Interaction of disulfiram with benzodiazepines

The disposition of chlordiazepoxide (50 mg, intravenously), diazepam (0.143 mg/kg, orally), and oxazepam (0.429 mg/kg, orally) were studied in normal and alcoholic men before and after chronic disulfiram administration. Decreases in the plasma clearance of chlordiazepoxide (54%, $p < 0.05$), diazepam (41%, $p < 0.05$), and their active N-desmethyl metabolites were observed. Oxazepam has no important active metabolites and its net disposition is minimally altered by disulfiram. Oxazepam disposition is unaffected by age and liver disease. These considerations together with that of the short half-life of oxazepam (median, 6.1 hr) suggest that oxazepam may be the drug of choice if benzodiazepine therapy is used for patients taking disulfiram.

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Disulfiram (Antabuse) has been used for over 25 years in aversive therapy for selected chronic alcoholic patients.[1, 2] It is upon the threat of an Antabuse reaction when alcohol is ingested, that the use of the drug is based. This reaction is probably due to accumulation of acetaldehyde associated with disulfiram inhibition of acetaldehyde oxidoreductase.[1, 2] Disulfiram also inhibits a variety of other enzymes, including dopamine-β-hydroxylase, xanthine oxidase, hexokinase, glyceraldehyde 3-phosphate dehydrogenase, and d-amino acid oxidase.[31] Disulfiram also inhibits hepatic microsomal drug metabolism in laboratory animals after both short- and long-term administration.[6, 8, 11, 14, 17, 19, 25, 31] In man, disulfiram prolongs the half-life ($t_{1/2}$) of antipyrine[28, 29] and can cause toxic accumulation of phenytoin[5, 20] and warfarin[21] through inhibition of hepatic mixed function oxidase-catalyzed hydroxylation.

The 1,4-benzodiazepines, diazepam and chlordiazepoxide, are frequently used in the treatment of chronic alcoholic subjects concurrently with disulfiram.[1, 2] Inhibition of benzodiazepine biotransformation could be associated with unexpectedly high drug concentrations and enhanced clinical effects. The major pathway of chlordiazepoxide and diazepam biotransformation is initial N-demethylation and eventual glucuronidation.[10] The nonglucuronide metabolites of each drug are pharmacologically active. Other metabolites

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have been identified in animals but are unimportant in man. In contrast, oxazepam has no quantitatively important active metabolites (<5%)[30] and is conjugated to a pharmacologically inactive glucuronide. In liver disease, conjugation appears to be influenced less than mixed-function oxidase activity, since the t½ of chlordiazepoxide, diazepam, and desmethyldiazepam but not oxazepam is prolonged.[13, 22, 24] Animal studies suggest that similar differential effects of inhibitors of drug metabolism may also exist.

In three separate experimental protocols we have determined the influence of chronic disulfiram administration on chlordiazepoxide, diazepam, and oxazepam disposition in normal subjects and chronic alcoholic subjects. The results are presented here in combination to facilitate comparison. The results suggest that oxazepam disposition is altered only minimally by disulfiram and that oxazepam will be the benzodiazepine with fewest dosing difficulties when administered with disulfiram to alcoholic subjects.

Methods

Participants in these studies were healthy, male subjects or male chronic alcoholic patients in an alcohol abuse rehabilitation program, with a past history of consuming at least 80 gm ethanol per day for at least 10 yrs. All subjects had a history, physical, SMA-12 hemogram, urinalysis, electrocardiogram, and urine drug screen. Subjects with active medical or surgical problems or any factors except smoking known to alter drug disposition were excluded. All chronic alcoholic patients had stopped drinking at least 2 wk prior to the study. In normal subjects, drug or alcohol intake was not permitted during and for at least 2 wk before the study. No chronic alcoholic patient was included if he had clinical or laboratory evidence of cirrhosis or hepatitis.

