who first described the action of the drug, did not have a method of ethchlorvynol assay, but they showed that neither liver damage nor nephrectomy prolonged the action of the drug in rats. Carr and Crampton (26), however, stated that the excision of 75% of the rat liver did prolong the sedative action of the drug. Cravey and Baselt (27) showed that, as a lipophilic drug, ethchlorvynol concentrates in adipose tissue; the studies of Cummins *et al.* (24) indicated that the decline in therapeutic levels of ethchlorvynol in serum is biphasic and fits a two-compartment model. The slow removal of ethchlorvynol from the body seen after therapeutic doses and overdosage may thus reflect localization of the drug in adipose and other tissues rather than a slow rate of biotransformation.

Using a GLC procedure, Cummins *et al.* (24) also showed that only 0.025% of a therapeutic dose of the drug was excreted in the urine over a period of 50 hr. after ingestion and that an average of 62% of this amount was conjugated as the gluconuride. The data presented here show that urinary excretion of unchanged drug likewise plays a small part in the excretion of ethchlorvynol after overdosage and suggest that the drug is extensively metabolized. That the metabolites may not contain the chlorine atom is indicated by the failure of Cummins *et al.* (24) to detect other compounds in the urine when using GLC with an electron-capture detector. The IR studies of the steam distillates of acidified urine indicate that alkene and alkyne bonds are retained in the metabolites, but their structures remain to be determined.

Inadequate knowledge of ethchlorvynol metabolism does not allow us to predict the pharmacological activity of the metabolites. Clearly, this will be an important factor in deciding on the appropriate course of treatment of acute overdosage. If the metabolites are inactive, then a dialysis procedure would provide an effective method of increasing elimination of unchanged drug and reducing the period of coma, since forced diuresis is not likely to lead to the excretion of a significant quantity of unchanged drug. However, if the metabolites are pharmacologically active, forced diuresis would be expected to promote their excretion and might provide a valuable treatment. In the absence of this information, while hemodialysis has been shown to be effective in removing unchanged drug, the value of forced diuresis is unproven.

The procedure described by Andryauskis *et al.* (14) provides a rapid and convenient qualitative screening method, but the present work suggests that where a quantitative assay for ethchlorvynol in biological fluids is required, the colorimetric and spectrophotometric procedures should be avoided. Instead, one of the methods depending on GLC assay (10, 17–24), preferably one that employs an internal standard (22 and this paper), should be used.

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