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MECHANISMS IN THE POTENTIATION AND INHIBITION OF PHARMACOLOGICAL ACTIONS OF HEXOBARBITAL AND ZOXAZOLAMINE BY GLYCOFUROL

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Abstract—When used as a solvent, glycofurol increased the sleeping and paralysis times of hexobarbital and zoxazolamine. These effects were correlated with increases in rates of absorption, decreases in volumes of distribution, decreases in average total body clearances and with *in vitro* inhibition of hepatic microsomal metabolism. In contrast, when glycofurol was administered in repeated doses (1 ml/kg, i.p., twice a day for 4 days), it decreased the sleeping and paralysis times of hexobarbital and zoxazolamine, probably as the result of their increased hepatic metabolism. Repeated doses of glycofurol also increased significantly cytochrome P-450, cytochrome b_5, NADPH cytochrome *c* reductase, and activities of ethylmorphine *N*-demethylase and benzopyrene hydroxylase, in liver microsomes.

Although water is considered the universal solvent and always the solvent of choice, its use as a solvent is sometimes not possible because drugs have limited solubility and undergo hydrolytic reactions [1]. Fixed oils are not always suitable solvents because they have been known to cause undesirable local tissue reactions, such as foreign body granulomas and occasionally nerve injury. As substitutes for fixed oils or water, some synthetic nonaqueous liquids are used as solvents in pharmaceutical preparations for parenteral administration. Because glycofurol (tetrahydrofurfuryl alcohol polyethylene glycol ether) possesses excellent properties as a solvent, it has been proposed as a vehicle for some water-insoluble medicinal compounds. Glycofurol is a mixture of 2-[(tetrahydrofurfuryl)oxy]ethanol and 2-{2[tetrahydrofurfuryl)oxy]ethoxy}-ethanol (Fig. 1).

The intravenous LD_{50} for glycofurol in the mouse is 3.78 g/kg or 3.5 ml/kg [1] and in rats (route not specified) is above 5 ml/kg †. Spiegelberg *et al.* [2] reported that glycofurol and propyleneglycol are tolerated equally well. In addition, glycofurol stabilizes solutions containing 3-pyridinecarbinol, acetylcholine chloride or pyridostigmine bromide, which are not stable in propyleneglycol. For these reasons, formulations containing glycofurol have been used commercially as vehicles for diazepam and its derivatives in several countries [3].

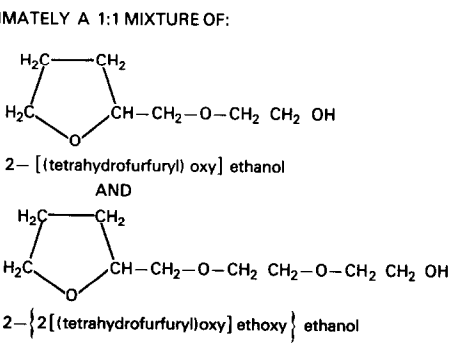

Fig. 1. Structure of glycofurol 75.

The pharmacological actions of glycofurol and its effects on the action of other drugs have not been studied extensively. This paper reports the influence of treatment of rats with glycofurol on sleeping and paralysis times and total body clearances of hexobarbital and zoxazolamine, as well as the relation to some effects on hepatic microsomes.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats weighing 150–200 g were used for all experiments. Commercial rat chow was available until 14–18 hr before the rats were killed. Tap water was available for drinking at all times. All animals were housed under conditions of controlled temperature and lighting.

Chemicals. Nicotinamide adenine dinucleotide phosphate, glucose 6-phosphate dehydrogenase, and cytochrome *c* were purchased from Sigma Chemical Co., St. Louis, MO. Zoxazolamine was purchased from Aldrich Chemical Co., Milwaukee, WI, and hexobarbital sodium was obtained from Winthrop Lab., New York, NY. Glycofurol 75 was kindly supplied by the developer (Hoffmann-La Roche, Nutley, NJ) through Dr. W. E. Scott. All other chemicals were of the highest grade available commercially.

Treatment. Glycofurol was administered by i.p. injection in the doses indicated in Results.

In vivo *metabolism.* Hexobarbital sleeping times

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† W. E. Scott, personal communication.

and zoxazolamine paralysis times were measured as the time between loss and recovery of the righting reflex after i.p. injection of 150 mg/kg of hexobarbital or 50 mg/kg of zoxazolamine. Plasma levels of hexobarbital were measured by the spectrophotometric assay of Cooper and Brodie [4]. Zoxazolamine levels were measured as described by Conney *et al.* [5].

Pharmacokinetic analysis. After i.v. administration of hexobarbital (75 mg/kg) or zoxazolamine (25 mg/kg), plasma drug levels were fitted to the curve described by the following biexponential equation:

$$C = A_0 e^{-\alpha t} + B_0 e^{-\beta t}$$

in which A_0 and B_0 are the hypothetical intercepts with the ordinate axis for the α- and β-phases of the concentration of drug in plasma, and α and β are hybrid rate constants of these phases.