Group I: Chlordiazepoxide. Fourteen normal subjects (median age, 24.5; range, 21 to 31 yr; median weight, 72.2, range 60.0 to 96.5 kg) received 50 mg of chlordiazepoxide HCl intravenously over 10 min. Venous blood samples were drawn through an indwelling cannula placed in the contralateral forearm or by separate veinpuncture, prior to drug administration, at the termination of the infusion and over 48 hr after infusion. Plasma concentrations of chlordiazepoxide, and its metabolites, desmethylchlordiazepoxide and demoxepam, were determined by a spectrophotofluorometric assay.[26]

At least 1 mo later, 6 of the same subjects received 0.5 gm disulfiram orally each night for 14 days. Compliance was checked by pill counts. Twelve hours after the last dose of disulfiram, chlordiazepoxide was administered.

Eight chronic alcoholic patients (median age, 41; range, 33 to 54 yr; median weight, 72.7; range 57 to 104 kg) administered disulfiram for at least 14 days as part of treatment, were given a single intravenous dose of chlordiazepoxide and samples were drawn and analyzed as indicated above.

Group II: Diazepam. Six normal subjects (median age, 25.5; range, 21 to 30 yr; median weight, 71.8; range, 67.3 to 88.5 kg) received diazepam, 0.143 mg/kg, orally after a 6-hr fast. Fasting was continued for 2 hr after drug administration. Blood samples were taken over 120 hr.

The first night after the 120-hr blood sample, disulfiram (0.5 gm orally each night) was given for 12 days and the identical diazepam dose readministered. Disulfiram was discontinued 108 hr after the second diazepam dose. Two chronic alcoholic subjects (aged, 35 and 51 yr; weight, 67.7 and 76.1 kg) received diazepam and disulfiram by an identical protocol as the normal subjects. Serum diazepam and its active metabolite desmethyldiazepam were assayed by electron-capture gas chromatography.[32]

In order to maximize compliance to the prescribed regimen, subjects were required to telephone the Clinical Research Unit at the time they ingested the disulfiram dose each evening.

Group III: Oxazepam. Five normal subjects (median age, 25; range, 23 to 28 yr; median weight, 73.7; range, 71.0 to 82.8 kg) and 2 chronic alcoholic subjects (age 57 and 28; weight, 83.6 and 89.4 kg) received oxazepam, 0.429 mg/kg orally, before and after disulfiram in a study format identical to that used for diazepam except that disulfiram was discontinued 24 hr after the second oxazepam dose. Blood samples were taken over 36 hr. Plasma oxazepam concentrations were determined as for diazepam, except that benzene:ether (5:1) was used as the extracting solvent and desmeth-

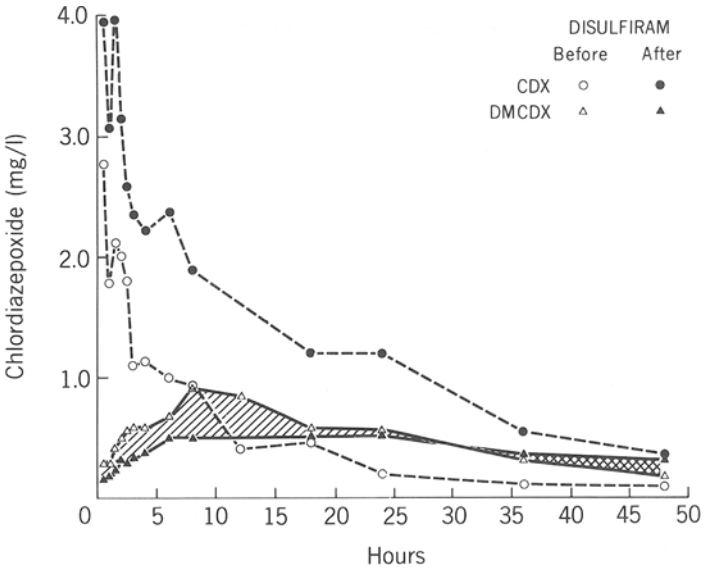

Fig. 1. Plasma concentrations of chlordiazepoxide and desmethylchlordiazepoxide in a normal subject before and after disulfiram administration.

