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EFFECT OF ETHANOL ON RATES OF ELIMINATION AND METABOLISM OF ZOXAZOLAMINE, HEXOBARBITAL AND WARFARIN SODIUM IN THE RAT*

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Abstract—Male rats were fed single doses of ethanol or isocaloric quantities of glucose, in solution, by stomach tube following a 6 hr fast and were injected with zoxazolamine, warfarin sodium or hexobarbital 2 or 17 hr later. Other rats were pair-fed a nutritionally adequate synthetic diet containing ethanol or isocaloric quantities of sucrose for 7–11 days, and were treated with drugs following a 17 hr fast. Plasma concentrations of each drug were determined at various intervals of time following the injections. Administration of one dose of ethanol significantly increased the rates of elimination of zoxazolamine and of hexobarbital from the circulation 17 hr later, but the rate of warfarin elimination was unchanged. Chronic ethanol feeding also increased the rate of zoxazolamine elimination; but this increase was not greater than that following acute ethanol feeding; warfarin elimination was again not altered. Rates of metabolism of the drugs by liver microsomes *in vitro* 17 hr after a single dose of ethanol or glucose mirrored the effects on elimination *in vivo*; they were significantly increased in the alcohol-treated rats in the case of zoxazolamine and hexobarbital, but not changed in the case of warfarin. The rates of elimination of zoxazolamine and warfarin from the circulation were significantly reduced in rats fed ethanol 2 hr before. It is concluded that acute ethanol intake may increase the rates of elimination of some drugs when the alcohol is no longer present, as a consequence of microsomal enzyme induction, and may decrease rates of drug elimination when alcohol is present *in vivo*, probably as a result of inhibition by ethanol of microsomal drug-metabolizing enzymes.

THE IMPORTANCE of drug interactions is being increasingly recognized. Ethanol is of particular interest in this respect because of the high consumption of alcoholic beverages, the high incidence of alcoholism and the frequency with which alcohol† is consumed by people who are taking other medications.[1]

It is well known that a number of drugs and chemical agents are able to induce hepatic microsomal enzymes that are responsible for the bio-transformation of other drugs.[2] Generally, the former are themselves metabolized by microsomal enzymes of liver. When however, drugs metabolized by the same microsomal enzyme system are simultaneously available to the latter, they may inhibit each other's metabolism.[3] Thus, when drug elimination *in vivo* is largely dependent on bio-transformation, one drug may increase or decrease respectively, the rate of elimination of another.

It has been found recently that ethanol is not only oxidized to acetaldehyde by hepatic alcohol dehydrogenase, a soluble enzyme present in cell cytoplasm, but also to some degree *in vitro*, by liver microsomes.[4–7] The nature of the enzymes involved

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† The words *ethanol* and *alcohol* are used interchangeably in this report.

in this oxidation has been subject to dispute;[4–6,8–10] so has the significance of such oxidation in the overall metabolism of ethanol *in vivo*.[5,6,8,9,11–13] Nevertheless, it has been reported that chronic administration of alcohol increases the activities of liver microsomal enzymes responsible for the oxidation of ethanol,[5] for the hydroxylation of aniline,[14,15] pentobarbital[15] and 3,4-benzpyrene,[15] and for the reduction of *p*-nitrobenzoic acid,[14] in rats, and for the hydroxylation of pentobarbital[15] in man. In addition, chronic consumption of alcohol has been found to increase the rate of elimination of ethanol,[16,17] pentobarbital[17,18] meprobamate[17] and tolbutamide[19] from the circulation in man and of meprobamate[17] from that in the rat.

The present investigation was carried out to determine: (a) the effect of administering a single dose of ethanol on the rates of elimination of certain drugs that are almost completely dependent on hepatic microsomal enzymatic metabolism for their elimination and, (b) whether any observed alteration in rates of elimination *in vivo* promoted by alcohol could be correlated with changes in hepatic microsomal metabolism of these agents *in vitro*.