The half-lives of the α-phase [$T_{1/2}(\alpha)$] and the β-phase [$T_{1/2}(\beta)$] were calculated from the semilogarithmic plots by the method of residuals previously described by Gillette [6, 7]. Clearance (Cl) is the relationship: $Cl = (Dose_{i.v.}/AUC)$ in which AUC is the area under the curve from $t = 0$ to $t = \infty$. The volume of distribution in the central compartment (V_{dc}), the kinetic volume of distribution ($V_{d\beta}$) and the volume of distribution at equilibrium ($V_{d(eq)}$) were also calculated as explained by Gillette [6, 7].

Tissue preparation and in vitro *metabolism.* Animals were killed by decapitation; livers were removed, washed in saline and homogenized in 3 vol. of 20 mM Tris–1.15% KCl (pH 7.4). The homogenate was centrifuged for 20 min at 9000 *g* in a Sorvall centrifuge and the supernatant fraction was centriguged for 60 min at 105,000 *g* in a Beckman model L5-65 preparative ultracentrifuge. The microsomal pellets were washed by resuspension in the Tris–KCl buffer and were recentrifuged at 105,000 *g* for 4B min. The washed microsomal pellets were resuspended in 1 vol. of 0.05 M phosphate buffer (pH 7.4). The incubation mixture contained liver microsomal protein, an NADPH-generating system (NADP, 0.43 mM; glucose 6-phosphate, 12.6 mM; and glucose 6-phosphate dehydrogenase, 1 unit); $MgCl_2$, 3.3 mM; ethylenediamine tetra-acetic acid, 0.3 mM; and phosphate buffer (pH 7.4), 75 mM. The concentration of liver microsomal protein in the reaction mixture was 2 mg/ml. Reactions were carried out at 37° in a Dubnoff shaking machine, in air for 10 min.

N-demethylation of ethylmorphine was assayed by measuring the formation of formaldehyde using the method described by Nash [8]. Hydroxylation of hexobarbital was determined by the method of Cooper and Brodie [4].

Hydroxylation of zoxazolamine was assayed using the method described by Conney *et al.* [5]. Hydroxylation of 3,4-benzopyrene was assayed fluorometrically as described by Gelboin and Blackburn [9].

The amount of hepatic microsomal cytochrome P-450 was measured in an Aminco-Chance Dual wavelength spectrophotometer as described by Omura and Sato [10]. NADPH cytochrome *c* reductase was measured as described by Phillips and Langdon [11]. Cytochrome b_5 was measured as previously described by Sasame *et al.* [12]. Protein concentrations were measured by the method of Lowry *et al.* [13] with bovine serum albumin as a protein standard.

Statistical evaluation. The statistical significance of differences between control and treated groups was analyzed by Student's *t*-test or by the paired *t*-test [14], in the analysis of curves showing concentrations changing with time.

RESULTS

In vivo *effects of glycofurol used alone.* Doses of glycofurol from 0.5 to 2.5 ml/kg did not produce any obvious effects on the behavior of rats. However, administration of 5 ml/kg produced a mild ataxia and reduced exploratory behavior. The righting reflex was not abolished. These effects lasted only a few minutes.

In vivo *effects of glycofurol as a solvent for hexobarbital and zoxazolamine.* Glycofurol prolonged the

Table 1. Effect of glycofurol as a solvent on hexobarbital sleeping times and plasma levels upon awakening

Treatment*	Sleeping times† (min)	Hexobarbital levels on awakening†	
		Plasma (μg/ml)	Brain (μg/g)
Hexobarbital in saline	41.0 ± 2.9	58.7 ± 4.2	46.3 ± 4.3
Hexobarbital in 12.5% glycofurol	59.4 ± 4.9‡	45.7 ± 6.1	41.1 ± 4.2
Hexobarbital in 25% glycofurol	80.0 ± 7.5§	55.0 ± 3.2	48.9 ± 5.5
Hexobarbital in 50% glycofurol	121.5 ± 8.7§	48.2 ± 5.8	55.8 ± 4.7
Hexobarbital in 75% glycofurol	147.3 ± 8.6‖	43.3 ± 5.7	58.4 ± 7.4
Hexobarbital in 100% glycofurol	238.3 ± 9.4‖	53.3 ± 4.6	48,8 ± 5.5

*Hexobarbital sodium (150 mg/kg) was injected i.p. in a volume of 5 ml/kg. The solvent was varied as indicated.

†Values are expressed as the means ± S. E. of determinations on four male Sprague–Dawley rats (150–200 g).

‡$P < 0.05$, with respect to control (hexobarbital sodium in saline).

§$P < 0.01$, with respect to control.

‖$P < 0.001$, with respect to control.