yldiazepam was used as the internal standard.[7]

Pharmacokinetics. Using weighted (reciprocal of the square root of the concentration) nonlinear least-squares regression analysis, postinfusion plasma concentrations of chlordiazepoxide were fitted to the linear sum of the smallest number of exponential terms (in most cases two) necessary to describe the data points adequately.[16, 27] The following pharmacokinetic parameters were calculated: apparent volume of distribution (V_d) by the "area" method, apparent elimination half-life ($t_{1/2}(\beta)$), and total body clearance. Total body clearance was calculated from the dose and area under the curve (AUC) integrated to infinity.

Diazepam and oxazepam were administered orally. In consequence, it is inappropriate to calculate pharmacokinetic parameters such as apparent volume of distribution and clearance in which exact knowledge of the systemically available dose is required. Accordingly, $t_{1/2}(\beta)$ (determined from the log-linear apparent elimination phase), "nominal apparent volume of distribution," and "nominal clearance" have been calculated.

After characterization of the pharmacokinetic profile of each subject, it is possible to report intercompartmental rate constants; we have chosen not to do so. The numerical values of these parameters are dependent on the sampling times, the number of samples in each pharmacokinetic phase, and the configuration of the pharmacokinetic model. In addition, the assumption of monotonic log-linear elimination may not be valid. This is particularly well illustrated with diazepam in which marked fluctuations in the plasma concentrations occur within a 24-hr period during the apparent elimination phase.[23]

Statistics. Comparison between the before and after conditions between groups were made with the nonparametric rank sign and Mann-Whitney tests.

Results

Comparison of liver function tests of the normal subjects and chronic alcoholic subjects indicated that while albumin, prothrombin time, serum glutamic oxaloacetic transaminase, GGTP, and alkaline phosphatase were marginally different there were no important differences between groups. The median age of the chronic alcoholic subjects (41) was significantly greater than that of the normal subjects (25) ($p < 0.01$) but not sufficiently different to cause important differences in drug clearance as affected by age.[13, 15, 22]

The four potential effects of disulfiram to be considered are on relative bioavailability, $t_{1/2}$, apparent volume of distribution, and clearance.

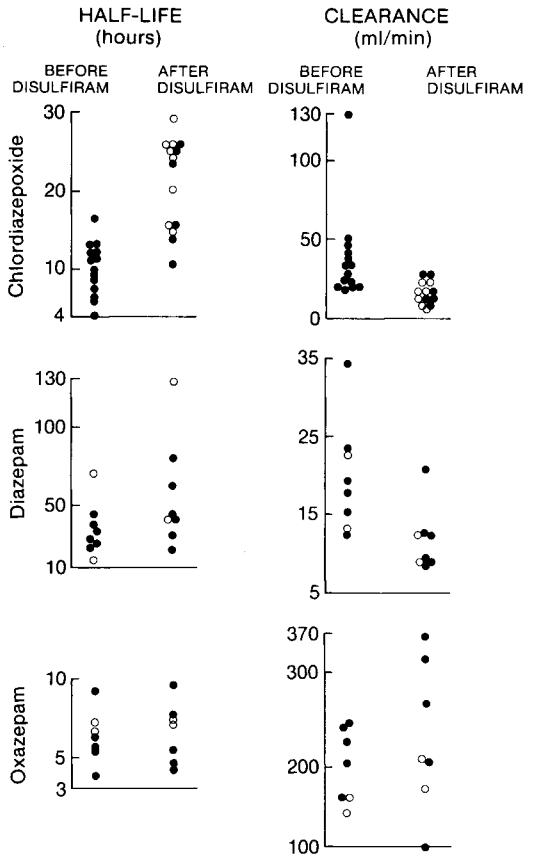

Fig. 2. Apparent elimination half-lives and total body clearances in normal *(closed circles)* and alcoholic *(open circles)* subjects before and after disulfiram administration. In the cases of the orally administered diazepam and oxazepam the clearances are nominal.

