Serum and Urine Levels of Ethchlorvynol in Man

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Abstract □ Serum and urine levels of ethchlorvynol were determined by gas chromatography using electron-capture detection. The method is sensitive to 0.25 mcg./ml. and reproducible within 2% relative standard deviation. In 25 hr., 0.025% of the total dose is excreted unchanged or as the glucuronide. Peak serum levels of 2.5, 6.5, or 8.0 mcg./ml. are seen at 1 hr. after administration of 200, 500, or 750-mg. capsules, respectively. The decline of the serum levels is biphasic, with disappearance constants of 0.5 and 0.03 $hr.^{-1}$. When the data are interpreted in terms of a two-compartment model, the rate constant for elimination is calculated to be 0.13 $hr.^{-1}$. The ratio of the amount of ethchlorvynol in the tissue compartment to that in the central compartment is 3.5 : 1.

Keyphrases □ Ethchlorvynol levels—urine, serum □ Absorption half-life—ethchlorvynol □ Kinetics—ethchlorvynol absorption, excretion □ GLC, electron-capture detection—analysis

Wallace *et al.* (1) reported therapeutic blood levels and 24-hr. urinary excretion levels of ethchlorvynol,[1] a mild nonbarbiturate hypnotic-sedative, following the ingestion of a 500-mg. capsule. In their method of analysis, ethchlorvynol was destructively hydrolyzed to a carbonyl derivative which was isolated by steam distillation, converted to a semicarbazone, and determined spectrophotometrically. Because of the hydrolysis step, this method could lack specificity and might not differentiate between parent drug and similar metabolites. In addition, blood levels were not determined later than 6 hr. after dosing, which is an insufficient length of time for detailed studies of the kinetics of distribution and metabolism of ethchlorvynol.

Robinson (2) recently published a specific gas chromatographic method which is sensitive enough to determine peak therapeutic and overdosage blood levels of ethchlorvynol. This method, however, can determine only the peak levels with normal dosage and not the lower concentrations observed at later times.

In the study described here, a gas chromatographic method using electron-capture detection (ECD) was developed. The chlorine of ethchlorvynol imparts enough electronegativity to the molecule so that serum levels of the drug are easily detected up to 48 hr. following a single 500-mg. dose. Serum levels on three dosages, 200, 500, and 750 mg., as well as the kinetic interpretation and 24-hr. urinary excretion data, are presented. The urine samples were analyzed both before and after enzymatic hydrolysis in an attempt to determine the extent of conjugation.

EXPERIMENTAL

Apparatus—A model 1200 Varian-Aerograph gas chromatograph equipped with a tritium foil electron-capture detector was used in this study. The column was stainless steel, 1.52 m. × 0.32 cm. (5 ft. × 0.125 in.) o.d., with 5% NPGS on AW-DMCS 100/120 mesh diatomite aggregate.[2] The carrier gas (nitrogen) flow was

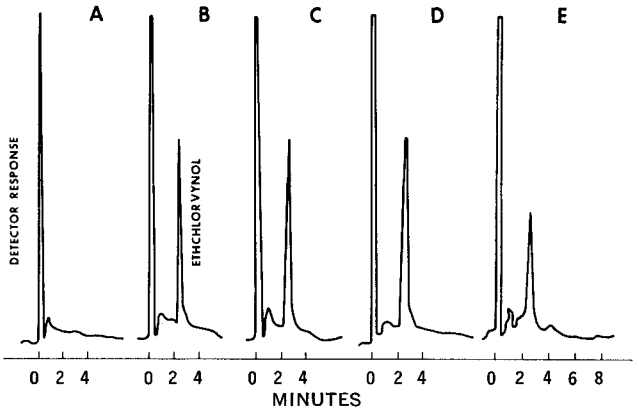

Figure 1—*Chromatograms of: (A) benzene extract from 2.0 ml. of control serum; (B) benzene extract from 2.0 ml. serum containing 3.0 mcg./ml. of ethchlorvynol; (C) benzene extract from 2.0 ml. aqueous solution containing 3.0 mcg./ml. of ethchlorvynol; (D) ethchlorvynol solution at a concentration of 3.0 mcg./ml. in benzene; and (E) benzene extract of serum from a treated subject.*

50–60 ml./min. with injector temperature of 150°, oven temperature of 120°, and a detector temperature of 210°. All quantitation was obtained with a model 480 Varian-Aerograph digital integrator.

Extractions were carried out in 20-ml., 16 × 150-mm. culture tubes fitted with Teflon-lined caps.