MATERIALS AND METHODS

Plasma drug levels after acute ethanol administration. Male Sprague–Dawley rats, 220–260 g in weight, maintained on Agway rat and mouse diet pellets, were deprived of food but permitted water *ad libitum* for 6 hr before being fed a single dose of ethanol or glucose by stomach tube. Ethanol was administered in a dose of 4·71 g/kg of body weight as a 50% solution, v/v, and glucose was given to the controls in isocaloric quantities in the same volumes of solution as the alcohol. The test drugs were administered 17 hr later. Rats were lightly anesthetized with ether before i.v. administration. Zoxazolamine,* in an acidified solution containing 5 mg/ml, was injected into the femoral vein in a dose of 25 mg/kg; warfarin sodium, in a solution containing 3 mg/ml, was injected in a dose of either 1·5 or 3 mg/kg, similarly; and hexobarbital, in alkaline solution, was injected i.p. in a dose of 100 mg/kg. The animals were anesthetized with ether at various intervals of time after the injections, and samples of blood were withdrawn from their venae cavae and placed into heparinized centrifuge tubes. One sample of blood was removed from each rat, five to seven control and five to seven ethanol-fed rats being used in each experiment. After centrifugation of the blood, the plasmas were analyzed for unchanged drug.

Zoxazolamine, hexobarbital and warfarin were determined by the methods of Burns *et al.*,[2] Cooper and Brodie[21] and O'Reilly *et al.*,[22] respectively.

In one acute experiment, two groups of rats were given doses of ethanol or glucose, as described above, on two consecutive days, fasted for 17 hr, then treated with zoxazolamine as described. In another series of experiments, rats were fasted for 17 hr then given single doses of ethanol or glucose. Two hr later the animals were injected with zoxazolamine, hexobarbital or warfarin sodium as described. Samples of blood were withdrawn at various intervals of time thereafter and analyzed for unchanged drug.

Plasma drug levels after chronic ethanol administration. Groups of male rats, 200–220 g in weight, were pair-fed nutritionally adequate synthetic diets containing ethanol or isocaloric quantities of sucrose in place of the ethanol for periods of 7–11 days. The diets, offered as aqueous suspensions in Richter drinking tubes, were modifications of

* Zoxazolamine was kindly supplied to us by McNeil Laboratories, Inc.

those employed by De Carli and Lieber,[23] and supported growth of the animals. The composition of the control diet was as follows: 102 g of diet contained 45·90 g sucrose, 2·20 g vitamin mix in glucose (Diet Fortification Mix, Nutritional Biochemicals Co.), 22·30 g Wesson oil, 22·80 g enzymatically prepared casein hydrolysate (Nutritional Biochemicals), 4·80 g Hegsted salt mix and 4·00 g tragacanth. Each unit of the 102 g diet was suspended in water to give a total volume of 483 ml, which yielded 1 calorie/ml. The concentrations of ethanol in the diets of the experimental rats were such that ethanol contributed 21, 28 and 35 per cent of the total calories on days 1 and 2, 3 and 4, and 5, respectively. Food was removed from the rats at the end of the experimental feeding period and the test drugs were injected 17 hr later. The plasmas, obtained at various intervals of time after the injections, were analyzed for unchanged drug. Concentrations of drug in plasma were plotted against time on semi-log paper. Half-lives ($T_{1/2}$) were obtained from the straight line plots and elimination constants calculated from the half-lives ($k_e = 0{\cdot}693/T_{1/2}$).

Liver microsomal drug metabolism. Male rats, 220–250 g, were fasted for 6 hr then given single doses of ethanol or glucose orally, as described, and killed 17 hr thereafter by decapitation. The livers were rapidly removed, washed with ice-cold homogenizing medium, blotted with filter paper, weighed and sectioned. Small sections, removed for protein determination were weighed and frozen. The livers were then homogenized in ice-cold homogenizing medium composed of 0·25 M sucrose in 0·05 M phosphate buffer at pH 7·4, by means of a Lourdes blendor running for 1 min at 50 V.

The homogenates were centrifuged at 20,000 *g* at 4° for 30 min, and the supernatants obtained centrifuged at 100,000 *g* for 1 hr in a Spinco model L ultracentrifuge. The surfaces of the pellets obtained after discard of the supernatants were washed with small quantities of cold homogenizing medium and the tubes drained. In the first experiments with zoxazolamine, the microsomal pellets were resuspended in homogenizing medium and centrifuged at 100,000 *g* a second time. In subsequent experiments the extra washing was omitted, since it was determined that the results were not significantly affected by the added washing step. All of the microsomes obtained from each rat liver were resuspended in ice-cold homogenizing fluid and make up to a definite final volume.