Table 2. Effects of glycofurol as a solvent on zoxazolamine paralysis times and plasma and brain levels upon recovery of righting reflex

Treatment*	Paralysis time† (min)	Zoxazolamine levels on recovery of righting reflex†	
		Plasma (μg/ml)	Brain (μg/g)
Zoxazolamine in acidified H_2O (pH 2.0)	108.7 ± 8.4	15.5 ± 1.6	39.8 ± 2.5
Zoxazolamine in 12.5% glycofurol	144.6 ± 10.7‡	12.2 ± 2.3	36.5 ± 5.1
Zoxazolamine in 25% glycofurol	198.0 ± 21.7§	14.1 ± 3.2	39.0 ± 4.2
Zoxazolamine in 50% glycofurol‖	Died in 30–90 sec	(39.3 ± 4.4)§	(94.7 ± 4.5)§

*Zoxazolamine (50 mg/kg) was injected i.p. in volume of 5 ml/kg. The solvent was varied as indicated.
†Values are expressed as the mean ±S. E. of determinations on four male Sprague–Dawley rats (150–200 g).
‡$P < 0.05$, with respect to control (zoxazolamine in acidified water).
§$P < 0.001$, with respect to control.
‖Values in parentheses represent zoxazolamine levels at the time of death from respiratory arrest.

sleep induced by hexobarbital (Table 1). Despite the prolongation of sleeping time, plasma and brain levels of hexobarbital on awakening were not significantly different from those measured with hexobarbital in saline. Similar results were observed with zoxazolamine (Table 2). It is unlikely that the transient motor incoordination after the injection of 5 ml/kg of glycofurol alone would interfere with the measurement of much longer paralysis times of zoxazolamine or sleeping times of hexobarbital.

When the formulation contained 12.5 or 25% glycofurol the paralysis times were increased from 33 to almost 100 per cent with no change of zoxazolamine levels in plasma and brain upon recovery of the righting reflex. With zoxazolamine in 50% glycofurol, all animals died in 30–90 sec, and the zoxazolamine levels in plasma and brain at the time of death were about two or three times the levels determined in the control rats upon recovery of the right reflex. Autopsies, immediately after irreversible respiratory arrest, showed that their hearts were still beating.

Effects of glycofurol as a solvent on the plasma and brain levels of hexobarbital and zoxazolamine. The levels of hexobarbital in plasma and brain were determined at 3, 6, 12, 30, 60 and 120 min after injection with hexobarbital (150 mg/kg, i.p., 5.0 ml/kg) dissolved either in saline or in 25% glycofurol (Fig. 2). From 3 to 120 min, the plasma concentration of hexobarbital was 40–80 per cent higher in rats which received hexobarbital in 25% glycofurol than in rats which received hexobarbital in saline. Brain levels of hexobarbital were also higher in rats which received hexobarbital in 25% glycofurol.

Brain levels of zoxazolamine in rats which received the compound in 25% glycofurol were about twice those in control rats at all times tested (Fig. 3). Plasma levels were also about 66 per cent higher, except at 30 min. Moreover, the maximal concentra-

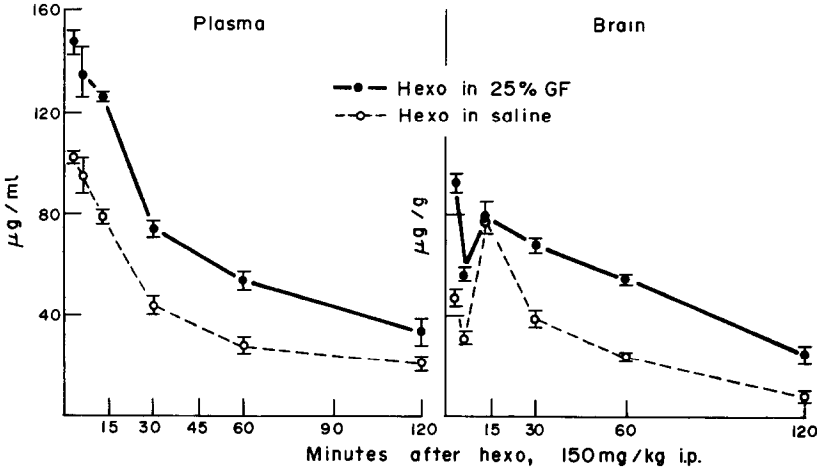

Fig. 2. Disappearance curves of hexobarbital in plasma and brain. Hexobarbital sodium (150 mg/kg) was injected i.p. in a volume of 5 ml/kg of saline or 25% glycofurol (GF). Thus, the dose of glycofurol was 1.25 ml/kg, i.p. Each point represents the mean ±S. E. of determinations on four rats. The curves for hexobarbital (Hexo) in 25% glycofurol are significantly higher ($P < 0.05$) than the curves for Hexo in saline, as shown by the *t*-value calculated from the paired *t*-test applied to the differences in the measured values.