Reagents—Spectrograde benzene was used for all extractions. Glusulase enzyme[3] was used for urine hydrolysis.

Drug Administration—The experimental groups consisted of equal numbers of normal adult males and females (24 on the 200-mg. dose and 8 on the 500 and 750-mg. doses). The same subjects were used for the 500 and 750-mg. doses. The weights of the subjects ranged from 100 to 225 lb., and ages ranged from 20 to 51 years. They fasted 12 hr. prior to and 2 hr. subsequent to administration of ethchlorvynol. The drug was given in soft gelatin capsules. Urine was refrigerated as voided and frozen after a 24-hr. collection was completed.

Serum Analysis—Serum was stored frozen until analysis. Four milliliters of serum, obtained from subjects on a 200-mg. dose, or 2.0 ml. of serum, from those on a 500 or 750-mg. dose, was analyzed. In all cases the serum was diluted to 8.0 ml. with distilled water and extracted for 10 min. with 2.0 ml. of benzene. Following centrifugation, 5.0 μl. of the benzene extract was injected into the chromatograph. Standard curves containing the appropriate amount of serum were run with each set of samples analyzed. Typical chromatograms of serum extracts are presented in Fig. 1.

Urine Analysis without Hydrolysis—Five milliliters of urine plus 1.0 ml. of 0.1 *M* acetate buffer (pH 5) was extracted for 10 min. with 2.0 ml. of spectro-benzene. Following centrifugation, 4.0 mcg. was analyzed. As with serum, standard curves were run with ethchlorvynol added to control urine.

Urine Analysis following Enzymic Hydrolysis—Five milliliters of urine plus 1.0 ml. of 0.1 *M* acetate buffer (pH 5) was incubated with 20 μl. of glusulase for 48 hr. at 38° in a Dubnof shaker. To minimize microbial degradation, 0.5 ml. of toluene was added to each tube. Standards of ethchlorvynol added to control urine were also carried through the entire hydrolysis process. These standards indicated minimal degradation of ethchlorvynol during the hydrolysis. Following hydrolysis the samples were extracted and analyzed according to the procedure for the nonhydrolyzed urine samples. A chromatogram of a urine extract is not presented because of its similarity to that of serum.

[1] Placidyl, Abbott Laboratories.
[2] Chromosorb W, Johns-Manville.
[3] Endo Laboratories.

Table I—Total 24-hr. Urinary Excretion of Ethchlorvynol following the Oral Administration of a 500-mg. Capsule

Subject, Sex	Collection Interval, hr.	Volume, ml.	mcg./Total Volume (not Hydrolyzed)	mcg./Total Volume (after Hydrolysis)	Percent Conjugated	Percent Total Dose Excreted
No. 1, male	0–4	224	341	332	0	0.007
	4–8	188	180	259	69	0.005
	8–12	188	128	226	56	0.005
	12–24	204	131	375	35	0.007
Total	0–24	804	780	1192	65	0.024
No. 2, male	0–4	148	314	284	0	0.006
	4–8	150	174	246	71	0.005
	8–12	190	175	403	43	0.008
	12–24	198	83	309	27	0.006
Total	0–24	686	746	1242	60	0.025

Table II—Kinetic Constants Calculated from Serum Levels of Ethchlorvynol following a Single Oral Dose

	Dose, mg. 200	500	750
α (hr.$^{-1}$)	0.530 ± 0.197	0.532 ± 0.043	0.450 ± 0.068
β (hr.$^{-1}$)	0.0274 ± 0.0061	0.0353 ± 0.0017	0.0218 ± 0.0032
V_1 (l)	59.3 ± 10.9	51.4 ± 2.3	60.7 ± 5.1
k (hr.$^{-1}$)	2.80 ± 0.83	2.04 ± 0.16	2.02 ± 0.31
k_{-1} (hr.$^{-1}$)	0.115 ± 0.028	0.132 ± 0.007	0.0962 ± 0.0146
k_1 (hr.$^{-1}$)	0.321	0.293	0.273
k_2 (hr.$^{-1}$)	0.127	0.142	0.102
V_2 (l)	164.	114.	172.