Aliquots of the microsomal suspensions were incubated in the presence of zoxazolamine, hexobarbital or warfarin to determine the rates of metabolism of these drugs *in vitro*. Two-tenths of 1 ml of each microsomal suspension was pipetted into each of several 60-ml glass-stoppered tubes, which had been placed in a 37° water bath 5 min before, and which contained the following: 0·4 ml of 0·1 M KH_2PO_4–K_2HPO_4 buffer at pH 7·4 in 0·4 M KCl solution, 0·1 ml of 0·05 M $MgCl_2$ solution, 0·1 ml of 0·05 M glucose 6-phosphate solution, 0·1 ml of TPN solution containing 1 mg TPN, 0·1 ml of glucose 6-phosphate dehydrogenase solution possessing 42 units of enzyme activity/ml, and 0·5 ml of a solution of the drug. The total volume was 1·5 ml. The quantities of drug added to each tube were: 50 μg zoxazolamine, 60 μg warfarin sodium or 60 μg hexobarbital. After addition of the microsomes, the tubes were incubated for various periods of time, after which the reactions were stopped. This was accomplished by adding 1·0 ml of 0·5 N NaOH to the zoxazolamine-containing tubes, 1·5 ml of 1 N HCl to the warfarin tubes, and 1·0 ml of pH 5·5 phosphate buffer to the tubes containing hexobarbital, which were then immediately frozen by immersion in an acetone–dry ice bath and kept frozen until analysis.

Analyses of incubation media for drug concentrations were carried out as described for plasma. For each liver, the concentration of drug was plotted against time of incubation on semi-log paper, and the metabolism rate constants, k_m, were calculated from the straight lines obtained ($k_m = 2{\cdot}303/t \log C_0/C_t$, where C_0 is the concentration of unchanged drug at zero time and C_t the concentration at time t).

Aliquots of the microsomal suspensions as well as sections of wet liver tissue were analyzed for protein content. Five ml of 10% trichloroacetic acid (TCA) was added to the sample of microsomal suspension or to the section of intact liver. The latter was then homogenized. After centrifugation, the protein residues were washed with 5% TCA at room temperatures, with TCA at 90° for 30 min, with 3:1 alcohol-ether and with ether. The proteins were then dried, dissolved in 10 ml of 3% NaOH at 37° and 1-ml aliquots of these solutions assayed for protein content by the Biuret method according to Gornall *et al.*,[24] the optical density being read at 540 nm. Correction was made for the slightly yellow color of the NaOH solution after protein dissolution by determination of the optical density of aliquots of the solution at 540 nm and subtracting the values obtained from those observed after addition of Biuret reagent.

Rates of microsomal metabolism of each drug were expressed in terms of the rate constant, k_m, per total liver wet weight, per gram of liver protein and per milligram of microsomal protein.

RESULTS

Rates of elimination from plasma—zoxazolamine. Linear relationships were obtained when the logarithms of plasma zoxazolamine concentrations were plotted against time over a 3-hr period after i.v. injection of the drug, showing that first-order elimination kinetics prevailed *in vivo* and there was a uniformity of effect in different

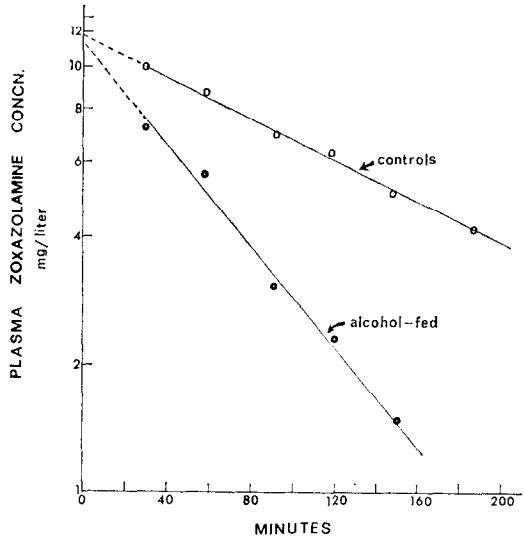

FIG. 1. Effect of ethanol on the rate of elimination of zoxazolamine *in vivo* from the plasma of rats. Rats were injected i.v. with 25 mg/kg zoxazolamine, 17 hr after they had been given one dose of ethanol (4·71 g/kg) or an isocaloric quantity of glucose by stomach tube and 23 hr after food had been withdrawn. Samples of blood were withdrawn from the rats after various intervals of time following zoxazolamine injection and analyzed for unchanged drug.