For chlordiazepoxide, which was administered intravenously, the bioavailability is 100%.

Fig. 1 shows one normal subject's plasma chlordiazepoxide concentrations against time, before and after disulfiram. After disulfiram, the early chlordiazepoxide concentrations are higher and desmethylchlordiazepoxide lower, but after 30 hr, desmethylchlordiazepoxide concentrations become higher than before disulfiram. Analysis of normal chlordiazepoxide subjects indicates significant ($p < 0.01$) effects of disulfiram on chlordiazepoxide (higher) and desmethylchlordiazepoxide (lower) concentrations. In all subjects the time to peak of desmethylchlordiazepoxide (9.0 ± 1.0 hr) was delayed after disulfiram (17.0 ± 2.41 hr). Four of the normal subjects had demoxepam detected in the plasma at 24 hr only after disulfiram and the other 2 had higher desmoxepam concentrations after disulfiram. In the 8 chronic alcoholic subjects on disulfiram, demoxepam was detected.

Table I. *Chlordiazepoxide median subject pharmacokinetic parameters*

Group	*t½(β) (hr)*	*Vd (L)*	*Clearance (ml/min)*
Group*			
1a	10.6	25.6	29.7
1b	19.5	26.0	13.7
1c	24.4	23.2	12.3
p (1a − 1b)†	<0.01	NS‡	<0.05
p (1a − 1c)	<0.01	NS	<0.05
p (1b − 1c)	NS	NS	NS

*Group 1a: 14 normal male subjects before disulfiram administration; Group 1b: 6 normal male subjects after disulfiram administration; Group 1c: 8 male alcoholic subjects after disulfiram administration.

†Significance was determined by the Mann-Whitney U test.

‡Not significantly different.

All kinetic data are summarized in Tables I, II, and III and Fig. 2. In normal subjects, disulfiram prolonged the median chlordiazepoxide t½(β) by 84% and chronic alcoholic patients without serious liver disease have a similar prolonged median t½ (24.4 hr). Disulfiram decreases chlordiazepoxide clearance significantly in both treated groups (Table I).

The pattern of diazepam absorption was not significantly altered by disulfiram; median peak plasma diazepam concentrations occurred at 0.88 hr (566 μg/L) before and at 0.75 hr (548 μg/L) after disulfiram treatment. Disulfiram increased the terminal half-life of diazepam by 37% and reduced its nominal clearance by 41%, without significantly altering the nominal V_d (Table II). The area under the curve of desmethyldiazepam (median, 7.63 μg hr/ml) was significantly ($p < 0.05$) increased (median 8.25 μg hr/ml) after disulfiram administration.

The disposition of oxazepam (Table III) was not altered significantly by disulfiram. The median time to peak oxazepam plasma concentration was 2.0 hr both before and after disulfiram administration, although disulfiram reduced ($p < 0.05$) the median peak concentration from 432 to 358 μg/L (17%). This suggests an effect on oxazepam bioavailability. Five of the 7 subjects had increased oxazepam t½ after disul-

Table II. *Diazepam median subject* pharmacokinetic parameters*

	t½ (β)	*Nominal Vd (L)†*	*Nominal clearance (ml/min)†*
Before disulfiram	30.2	45.3	17.9
After disulfiram	41.4	42.4	10.5
p‡	<0.05	NS§	<0.05

*Six normal and 2 alcoholic subjects.
†Nominal since bioavailability is unknown.
‡Significance was determined by a rank sign test.
§Not significantly different.