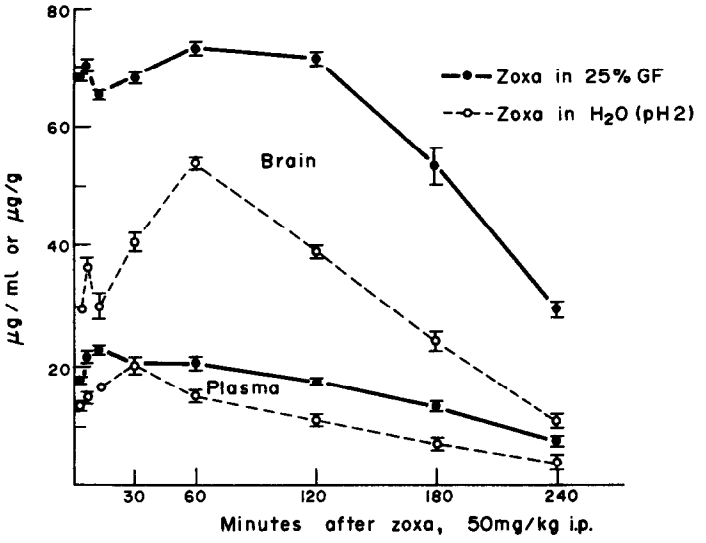

Fig. 3. Disappearance curves of zoxazolamine in plasma and brain. Zoxazolamine (Zoxa) (50 mg/kg) was injected i.p. in a volume of 5 ml/kg of acidified water or 25% glycofurol (GF). Thus, the dose of GF was 1.25 ml/kg, i.p. Each point represents the mean ±S. E. of determinations on four rats. The curves for Zoxa in 25% GF are significantly higher ($P < 0.05$) than the curves for Zoxa in acidified water.

tion of the drug in plasma occurred sooner in animals receiving glycofurol than in those receiving saline.

Pharmacokinetic analysis of plasma concentrations of hexobarbital and zoxazolamine. Glycofurol (1.25 ml/kg) or saline was injected i.p. into rats, and hexobarbital (75 mg/kg) was injected i.v. (Fig. 4). Glycofurol did not alter the initial plasma concentration of hexobarbital, indicating that it did not alter the volume of the central pool (Table 3). However, glycofurol markedly altered the subsequent distribution of hexobarbital. As shown in Table 3, glycofurol decreased the half-life [$T_{\frac{1}{2}}$ (α)] of the α-phase and the intercept A_0, thereby decreasing the area under the curve of the phase. In other terms, glyco-

Table 3. Pharmacokinetic parameters calculated for disposition of hexobarbital and zoxazolamine*

		Hexobarbital		Zoxazolamine	
Parameter	Units	Control	Glycofurol	Control	Glycofurol
$T_{\frac{1}{2}}(\alpha)$	min	14	8		
A_0	$\mu g\ ml^{-1}$	92	68		
α	min^{-1}	0.495	0.0866		
$T_{\frac{1}{2}}(\beta)$	min	236	112	144	196
B_0	$\mu g\ ml^{-1}$	3.2	24	13.3	15.8
β	min^{-1}	0.00294	0.00619	0.0048	0.0035
V_{dc}	liter kg^{-1}	0.79	0.83	1.48	1.33
$V_{d\beta}$	liter kg^{-1}	8.66	2.60	1.91	1.60
$V_{d(eq.)}$	liter kg^{-1}	3.53	2.23		
AUC†	$\mu g\ min\ ml^{-1}$	2947	4662	2733	4459
Cl	$ml\ kg^{-1}\ min^{-1}$	25.5	16.1	9.15	5.61

*Each point in Fig. 4 is the mean of determinations on four rats. A different group of rats was used for each point; thus 28 rats were used for each curve. The dose of hexobarbital sodium was 75 mg/kg, i.v. The dose of zoxazolamine was 25 mg/kg, i.v. The "control" rats received saline (1.25 ml/kg, i.p.) in addition to hexobarbital or zoxazolamine i.v. The "glycofurol" rats received glycofural (1.25 ml/kg, i.p.) in addition to hexobarbital or zoxazolamine i.v. The model is represented by the equation:

$$C = A_0 e^{-\alpha t} + B_0 e^{-\beta t}$$

Definitions of the parameters and methods for their determinations are given in Materials and Methods.

†Calculated from $t = 0$ to $t = \infty$ as $\frac{A_0}{\alpha} + \frac{B_0}{\beta}$ for hexobarbital and

$$\sum_{0}^{60\ min} \text{trapezoids} + \int_{t=60\ min}^{t=\infty} B_0 e^{-\beta t}\, dt \text{ for zoxazolamine.}$$