rats. The variation in data between groups of rats in experiments performed on different days was greater than that among the rats in any one experiment performed on the same day for reasons that are not clear. In any given experiment the deviations from linearity of the individual points in the plots were remarkably small. The plot of a typical experiment in which ethanol or glucose was administered 17 hr before zoxazolamine is shown in Fig. 1 and the results of the experiments are given in Table 1. At the time of zoxazolamine administration, all of the ethanol had been eliminated by the rats according to the rate of ethanol metabolism[25] or elimination[26] in these animals. It

TABLE 1. EFFECT OF ONE DOSE OF ETHANOL ON THE RATE OF ELIMINATION OF DRUGS FROM THE CIRCULATION OF RATS 17 HR LATER*

Drug	Expt.	$k_e \times 10^3$† Glucose	Ethanol	P
Zoxazolamine	1	4·91 (6)‡		
	2	4·34 (6)		
	3	5·50 (5)	13·9 (5)	
	4	4·98 (6)	7·77 (6)	
	5	7·13 (6)	12·6 (6)	
	6	4·01 (7)	7·30 (7)	
	7	5·67 (5)	11·3 (5)	
	8	3·82 (6)	11·4 (6)	
	9	4·91 (6)	12·8 (6)	
		5·03	11·01	
		± 0·334§	± 0·958	<0·01
Hexobarbital	1	25·7 (7)		
	2	25·6 (4)		
	3	28·6 (5)	45·3 (5)	
	4	27·3 (6)	32·7 (6)	
	5	21·0 (5)	35·0 (5)	
	6	21·0 (5)	35·5 (5)	
		24·9	37·1	
		± 1·30	± 2·79	<0·01
Warfarin	1	1·94 (7)	1·93 (8)	
	2	2·56 (6)	2·59 (6)	
	3	1·74 (5)	2·09 (6)	
	4	2·68 (6)	1·78 (6)	
	5	1·49 (6)	1·42 (6)	
		2·08	1·92	
		± 0·232	± 0·192	>0·1

* Ethanol was given orally in a dose of 4·71 g/kg and glucose was administered in isocaloric quantities in the same fashion. Zoxazolamine, hexobarbital and warfarin sodium were administered in doses of 25 mg/kg (i.v.), 100 mg/kg (i.p.) and 3 mg/kg (i.v.), respectively, 17 hr after ethanol or glucose. Blood samples were withdrawn at approximately 30-min intervals over a period of 3 hr after zoxazolamine injection, at 10–12 min intervals over a period of 1 hr, starting at 30 min after injection when hexobarbital was given, and at hourly intervals over a period of 6 hr after warfarin administration. Plasmas were analyzed for unchanged drug and k_e values calculated from half-lives obtained from plots of plasma concentration vs. time.

† Elimination rate constant $k_e = 0{\cdot}693/T_{1/2}$.

‡ Numbers in parentheses refer to number of rats.

§ Mean ± standard error of mean.

was observed that rats pretreated with only one dose of ethanol eliminated zoxazolamine from their circulations about twice as rapidly as did the glucose-pretreated controls.

To determine whether repeated administration of alcohol would enhance the difference in rate of zoxazolamine elimination between alcohol-treated and control rats, the animals were given alcohol or glucose by stomach tube on two consecutive days and then injected with zoxazolamine after a 17-hr fast. The results were the same as when single doses were administered. Rats were then pair-fed synthetic diets containing ethanol or sucrose as described in Methods. The animals on the diet containing ethanol consumed between 9·63 and 10·97 g ethanol/kg of body weight/day. The ethanol-fed rats gained weight at the same rate as did the controls during the 7-day experimental feeding period. It was found that prolonged intake of ethanol did not result in a greater increase in the rate of zoxazolamine elimination from the circulation than that induced by a single dose of alcohol (Table 2).

TABLE 2. EFFECT OF CHRONIC ADMINISTRATION OF ETHANOL ON THE RATES OF ELIMINATION OF ZOXAZOLAMINE AND WARFARIN SODIUM FROM THE CIRCULATION OF RATS*

Drug	Expt.	Days on diet	$k_e \times 10^3$† Sucrose	Ethanol
Zoxazolamine	1	7	5·82 (10)‡	8·64 (10)
Warfarin	1	10	2·44 (5)	2·47 (5)
	2	11	3·03 (5)	3·22 (5)

* Rats were pair-fed synthetic diets containing ethanol, or sucrose isocalorically substituted for the ethanol. Zoxazolamine or warfarin sodium was administered i.v. in doses of 25 mg/kg and 3 mg/kg, respectively, 17 hr after food was removed from the rats following the last day of pair-feeding. Blood samples were withdrawn at approximately 30-min intervals over a period of 3 hr after zoxazolamine injection and at hourly intervals over a period of 6 hr after warfarin administration. Plasmas were analyzed for unchanged drug and k_e values calculated from the half-lives obtained from plots of plasma concentration vs. time.