Kinetic Analysis of the Serum Levels—The time course of the rise and fall of the mean serum levels was analyzed by using the computer program NONLIN.[4] This program calculates a statistical "best fit" in the least-squares sense to a nonlinear function. In this case, the equation fit was that for two-compartment distribution following oral absorption (3):

$$C_1 = \frac{kD}{V_1}\left[\frac{(k_{-1}-\alpha)}{(k-\alpha)(\beta-\alpha)}e^{-\alpha t} + \frac{(k_{-1}-\beta)}{(k-\beta)(\alpha-\beta)}e^{-\beta t} + \frac{(k_{-1}-k)}{(\alpha-k)(\beta-k)}e^{-\beta t}\right] \quad \text{(Eq. 1)}$$

In this equation, C_1 is the concentration of drug in the central compartment (serum level), k is the rate constant for absorption, D is the dose, V_1 is the volume of the central compartment, k_{-1} is the rate constant for transfer from the tissue to the central compartment, α is the exponential term for the first phase of decline of serum levels, β is the similar term for the terminal phase of decline of serum levels, and t is the time after dosing at which the sample was taken.

RESULTS AND DISCUSSION

Serum Assay for Ethchlorvynol—From Fig. 1 it is apparent that excellent recovery of ethchlorvynol was obtained with the benzene extraction. Based on integrated values and comparison to a standard benzene solution, it was calculated that 93.8 and 95.8% of the total drug were recovered from serum and aqueous solutions, respectively. From Part E of Fig. 1, it is also apparent that only one peak resulting from ethchlorvynol is detectable in the serum extracts, thus demonstrating the selectivity of ECD.

Linear standard curves were obtained from 0.25 to 5.0 mcg./ml. when 2.0 ml. of serum was analyzed. When 4.0 ml. of serum was analyzed, the range of linearity was from 0.25 to 4.0 mcg./ml. If the responses approached or exceeded the range of linearity, dilutions were made so that responses were obtained in the range of linearity.

Precision of the Assay—Ten replicate analyses of serum samples to which 5.0 mcg./ml. of ethchlorvynol had been added yielded a standard deviation of ±2.13%.

Urine Levels—The data in Table I show that an average of only 0.025% of a dose of ethchlorvynol is excreted unchanged in 24 hr. Approximately 62% of this amount is conjugated to glucuronic acid or sulfate. Thus, ethchlorvynol is apparently metabolized extensively. These recoveries are much lower than those reported by Wallace *et al.* (1).

In this assay, only ethchlorvynol was measured. There were no additional peaks in either serum or urine, which suggests that during metabolism ethchlorvynol is converted to either a benzene-insoluble compound or a nonchlorinated compound. Either type of metabolite could be confused with ethchlorvynol by the Wallace *et al.* method (1). The lack of ECD of any other compound suggests that during metabolism the chlorine atom is lost.

Serum Levels—The mean serum concentrations of ethchlorvynol at various times after dosing are shown in Fig. 2. Twenty-four subjects were averaged for the 200-mg. dose; eight for the 500 and 750-mg. doses. These data show that ethchlorvynol is rapidly absorbed, with the peak serum level occurring 1 hr. after dosing. The initial decline of the serum concentration is also rapid, since at 4 hr. the concentration is less than half that at 1 hr. The second phase of the blood level decline is much slower.

Wallace *et al.* (1) reported whole blood levels, after a 500-mg. capsule, somewhat lower than the serum levels in Fig. 1. They did not see the biphasic decline since their last sample was taken at 6 hr. after dosing.

The data observed do not fit a single-compartment distribution model; the actual serum levels at 24 hr. are 10-fold higher than those predicted by this model.

The parameters calculated by using the two-compartment model are collected in Table II. The terms not previously defined are:

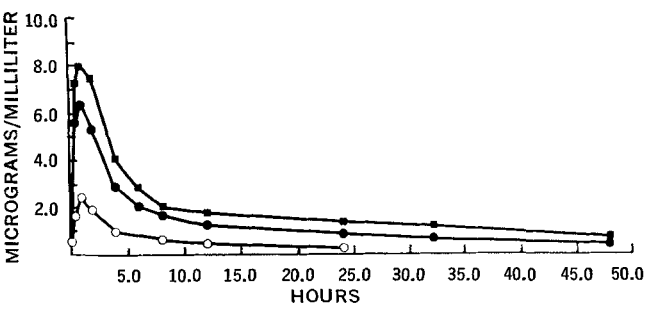

Figure 2—*Human serum levels of ethchlorvynol. Key:* ○, *average value for 24 subjects following a single 200-mg. dose;* ●, *average value for 8 subjects following a single 500-mg. dose; and* ■, *average value for 8 subjects following a single 750-mg. dose.*

[4] Dr. Carl M. Metzler of The Upjohn Co., Kalamazoo, Mich., furnished this program.