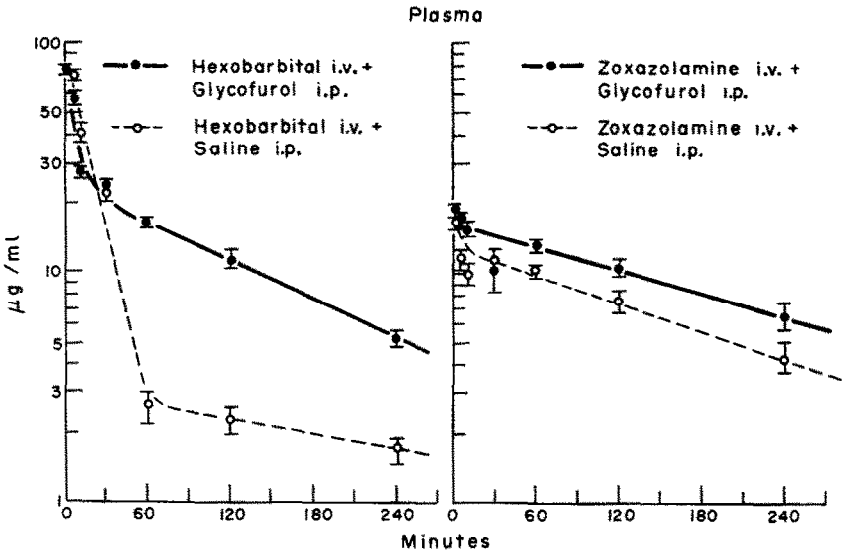

Fig. 4. Semilogarithmic plots of plasma concentrations of hexobarbital (Hexo) and zoxazolamine (Zoxa) as a function of time. Hexo (75 mg/kg) or Zoxa (25 mg/kg) was injected i.v. and GF (1.25 ml/kg) was injected i.p. 4 sec after one of these drugs. The control rats received the same amount of saline i.p. Each point represents the mean ± S. E. of determinations on four rats.

furol increased the rate of distribution of hexobarbital. Moreover, glycofurol also decreased the half-life of the β-phase about 50 per cent, but the intercept of this phase (B_0) was increased 6-fold. Despite the decrease in half-life, the area under the curve of the β-phase was increased almost 3-fold. These effects of glycofurol on the α- and β-phases resulted in a decrease in the total body clearance of hexobarbital of about 37 per cent and a decrease in the kinetic volume of distribution ($V_{d\beta}$) of 70 per cent.

When glycofurol was injected i.p. and zoxazolamine was injected i.v., glycofurol had a relatively small effect on the initial concentration of zoxazolamine. Thus, glycofurol had little or no effect on the volume of the central pool. However, the α-phase was too small to measure accurately. Indeed, virtually all of the AUC was associated with the β-phase.

Glycofurol increased the terminal half-life about 30 per cent and the intercept B_0 almost 20 per cent; the clearance and kinetic volume of distribution were decreased 37 and 14 per cent respectively.

Effects of a single dose of glycofurol on the microsomal mixed-function oxidase system. There were no significant differences at 3, 6, 12, and 24 hr after a

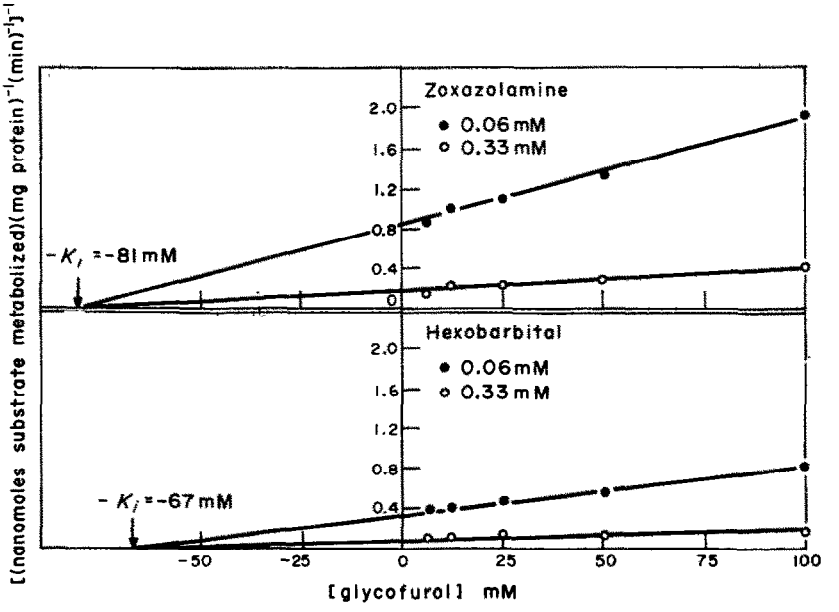

Fig. 5. Dixon plots showing noncompetitive inhibition by glycofurol of hexobarbital and zoxazolamine metabolism. Each point represents the mean of four determinations.

Table 4. Effects of repeated doses of glycofurol on hexobarbital sleeping times and zoxazolamine paralysis times, and plasma and brain levels on awakening or recovery of the righting reflex

Treatment*	Sleeping times or paralysing times (min)	Hexobarbital or zoxazolamine levels on recovery of righting reflex	
		Plasma (μg/ml)	Brain (μg/ml)
Saline + hexobarbital	49.5 ± 5.2	45.5 ± 4.0	43.0 ± 5.3
Glycofurol + hexobarbital	17.6 ± 2.2†	35.0 ± 4.6	50.0 ± 3.4
Saline + zoxazolamine	94.2 ± 8.7	12.1 + 2.8	43.5 + 3.1
Glycofurol + zoxazolamine	65.4 ± 3.5‡	5.47 ± 1.8‡	27.6 + 3.5‡

*Rats received 1.0 ml/kg of saline or glycofurol twice daily for 4 days. Hexobarbital (150 mg/kg, i.p.) or zoxazolamine (50 mg/kg, i.p.) was injected 14–18 hr after the last dose of saline or glycofurol. Values are expressed as the means ± S. E. of determinations on eight male Sprague–Dawley rats (150–200 g).