† Elimination rate constant $k_e = 0{\cdot}693/T_{1/2}$.

‡ Numbers in parentheses refer to number of rats.

It was observed in all of the above experiments, that when the plots of zoxazolamine concentration vs. time were extrapolated to zero time, the same initial plasma concentrations of the drug were obtained for ethanol-fed and control rats, indicating that plasma volumes were the same in both groups of animals at the time of zoxazolamine injection.

It was thought that if the increased rate of elimination of zoxazolamine following previous administration of alcohol were due to induction by alcohol of the microsomal enzymes responsible for zoxazolamine metabolism, then the presence of alcohol in relatively high concentration might inhibit zoxazolamine metabolism, and this would be reflected by a decreased rate of elimination. To test this hypothesis, zoxazolamine was injected into rats 2 hr after the oral administration of ethanol or glucose

TABLE 3. EFFECT OF ONE DOSE OF ETHANOL ON RATES OF ELIMINATION OF ZOXAZOLAMINE AND WARFARIN SODIUM FROM THE CIRCULATION OF RATS 2 HR LATER*

Drug	Expt.	$k_e \times 10^3$† Glucose	Ethanol	P
Zoxazolamine	1	5·59 (5)‡	2·72 (5)	
	2	4·99 (6)	1·24 (6)	
	3	5·19 (6)	2·51 (6)	
		5·26§	2·16	
		± 0·176	± 0·462	<0.01
Warfarin	1	0·984 (6)	0·625 (6)	
	2	1·47 (5)	0·763 (5)	
	3	1·52 (6)	0·550 (6)	
		1·33	0·646	
		± 0·171	± 0·062	<0·01

* Ethanol was given orally in a dose of 4·71 g/kg and glucose was administered in isocaloric quantities in the same fashion. Zoxazolamine and warfarin sodium were administered i.v. in doses of 25 and 3 mg/kg, respectively, 2 hr after ethanol or glucose administration. Blood samples were withdrawn at approximately 30-min intervals over a period of 3 hr after zoxazolamine injection and at hourly intervals over a period of 6 hr after warfarin injection. Plasmas were analyzed for unchanged drug and k_e values calculated from the half-lives obtained from plots of plasma concentration vs. time.

† Elimination rate constant $k_e = 0{\cdot}693/T_{1/2}$.

‡ Numbers in parentheses refer to number of rats.

§ Mean ± standard error of mean.

following a 17-hr fast. The results, given in Table 3, show that when alcohol is present *in vivo* after a single dose, zoxazolamine is eliminated from the circulation at only half the rate occurring in glucose-fed control rats.

Hexobarbital. After hexobarbital was injected i.p. 17 hr after the administration of a single dose of ethanol or glucose, a half-hour was allowed to elapse for absorption and equilibration of the drug in tissues. Samples of blood were withdrawn after various intervals of time during the next hour. When plasma hexobarbital concentrations were plotted against time on semi-log paper, first-order kinetics were again observed. The results given in Table 1 show that the rate of elimination of hexobarbital from the plasma was about 50 per cent greater in alcohol-treated rats than in controls. Experiments in which hexobarbital was administered shortly after ethanol administration were not successful because of the additive effects of the two drugs on the central nervous system and the consequent high mortality rate with the dose of hexobarbital employed.

Warfarin sodium. In contrast to the finding with zoxazolamine and hexobarbital, rats given alcohol 17 hr before the i.v. injection of warfarin did not eliminate warfarin from their circulation more rapidly than did the glucose-treated controls over a period of 6 hr following warfarin injection. The results are given in Table 1.