Table III. *Oxazepam median subject* pharmacokinetic parameters*

	t½ (β) (hr)	*Nominal Vd (L)†*	*Nominal clearance (ml/min)†*
Before disulfiram	6.05	91.7	205
After disulfiram	7.07	128.7	210
p‡	NS§	NS	NS

*Five normal and 2 alcoholic subjects.
†Nominal since bioavailability is unknown.
‡Significance was determined by a rank sign test.
§Not significantly different.

firam treatment but the median increase in the 7 subjects was only 17% (not significant).

Discussion

These studies demonstrate that disulfiram, markedly changes the pharmacokinetic profiles of chlordiazepoxide and diazepam while only minor effects on oxazepam disposition were demonstrated. The clearance of chlordiazepoxide and the nominal clearance of diazepam are significantly decreased. All subjects received disulfiram in a pattern typical of the actual therapeutic use of the drug. Close agreement between disulfiram-treated normal subjects and alcoholic subjects receiving disulfiram suggests that studies in normal subjects are adequate predictors of disulfiram effects in the population of clinical interest.

The clinical importance of the inhibition of chlordiazepoxide and diazepam biotransformation is not proved by this study but is predictably important because increasing chlordiazepoxide, diazepam, and desmethyldiazepam doses (and, therefore, plasma concentrations[32]) are correlated with increased sedation.[3] Since steady-state drug concentrations are directly dependent on t½ and inversely dependent on clearance, more extensive accumulation of chlordiazepoxide and diazepam will occur during long-term therapy. However, not all patients receiving chlordiazepoxide, diazepam, or chlorazepate (which is rapidly converted to desmethyldiazepam) in combination with disulfiram will have equally important accumulation of parent drug or metabolites since there is considerable interindividual variation in the extent of disulfiram effects. Disulfiram treatment increased chlordiazepoxide t½ between 8.2% and 52.8% in the normal subjects. The median increase in chlordiazepoxide and diazepam t½(β), respectively, of 84% and 37% after disulfiram suggests that mean steady-state and the time to reach steady-state will be proportionately greater and of longer duration. Furthermore, both chlordiazepoxide and diazepam have active metabolites which contribute to the overall clinical effect. Dasberg and associates[4] have suggested that desmethyldiazepam levels above 300 μg/L might cause worsening of certain symptoms of anxiety and this would further complicate the diazepam/disulfiram drug interaction. The importance of the interaction may in part be offset by the development of chronic tolerance to benzodiazepines. Patients

who will have clinically important potentiation cannot be identified in advance. Important sedation would be particularly likely to occur and could be detected by careful observation of the patient upon initiation of chlordiazepoxide or diazepam administration to patients on disulfiram.

The Boston Collaborative Drug Surveillance Program[3] reports drowsiness in 4.5% of 2,623 patients receiving diazepam and 7.2% of the 2,086 patients receiving chlordiazepoxide. Drowsiness is among the most common side effects of disulfiram, and in our study 63% of the normal subjects and 79% of the alcoholic subjects reported drowsiness as a disulfiram side effect. There is, therefore, potential for pharmacodynamic as well as pharmacokinetic interactions to occur between these two drugs.

The pharmacokinetics of the demethylated metabolites of chlordiazepoxide and diazepam after disulfiram are complex because the levels depend on the concentration of the parent compound, the rate constant for conversion (demethylation) to its metabolite, the apparent volume of distribution of the desmethyl metabolite, and the rate of conversion to demoxepam and oxazepam, respectively. Inhibition of desmethylchlordiazepoxide, demoxepam, and desmethyldiazepam metabolism by disulfiram is likely to occur. Supporting evidence includes: (1) many subjects had higher AUC for desmethyl after disulfiram, (2) the time to peak for desmethyl was delayed, and (3) more demoxepam was measurable after disulfiram. In addition, separate studies with suspended, isolated rat hepatocytes[12] and rabbit liver microsomal preparations show inhibition of diazepam and desmethyldiazepam metabolism by in vitro addition of disulfiram.[18] Animal studies suggest that there may be differences in the mechanism of disulfiram after short- and long-term administration.[31] In any case the consequence of the two durations of exposure is inhibition of metabolism.