†$P < 0.001$, with respect to rats given repeated doses of saline + hexobarbital.

‡$P < 0.01$, with respect to rats given repeated doses of saline + zoxazolamine.

single dose (4 ml/kg, i.p.) of glycofurol or saline in: liver weight, microsomal protein, cytochrome P-450, cytochrome b_5, NADPH cytochrome *c* reductase, and metabolism of hexobarbital and zoxazolamine. However, incubation of liver microsomes from untreated rats with varying concentrations of glycofurol, in addition to the substrate, hexobarbital or zoxazolamine, showed noncompetitive inhibition (Fig. 5) of the substrate metabolism. The substrate concentrations chosen for the graphs in Fig. 5 are within the range of actual plasma concentrations for hexobarbital (Fig. 2). The lower concentration of zoxazolamine in Fig. 5 (0.06 mM) is also within the range of actual plasma concentrations (Fig. 3) and the higher concentration (0.33 mM) is lower than some of the measured brain concentrations (Fig. 3). Calculations based on Fig. 4 indicate that a concentration of about 25 mM glycofurol in the extracellular fluid would be expected to inhibit the metabolism of either zoxazolamine or hexobarbital about 25 per cent. It is possible that this concentration of glycofurol may be achieved in plasma after injection of the dose used to obtain the data on Figs. 2 and 3 and that if the diluent is rapidly absorbed, it is distributed with the extracellular fluid and is eliminated slowly. However, no attempt was made to substantiate these relationships.

In vivo *effects of hexobarbital and zoxazolamine after repeated doses of glycofurol.* Rats received glycofurol (1 ml/kg, i.p.) twice a day for 4 days; 12–18 hr after the last dose, hexobarbital (150 mg/kg in 5 ml saline) or zoxazolamine (50 mg/kg in 5 ml acidified water) was injected i.p. and the sleeping and paralysis times were measured (Table 4). There was a 65 per cent decrease of hexobarbital sleeping time in the glycofurol-treated groups, although there was no difference statistically between the groups in hexobarbital levels in plasma or brain upon awakening. The paralysis times elicited by zoxazolamine administration were 31 per cent lower in glycofurol-treated rats than in the control rats. But in contrast to the experiment with hexobarbital, there was an

Table 5. Effects of repeated doses of glycofurol on some microsomal parameters*

Treatment	Liver wt (g)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg microsomal protein)	Cytochrome b_5 (nmoles/mg microsomal protein)	NADPH cytochrome *c* reductase (nmoles cytochrome *c* reductase/mg microsomal protein/min)
Control (saline)	6.93 ± 0.21	17.33 ± 1.62	1.14 ± 0.09	0.41 ± 0.03	117 ± 11
Glycofurol					
0.1 ml/kg	6.42 ± 0.35	17.72 ± 0.72	1.15 ± 0.04	0.43 ± 0.01	221 ± 8 ‡
0.5 ml/kg	7.03 ± 0.18	17.33 ± 0.84	1.27 ± 0.07	0.55 ± 0.04†	231 ± 14‡
1.0 ml/kg	7.26 ± 0.21	18.50 ± 1.31	1.85 ± 0.09‡	0.55 ± 0.03†	217 ± 13‡
2.0 ml/kg	5.80 ± 0.81	22.83 ± 2.86	1.53 ± 0.08	0.62 ± 0.06‡	230 ± 6‡

*Male Sprague–Dawley rats (150–200 g) received 0.1 ml/kg of glycofurol or saline i.p. twice a day for 5 days or 0.5, 1.0 or 2.0 ml/kg of glycofurol or saline i.p. twice a day for 4 days. Measurements were made 14–18 hr after the last injection. Values are expressed as the means ± S.E. of determinations on six rats.

† $P < 0.01$, with respect to control.

‡ $P < 0.001$, with respect to control.

Table 6. Effects of repeated doses of glycofurol on hepatic microsomal metabolism of hexobarbital, zoxazolamine, ethylmorphine and benzopyrene

Treatment*	Hexobarbital (nmoles/mg/min)	Zoxazolamine (nmoles/mg/min)	Ethylmorphine (nmoles/mg/min)	Benzopyrene (% Δ transmittance/mg/10 min)
Control	5.76 ± 0.58	5.53 ± 0.59	8.3 ± 0.6	13.5 ± 1.1
Glycofurol	9.91 ± 0:59†	7.60 ± 0.22†	12.5 ± 1.5†	23.5 ± 1.3†

*Rats received 1 ml/kg, twice a day for 4 days, of saline or glycofurol. Measurements were made after the last injection. Values are expressed as the means ± S. E. of six determinations on male Sprague–Dawley rats (150–200 g).