To determine whether repeated administration of ethanol would have an effect, two experiments were performed in which rats were pair-fed synthetic diets containing ethanol or sucrose isocalorically substituted for ethanol for periods of 10 and 11 days. Again, as shown in Table 2, no significant difference was observed in the rate of

TABLE 4. EFFECT OF CONCENTRATION OF MICROSOMES *in vitro* ON RATE OF METABOLISM OF HEXOBARBITAL*

Ml microsomal suspension in incubation flask	$k_m \times 10^3$†	Ratio
0·1	13·25	1·00
0·2	26·53	2·00
0·3	39·97	3·02
0·4	57·49	4·34

* Microsomes prepared from the liver of a normal rat were suspended in 8·0 ml of homogenizing fluid. Aliquots of the suspension were added to the medium in which hexobarbital was present and incubation was carried out for 12·5 min. The total volume of incubation medium in all cases was 1·5 ml. Concentrations of hexobarbital in the incubation fluid, determined at 0 time and after 2, 4, 6, 8, 10 and 12·5 min of incubation, were plotted against time on semi-log paper and k_m values were calculated from these plots.

† Metabolism rate constant $k_m = 2{\cdot}303/t \log C_0/C_t$.

elimination of warfarin from the circulation when the latter was injected 17 hr before food had been removed from the animals.

The rate of elimination of warfarin was 50 per cent lower in alcohol-fed rats than in controls, however, when warfarin was administered 2 hr after feeding a single dose of ethanol or glucose following a 17-hr fast, as shown by the data in Table 3.

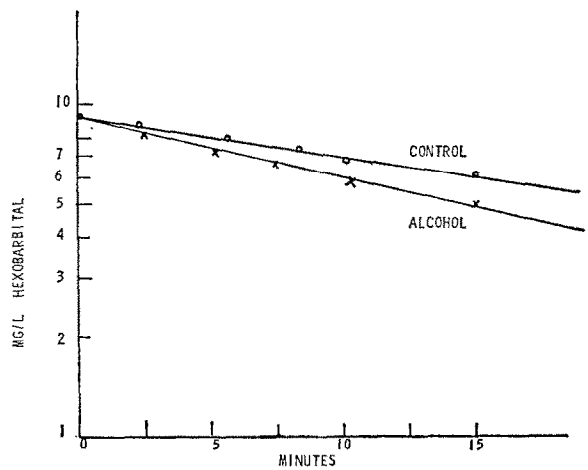

FIG. 2. Effect of ethanol on the rate of metabolism of hexobarbital *in vitro* by rat liver microsomes. Microsomes were isolated from livers of rats 17 hr after the animals received one dose (4·71 g/kg) of ethanol or an isocaloric quantity of glucose by stomach tube. The incubation medium (1·5 ml) contained 0·2 ml of microsomal suspension representing a fixed fraction of the total liver microsomes, 0·027 M phosphate buffer at pH 7·4, 0·011 M KCl, 0·0033 M $MgCl_2$, 0·0033 M glucose 6-phosphate, 1 mg NADP, 4·2 units of glucose 6-phosphate dehydrogenase activity and 60 μg hexobarbital.

Rate of hepatic microsomal metabolism. The rate of microsomal drug metabolism was found to be proportional to the concentration of microsomes in the incubation medium, as illustrated for hexobarbital in Table 4, and followed first-order kinetics during the incubation periods employed, as illustrated for hexobarbital in Fig. 2.

Zoxazolamine. Microsomes obtained from the livers of rats given a single dose of alcohol or glucose 17 hr before, then fasted, were incubated with zoxazolamine in an

TABLE 5. EFFECT OF ONE DOSE OF ETHANOL *in vitro* ON THE RATE OF METABOLISM OF DRUGS BY RAT LIVER MICROSOMES *in vitro* 17 HR LATER*

		Microsomal $k_m \times 10^3$†					
		Per total liver		Per gram of liver protein		Per milligram of microsomal protein	
Drug	Expt.	Glucose	Ethanol	Glucose	Ethanol	Glucose	Ethanol
Zoxazolamine	1	150	495	67·0	240	0·998	4·09
	2	156	351	73·6	173	0·857	2·30
	3	135	252	59·5	113	0·595	1·24
	4	161	419	85·2	206	1·26	3·04
	Mean	151	379	71·3	183	0·928	2·68
	± S. E.	± 5·0	± 59·7	± 5·45	± 27·1	± 0·139	± 0·603
		P < 0·01		P < 0·01		P < 0·05	
Warfarin	1	13·7	16·4	9·70	9·65	0·143	0·137
	2	11·7	17·3	7·74	10·10	0·082	0·112
	3	16·6	22·5	9·03	11·3	0·115	0·107
	4	15·6	22·3	11·4	13·6	0·125	0·160
	5	11·6	5·73	7·67	3·51	0·079	0·040
	Mean	13·8	16·9	9·11	9·63	0·109	0·111
	± S. E.	± 1·01	± 3·05	± 0·691	± 1·677	± 0·012	± 0·020
		P > 0·1		P > 0·1		P > 0·1	
Hexobarbital	1	1112	1622	577	970	6·70	9·20
	2	1476	2361	891	1311	8·10	12·30
	3	1229	1630	744	828	7·41	8·29
	4	1676	2221	937	1139	9·52	13·41
	5	1707	2070	918	1139	8·57	12·18
	6	1462	2910	898	1501	8·20	13·59
	Mean	1444	2136	828	1148	8·08	11·50
	± S. E.	96·8	± 198·5	± 57·4	± 97·5	± 0·395	± 0·908
		P < 0·01		P < 0·02		P < 0·01	