Inhibition by disulfiram of oxazepam glucuronidation is minimal. By inference from the present data it may be said that disulfiram exhibits independent inhibitory effects of variable magnitudes on both N-demethylation and C-hydroxylation by hepatic mixed-function oxidase as well as glucuronidation of the metabolites so formed.

The small inhibition of elimination of oxazepam by disulfiram is offset by a slightly reduced bioavailability. The net result is a similar AUC and nominal clearance. Since the $t_{1/2}(\beta)$ of oxazepam is considerably less than that of the other two benzodiazepines (Tables I to III) and since it has no important active metabolites, more accurate titration of dosage to clinical response should be possible on both short- and long-term coadministration with disulfiram.

From all the various studies with disulfiram it seems that it inhibits all microsomal metabolic reactions. It is therefore probable that there are many other drugs in which inhibition of drug metabolism by disulfiram is likely to be found and may be important in clinical use.

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References

1. Baekeland F, Lundwann KL: Engaging the alcoholic in treatment and keeping him there. *in* Kissin B, Begleiter H, editors: The biology of alcoholism, vol. 5. Treatment and rehabilitation of the chronic alcoholic. New York. 1977. Plenum Press. pp. 161-195.
2. Baekeland F, Lundwall L, Kissin B: Methods for the treatment of chronic alcoholism: A critical appraisal. *in* Gibbins RJ, Israel Y, Kalant H, Popham RE, Schmidt W, and Smart, RG, editors: Recent advances in alcohol and drug problems. vol. 2. New York. 1975. John Wiley and Sons. pp. 247-327.
3. Boston Collaborative Drug Surveillance Program: Clinical depression of the central nervous system due to diazepam and chlordiazepoxide in relation to cigarette smoking and age. N Engl J Med **288:**277-280, 1973.
4. Dasberg HH, van der Kleijn E, Guelen PJR, van Praag HM: Plasma concentrations of diazepam and of its metabolite N-desmethyldiazepam in relationship to anxiolytic effect. CLIN PHARMACOL THER **15:**473-483, 1974.
5. Dry J, Pradalier A: Intoxication par la phenytoine au cours d'une association therapeutique avec le disulfirame. Therapie **28:**799-802, 1973.
6. Giarman NJ, Flick FH, White JM: Prolongation