† $P < 0.01$, with respect to control.

approximately 50 per cent decrease in zoxazolamine levels in plasma and brain upon recovery of the righting reflex in glycofurol-treated rats.

Effects of repeated doses of glycofurol on the microsomal mixed-function oxidase system. Repeated administration of glycofurol to rats produced changes in the components of the mixed-function oxidase activity (Table 5). Liver weight and microsomal protein content were unaffected. However, levels of cytochrome P-450 were increased about 50 per cent when glycofurol was administered in doses of 1–2 ml/kg twice a day for 4 days. Cytochrome b_5 was increased about 40 per cent with repeated doses of 0.5 ml/kg up to 2.0 ml/kg; with all doses tested (0.1 to 2.0 ml/kg) NADPH cytochrome *c* reductase was increased about 90 per cent.

Treatment with 1 ml/kg of glycofurol, twice a day for 4 days, produced a significant increase in metabolism in both hexobarbital and zoxazolamine (Table 6). The 72 per cent increase in hexobarbital metabolism correlated well with the 64 per cent decrease in sleeping times (Table 4). Zoxazolamine metabolism was also increased about 37 per cent, corresponding to a 31 per cent decrease in the paralysis time (Table 4).

The induction by glycofurol also enhanced microsomal activity in *N*-demethylation and hydroxylation reactions. The per cent increases above controls were 50 per cent for ethylmorphine demethylation and 74 per cent for benzopyrene hydroxylase (Table 6).

DISCUSSION

When used as a solvent, glycofurol increased the sleeping and paralysis times of hexobarbital and zoxazolamine in several different ways. Glycofurol decreased the total body clearance of both drugs. Since glycofurol does not affect the initial concentrations of the drug in the central pool, it probably does not alter the binding of the drug to plasma proteins. Thus, glycofurol probably decreased the total body clearances of the drugs by decreasing their metabolism in the liver. In accord with this view, glycofurol caused noncompetitive inhibition of the metabolism of zoxazolamine and hexobarbital by liver microsomes. With a substrate concentration of 6×10^{-5} M, the Dixon plots suggest that the metabolism of zoxazolamine or hexobarbital may be inhibited by 25 per cent at glycofurol concentrations (25 mM) that might occur in the extracellular fluid after i.p. injection.

However, inhibition of liver microsomal enzymes is not the only mechanism by which glycofurol affected the plasma levels of the drugs. Glycofurol also decreased the kinetic volume of distribution ($V_{d\beta}$) of hexobarbital. Indeed, the decrease in $V_{d\beta}$ of hexobarbital was so great that the terminal half-life of hexobarbital was decreased even though the metabolism of the drug was inhibited. Calculation of the volume of distribution at equilibrium ($V_{d(eq)}$) of hexobarbital confirmed the view that glycofurol decreased the volume of the peripheral pool, but it also revealed that much of the decrease in $V_{d\beta}$ was due to the decrease in the clearance of hexobarbital. Although glycofurol also decreased the $V_{d\beta}$ of zoxazolamine, we could not determine whether the decrease was due solely to the decrease in the clearance of zoxazolamine or also to a decrease in $V_{d(eq)}$.

Although the increases in hexobarbital and zoxazolamine concentrations of plasma and brain after i.p. administration were correlated with increases in sleeping and paralysis times, it is noteworthy that the initial concentrations in the central compartment of both hexobarbital and zoxazolamine were considerably higher when the drugs were dissolved in glycofurol than when they were dissolved in saline. Since glycofurol did not change the volume of the central pool of the i.v. administered drugs, and since clearances of the drugs seem to be too slow to have a marked first-pass effect (hepatic blood flow rates in rats are usually about 50–150 ml kg^{-1} min^{-1}), it seems likely that glycofurol accelerated the absorption of the drugs. Although both drugs were injected as solutions, it is noteworthy that both are rather insoluble at pH 7.4. Thus, it is possible that glycofurol facilitated the uptake of zoxazolamine and hexobarbital by maintaining them in solution. However, inspection of the peritoneal cavities of the animals failed to reveal any gross precipitation of either drug when administered in an aqueous solution. Fouts [15] has suggested that zoxazolamine in aqueous acidic media is sometimes erratically absorbed, probably due to irritation of the surrounding tissues by the acidity.

Crankshaw and Raper [3] found that glycofurol produced hypnosis and depression of polysynaptic reflexes in mice. They suggested that the increased

potency of benzodiazepines dissolved in glycofurol is due to the solubilization of drug or to the synergism of its pharmacologic activity with that of glycofurol. Since we did not observe hypnosis in rats after doses of glycofurol up to 5 ml/kg, a species difference may exist.