* Microsomes prepared from livers of rats given a single dose of ethanol or glucose orally 17 hr before were incubated in phosphate buffer at pH 7·4 containing KCl, $MgCl_2$, TPN, glucose 6-phosphate, glucose 6-phosphate dehydrogenase and a drug, as indicated in Methods. Incubations were carried out in series of tubes for 30, 90 and 15 min when the drug present was zoxazolamine, warfarin and hexobarbital respectively. Tubes were removed periodically during the incubations and their contents were analyzed for unchanged drug. Metabolism rate constants were obtained from plots of log drug concentration vs. time, which showed first-order kinetics during the periods of incubation ($k_m = 2{\cdot}303/t \log C_0/C_t$ where C_0 = concn of drug at zero time and C_t = concn of drug after t minutes of incubation). These constants, expressing rates for given known concentrations of microsomes in the incubation media, were recalculated to give rate constants per: (a) total liver microsomes, (b) microsomes present in a quantity of liver containing 1 g protein and, (c) microsomes containing 1 mg protein.

† Metabolism rate constant = k_m.

NADPH-generating system, as described under Methods, for a period of 30 min. The microsomes from one alcohol-treated and one control rat were incubated at the same time in each experiment. One tube containing the incubation mix was removed from the incubator at zero time and one every 5 min thereafter, the contents of the tubes were later analyzed for unchanged zoxazolamine. The results of the experiments given in Table 5 indicate that hepatic microsomes from rats pretreated with one dose of ethanol metabolized zoxazolamine approximately 2·5 times as rapidly as did microsomes from glucose-treated controls.

Hexobarbital. Microsomes from rats fed a single dose of ethanol 17 hr before metabolized hexobarbital 40–50 per cent more rapidly than did microsomes from control animals during a 15-min incubation period *in vitro* (Table 5).

Warfarin sodium. In contrast to the effects of ethanol pretreatment on the rate of microsomal metabolism of zoxazolamine and hexobarbital, no alteration in the rate of metabolism of warfarin was observed when rats were fed a single dose of ethanol instead of glucose 17 hr before the determinations were made (Table 5). Incubations were carried out for a period of 1·5 hr because of the slow rate of bio-transformation of warfarin. During this time, there was a linear relationship between the logarithm of warfarin concentration and the time of incubation.

DISCUSSION

The results obtained with zoxazolamine and hexobarbital suggest that the acute intake of alcohol may enhance the rate of elimination of a drug taken subsequently, at a time when ethanol is no longer present in the body. Other investigators have reported that chronic ingestion of alcohol increases the rate of elimination of a number of other drugs in man and the rat.[16–19] However, chronic consumption of alcohol need not be more effective in this respect than acute alcohol intake, as shown by the failure of chronic alcohol feeding of rats to increase the rate of zoxazolamine elimination beyond that promoted by a single dose of alcohol, and the inability of chronic as well as acute ethanol administration to affect the rate of warfarin elimination when alcohol was no longer present *in vivo*.

That little if any alcohol remains in the rat 17 hr after administration of a dose of 4·71 g/kg is indicated by data such as those of Lester *et al.*,[25] who found that ethanol is oxidized in the rat at a rate of 300 mg/kg/hr (5·1 g/kg/17 hr) and by the observation of Klaassen[26] that blood alcohol levels in 150–200 g male rats were reduced at a rate of 44 mg/100 ml/hr so that alcohol was eliminated from the blood in about 9 hr after an oral dose of 3 g/kg.