k_1, the rate constant for transfer from the central to the tissue compartment; k_2, the rate constant for elimination by metabolism or excretion from the body; and V_2, the volume of the tissue compartment. From the standard deviations of the parameters, it can be seen that the data fit this model rather well. Discussions of the meaning of two-compartment distribution may be found in *References 4–6*. The central compartment is generally thought to consist of blood and highly perfused tissues such as heart, lung, liver, and kidney. The more poorly perfused tissue compartment includes muscle and skin. Because of the oily nature of ethchlorvynol, one would expect that it would be preferentially distributed into fat. Riegelman *et al.* (4) pointed out that, because of direct diffusion from tissues to fat, the effect of distribution of a drug into a fat compartment simply modifies the effective volume of the compartment from which the drug diffused. Thus the identity of the tissue compartment with any anatomical structure cannot be made.

The results point out very clearly that the slow decline of serum levels in the β-phase is not due to a low rate of metabolism but rather to extensive tissue localization of ethchlorvynol since k_2 is at least four times as large as β at each dose.

These data also demonstrate that ethchlorvynol is rapidly absorbed; from the k values, a half-life for absorption of approximately 0.3 hr. is calculated. Likewise the rate of metabolism is rather fast; a half-life of 5.6 hr. is seen. From serum levels in the β-phase, one may calculate f_c, the ratio of the amount of ethchlorvynol in the central compartment to the total amount in the body, and f_T, the similar value for the amount in the tissue compartment, from the following relations:

$$f_c = \frac{k_{-1} - \beta}{k_1 + k_2 - \beta} = 0.22 \qquad \text{(Eq. 2)}$$

$$f_T = 1.0 - f_c = 0.78 \qquad \text{(Eq. 3)}$$

$$f_T/f_c = 0.78/0.22 = 3.5 \qquad \text{(Eq. 4)}$$

This ratio is substantially higher than those for aspirin, griseofulvin, and spectinomycin which were reported by Gibaldi *et al.* (6) to be 1.1, 0.9, and 0.65, respectively.

CONCLUSIONS

Ethchlorvynol is extensively metabolized since only 0.025% of the dose is excreted unchanged or as the glucuronide.

Absorption of ethchlorvynol is very rapid, with an absorptive half-life of 0.3 hr.

The decline of serum levels is biphasic, which suggests a two-compartment model for distribution. The hybrid rate constants are 0.5 and 0.03 $hr.^{-1}$. From these the rate constant for elimination is calculated to be 0.13 $hr.^{-1}$. There is also evidence for extensive tissue localization of ethchlorvynol.

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Effect of Aging on Some Physical Properties of Hydrochlorothiazide Tablets

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Abstract □ Hydrochlorothiazide tablets were prepared using acacia, starch, and polyvinylpyrrolidone as granulating agents. The tablets were evaluated at room temperature and at elevated temperatures relative to changes in hardness, disintegration, and dissolution. Acacia is an unsatisfactory granulating agent because the values of the hardness, disintegration, and dissolution times are increased with aging; starch and polyvinylpyrrolidone are acceptable granulating agents because the physical properties of the tablets are essentially unchanged with aging. The changes in the physical properties of the tablets after short-term storage at elevated temperatures correlated with the changes upon aging for 1 year at room temperature. Thus, for the formulations used in this study, changes occurring after short-term storage at 37, 50, and 80° could be used to predict changes in physical properties during the normal shelf-life of the tablets at room temperature.

Keyphrases □ Hydrochlorothiazide tablets—age effect □ Aging, hydrochlorothiazide tablets—accelerated, physical stability □ Tablets, hardness, disintegration, dissolution—aging effect □ Granulating agents effect—physical stability, tablets

The compressed tablet is the most popular dosage form; for many pharmaceutical manufacturers, it comprises the majority of their products. As a part of research and development operations, the chemical stability of the medicinal compound in a tablet is routinely studied. Nevertheless, the manner in which medicinal compounds degrade in solid dosage forms is obscure, and the number of publications reporting degradation in solid dosage forms is not numerous (1–6).

In addition to demonstrating that the chemical nature of the medicinal compound will not change during the recommended life of the product, the manufacturer must consider possible changes in the physical properties of a tablet. A physically stable tablet should retain its original color, disintegration time, friability, hardness, shape, size, weight, and dissolution profile (7, 8).

If the dissolution of the medicinal compound from a tablet is slowed upon aging or storage of the tablet, the biological availability may be seriously affected because the medicinal compound would be less available for gastrointestinal absorption. It cannot be assumed that a rapid release of the medicinal compound from the tablet immediately after its production will be