After repeated doses of glycofurol, hexobarbital sleeping times and zoxazolamine paralysis times were decreased. These decreases were correlated with increases in microsomal metabolism. Glycofurol shares a number of effects on hepatic microsomes with the classical enzyme inducer, phenobarbital. Like phenobarbital [16], glycofurol (1 ml/kg twice a day for 4 days) increases the amount of cytochrome P-450 and the activities of NADPH cytochrome *c* reductase, hexobarbital side chain oxidases, zoxazolamine hydroxylase, benzopyrene hydroxylase and ethylmorphine *N*-demethylase. However, glycofurol differs from phenobarbital [16, 17] in that liver weight and microsomal protein (mg/g) are unchanged. The effects of glycofurol differ from those of 3-methylcholanthrene in that glycofurol induces NADPH cytochrome *c* reductase and cytochrome b_5, whereas 3-methylcholanthrene does not cause these changes. Neither 3-methylcholanthrene [18] nor glycofurol increases microsomal protein/g of liver.

Thus, the effects of glycofurol show some characteristics produced by two types of classical inducers of drug metabolism, but differ from the types of induction produced by other solvents such as propyleneglycol and dimethyl sulfoxide. Propyleneglycol pretreatment [19] does not change the levels of cytochrome P-450, but increases significantly the *in vitro* hepatic microsomal metabolism of aniline and *p*-nitroanisole and at the same time decreases aminopyrine demethylation significantly. Also dimethyl sulfoxide [20, 21] does not change the amount of cytochrome P-450, NADPH cytochrome *c* reductase and cytochrome b_5 in liver microsomes, although it significantly increases the hydroxylation of aniline and the *O*-demethylation of *p*-nitroanisole. Other solvents such as acetone and isopropanol [22] do not change the microsomal content of cytochrome P-450 or the activity of NADPH cytochrome *c* reductase, but increase significantly the *in vitro* hepatic microsomal metabolism of dimethylnitrosamine

Since the amount of glycofurol used in these studies is high, the clinical significance of the results is obscure. Nevertheless, our findings indicate that glycofurol is not inert when used as a solvent or cosolvent for experimental pharmacologic studies.

REFERENCES

1. A. J. Spiegel and M. M. Noseworthy, *J. pharm. Sci.* **52** 917 (1963).
2. H. Spiegelberg, R. Schlapfer, G. Zbinden and A. Studer, *Arzneimittel.-Forsch.* **6**, 75 (1956).
3. D. P. Crankshaw and C. Raper, *J. Pharm. Pharmac.* **23**, 313 (1971).
4. J. R. Cooper and B. B. Brodie, *J. Pharmac. exp. Ther.* **144**, 409 (1955).
5. A. H. Conney, N. Trousof and J. J. Burns, *J. Pharmac. exp. Ther.* **128**, 333 (1960).
6. J. R. Gillette, *J. Pharmacokinetics Biopharm.* **1**, 497 (1973).
7. J. R. Gillette, in *Handbook of Experimental Pharmacology* (Eds J. R. Gillette and J. R. Mitchell), Vol. 28/3, p. 35. Springer, New York (1975).
8. T. Nash, *Biochem. J.* **55**, 416 (1953).
9. H. V. Gelboin and N. R. Blackburn, *Cancer Res.* **24**, 356 (1964).
10. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
11. A. Phillips and R. Langdon, *J. biol. Chem.* **237**, 2652 (1962).
12. H. A. Sasame, S. S. Thorgeirsson, J. R. Mitchell and J. R. Gillette, in *Citochromes P-450 and b_5, Structure, Function and Interaction* (Eds D. Y. Cooper and O. Rosenthal), p. 435. Plenum Press, New York (1977).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. G. W. Snedecor and W. G. Cochran, in *Statistical Methods*, 6th Edn. p. 91, Iowa State, University Press, Iowa (1967).
15. J. R. Fouts, in *Methods in Pharmacology* (Ed. A. Schwartz), p. 287. Meredith Co., New York (1971).
16. T. E. Gram and J. R. Gillette, in *Fundamentals of Biochemical Pharmacology* (Ed. Z. M. Bacq), p. 571. Pergamon Press, New York (1970).
17. K. W. Bock, W. Frohling, H. Remmer and B. Rexer, *Biochim. biophys. Acta* **327**, 46 (1973).
18. G. J. Mannering, in *Selected Pharmacological Testing Methods* (Ed. A. Burger), p. 51. Marcel Dekker, New York (1968).
19. M. E. Dean and B. H. Stock, *Toxic. appl. Pharmac.* **28**, 44 (1974).
20. B. H. Stock, A. R. Hansen and J. R. Fouts, *Toxic. appl. Pharmac.* **16**, 728 (1970).
21. B. H. Stock and J. R. Fouts, *Biochem. Pharmac.* **20**, 1525 (1971).
22. I. G. Sipes, B. Stripp, G. Krishna, H. M. Maling and J. R. Gillette, *Proc. Soc. exp. Biol. Med.* **142**, 237 (1973).