It appears likely that the enhanced elimination of zoxazolamine and hexobarbital that we observed 17 hr after one dose of alcohol was due to an induction by the alcohol of the liver microsomal enzymes responsible for the metabolism of these drugs, since elimination of zoxazolamine and hexobarbital is accomplished almost entirely by such metabolism. The results of our experiments with liver microsomes add further support to this hypothesis. Thus 17 hr after alcohol or glucose administration it was observed that microsomal metabolism of zoxazolamine and hexobarbital *in vitro* occurred at a significantly greater rate in alcohol-fed rats than in glucose-fed controls. In the case of warfarin, the failure of alcohol to affect its rate of elimination *in vivo* was mirrored by the lack of effect on its rate of microsomal metabolism *in vitro*.

However, the possibility that alcohol activated rather than induced the microsomal enzymes involved cannot be eliminated, although the finding that chronic ethanol administration increased the concentration of liver microsomal protein as well as of cytochrome P-450 in rats[14] renders this unlikely.

It has been found that chronic alcohol administration also enhances the rate of microsomal drug metabolism in man and the rat. Thus the activities of microsomal enzymes carrying out the oxidation of ethanol,[5] aniline[14,15] and pentobarbital[15] and the reduction of *p*-nitrobenzoic acid[14] in rats, and the oxidation of pentobarbital[15] in man are significantly increased after chronic alcohol consumption.

The inductive effect of alcohol does not occur in the case of all drugs metabolized by hepatic microsomal enzymes, as shown by the failure of acute ethanol administration to increase the rate of elimination and the hepatic microsomal metabolism of warfarin in rats in our experiments, and the inability of chronic alcohol ingestion to alter benzpyrene hydroxylase activity in the liver of man.[15] Remmer and Schuppel[27] first reported that chronic alcohol administration to rats had no effect on the rate of hexobarbital metabolism *in vitro* by microsomal preparations from the livers of these animals, but later observed that such preparations did metabolize ethanol more rapidly than did similar preparations from controls not fed alcohol.[28] Kalent *et al.*[29] in contrast to the findings of Rubin and Lieber[15] with rat liver microsomes, observed no increase or even a smaller decrease in the rate of uptake of pentobarbital by slices of liver from rats fed ethanol chronically, in comparison with such slices from pair-fed controls receiving sucrose in place of alcohol. Perhaps rate of transport of the drug to the sites of metabolism was a limiting factor in the slice studies.

If ethanol is indeed oxidized by a hepatic microsomal enzyme system *in vivo* to some extent and also induces hepatic microsomal enzymes responsible for the metabolism of other drugs, then the presence of alcohol *in vivo* might be expected to retard the metabolism and elimination of some drugs metabolized by the same enzyme system, as a result of competition for the enzymes.[3] It was indeed found that when zoxazolamine or warfarin was administered 2 hr after a single dose of ethanol or glucose, the rates of elimination of these drugs from the circulation of rats, *in vivo*, were significantly slower in the ethanol-fed than in the control rats. Because of the high mortality occurring when alcohol and hexobarbital were administered 2 hr apart in the same doses as when they were given 17 hr apart, similar experiments with hexobarbital were abandoned. Our observations of decreased rates of elimination of warfarin and zoxazolamine in the presence of ethanol are in accord with the report of Rubin *et al.*,[3] that acute ethanol ingestion by humans significantly retarded the disappearance of pentobarbital and of meprobamate from the circulation. The same was true for pentobarbital in the case of rats. It was also found by these investigators that the addition of ethanol *in vitro* to rat liver microsomes inhibited aniline, pentobarbital and benzpyrene hydroxylase and aminopyrine and ethylmorphine demethylase activities. It is not too surprising that we found the rate of elimination of warfarin to be inhibited 2 hr after ethanol administration but not enhanced 17 hr later, since a few other agents have been reported to induce liver microsomal drug-metabolizing enzymes after a sufficient interval of time has elapsed following a single administration, but not to inhibit such enzymes shortly after they are administered.[2] The mechanisms of induction and inhibition may follow different paths or involve different sensitivities.

Taken together, these experiments suggest that acute as well as chronic alcohol

consumption may accelerate the rate of hepatic microsomal bio-transformations of a number of drugs and hence their rates of elimination, when little or no alcohol remains in the body, while the presence of alcohol *in vivo* may decrease the rates of elimination of some drugs as a consequence of inhibition of hepatic microsomal metabolism of the latter by ethanol. Clearly, alcohol intake may alter the pharmacological effectiveness of medications taken simultaneously with the alcohol or subsequently.

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