of thiopental anesthesia in the mouse by premedication with tetraethylthiuram disulfide (antabuse). Science **114:**35-36, 1951.
7. Giles HG, Fan T, Naranjo CA, Sellers EM: A simple electron capture gas-chromatographic analysis of oxazepam in plasma by determination of its thermal degradation product. Can J Pharm Sci **13:**64-65, 1978.
8. Graham WD, Carmichael GE, Allmark M: The in vivo potentiation of barbiturates by tetraethylthiuram disulfide. J Pharm Pharmacol **3:**497-500, 1951.
9. Greenblatt DJ: Antianxiety agents. *in* Miller RR, Greenblatt DJ, editors: Drug effects in hospitalized patients. New York. 1976. John Wiley and Sons. pp. 193-203.
10. Greenblatt DJ, Shader RI, Koch-Weser J, Franke K: Clinical pharmacokinetics of chlordiazepoxide. *in* Gottschalk LA, Merlis S, editors: Pharmacokinetics of psychoactive drugs: Blood levels and clinical response. Flushing, N. Y. 1976. Spectrum Publications. pp. 127-139.
11. Honjo T, Netter KJ: Inhibition of drug demethylation by disulfiram in vivo and in vitro. Biochem Pharmacol **18:**2681-2683, 1969.
12. Jeejeebhoy KN, Ho J, Greenberg GR, Phillips MJ, Bruce-Robertson A, Sodtke U: Albumin, fibrinogen and transferin synthesis in isolated rat hepatocyte suspensions. A model for the study of plasma protein synthesis. Biochem J **146:** 141-155, 1975.
13. Klotz U, Avant GR, Hoyumpa A, Schenker S, Wilkinson GR: The effects of age and liver disease on the disposition and elimination of diazepam in adult man. J Clin Invest **55:**347-359, 1975.
14. Lang M, Marselos M, Torronen R: Modifications of drug metabolism by disulfiram and diethyldithiocarbamate. I. Mixed-function oxygenase. Chem Biol Interact **15:**267-276, 1976.
15. MacLeod SM, Giles HG, Bengert B, Liu FF, Sellers EM: Age and gender related differences in diazepam pharmacokinetics. J Clin Pharmacol (In press.)
16. Marquardt DW: An algorithm for least-squares estimation of nonlinear parameters. J Soc Ind Appl Math **11:**431-441, 1963.
17. Marselos M, Lang M, Torronen R: Modifications of drug metabolism by disulfiram and diethyldithiocarbamate. II. D-glucuronic acid pathway. Chem Biol Interact **15:**277-287, 1976.
18. Martin PR, Billings BJ, Giles HG, Marshman JA, Sellers EM: Inhibition of drug biotransformation by disulfiram. Can Fed Biol Soc **20:**125, 1977.
19. Notten, WRF, Henderson PT: Effect of disulfiram on the urinary D-glucaric acid excretion and activity of some enzymes involved in drug metabolism in guinea-pig. Arch Int Pharmacodyn Ther **205:**199-208, 1973.
20. Olesen OV: Disulfiramum (Antabuse) as inhibitor of phenytoin metabolism. Acta Pharmacol Toxicol **24:**317-322, 1966.
21. O'Reilly RA: Interaction of sodium warfarin and disulfiram (Antabuse) in man. Ann Intern Med **78:**73-76, 1973.
22. Roberts RK, Wilkinson GR, Branch RA, Schenker S: Effect of age and cirrhosis on the disposition and elimination of chlordiazepoxide (Librium). Gastroenterology **73:**1243, 1977.
23. Sellers EM, Naranjo CA, Giles HG: Fluctuations of plasma diazepam and N-desmethyldiazepam concentrations. Proceedings of the seventh international congress of pharmacology, Paris. July 16-21. 1978, p. 937.
24. Shull HJ, Wilkinson GR, Johnson RF, Schenker S: Normal disposition of oxazepam in acute viral hepatitis and cirrhosis. Ann Intern Med **84:** 420-425, 1976.
25. Stripp B, Greene FE, Gillette JR: Disulfiram impairment of drug metabolism by rat liver microsomes. J Pharmacol Exp Ther **170:**347-354, 1969.
26. Schwartz MA, Postma L: Metabolic N-demethylation of chlordiazepoxide. J Pharm Sci **55:** 1358-1362, 1966.
27. Usanis RA: NLIN—Nonlinear least squares estimation of parameters (Library Services Series Document No. LSR-089-1). Research Triangle Park, N. C. 1972. Triangle Universities Computation Center.
28. Vesell ES, Passananti GT, Glenwright PA: Anomalous results of studies on drug interaction in man. Pharmacology **13:**481-491, 1975.
29. Vesell ES, Passananti GT, Lee CH: Impairment of drug metabolism by disulfiram in man. CLIN PHARMACOL THER **21:**785-792, 1971.
30. Walkenstein SS, Wiser R, Gudmundsen CH, Kimmel HB, Corradino RA: Absorption, metabolism, and excretion of oxazepam and its succinate half-ester. J Pharm Sci **53:**1181-1186, 1964.
31. Zemaitis MA, Greene FE: Impairment of hepatic microsomal drug metabolism in the rat during daily disulfiram administration. Biochem Pharmacol **25:**1355-1360, 1976.
32. Zingales IA: Diazepam metabolism during chronic medication, unbound fraction in plasma, erythrocytes and urine. J Chromatog **75:**55-78, 1973.