Perfusion of the Isolated Rat Liver with Phenothiazines (36697)

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Previous studies have demonstrated that exposure of rabbit liver slices (1) and of suspensions of "liver" cells (Chang) grown in tissue culture (2) to CPZ leads to leakage of enzymes into the medium. Promazine (PZ), which in contrast to CPZ rarely produces hepatic injury in man (3), led to no effect (1) or to a lesser effect (2). A further study (4), using suspensions of cells from tissue culture, compared the relative cytotoxicity of several phenothiazine compounds. A trifluoromethyl ($-CF_3$) group, and to a lesser degree a chloride (–Cl) substituent in position R_2 appeared to increase the "cytotoxic" potential of the phenothiazine compounds. These findings parallel adverse effects of phenothiazines in other *in vitro* models (inhibition of motility of protozoa (5), hemolysis (6).

Studies on the perfused rat liver in this laboratory (7) have demonstrated that CPZ leads to a decrease in bile flow, hepatic perfusion flow, and in biliary excretion of sulphobromophthalein (BSP). In the present study the *ex vivo* perfused rat liver was utilized to compare effects of several phenothiazines on perfusate flow, bile production, and removal and excretion of BSP.

Materials and Methods. Preparations. Unfasted female rats (Sprague-Dawley) weighing 250–350 g were prepared for *ex vivo* perfusion of their livers as described previously (7). The medium for perfusion was a cell-free solution of Krebs buffer (pH 7.4) freshly prepared before each experiment. Sufficient bovine albumin (35% solution, Pentex Biochem.) was added to make a 2.5% concentration. In addition, the perfusate contained 240 mg/100 ml of glucose and 3000 units of heparin (Liquaemin, Organon).

Drugs. Promazine (PZ), triflupromazine (TFPZ) and trifluoperazine (TRPE) were supplied by Smith, Kline and French Laboratories. The drugs were each dissolved in 1.0 ml of saline and added to the perfusate to provide concentrations of 1.0×10^{-4}, 2.5×10^{-4} and 5.0×10^{4} mole/liter, respectively. They were added after a period of system equilibration that lasted for 30 min. In control experiments 1.0 ml of saline was added instead of the drugs. Measurements of rates of bile and perfusate flows were taken during the subsequent 30 min, after which sulphobromophthalein (BSP), in a concentration of 10 mg/100 ml of perfusate was added; and the determinations were continued for an additional 45 min. Samples (0.2 ml) of perfusate were drawn at intervals for the determination of BSP removal by the liver. Bile samples (20 μl) containing excreted BSP were collected at intervals for the assay of biliary BSP content, as previously reported (7).

The rate from a total of 51 perfusions were analyzed for variance of parameters. Student's t was used to test statistical significance of differences between means (8).

Results. All three drugs, at concentrations used, decreased BSP removal from the perfusate significantly. At the lowest concentration (1×10^{-4} M), PZ interfered less than did the other two, and the rate of removal of BSP from perfusate did not differ significantly from that of the control, until 35 min had elapsed after addition of the dye. At the highest level of drug (5×10^{-4} M), however, PZ exerted more of an adverse effect on BSP removal than did the other two phenothiazines (Fig. 1).

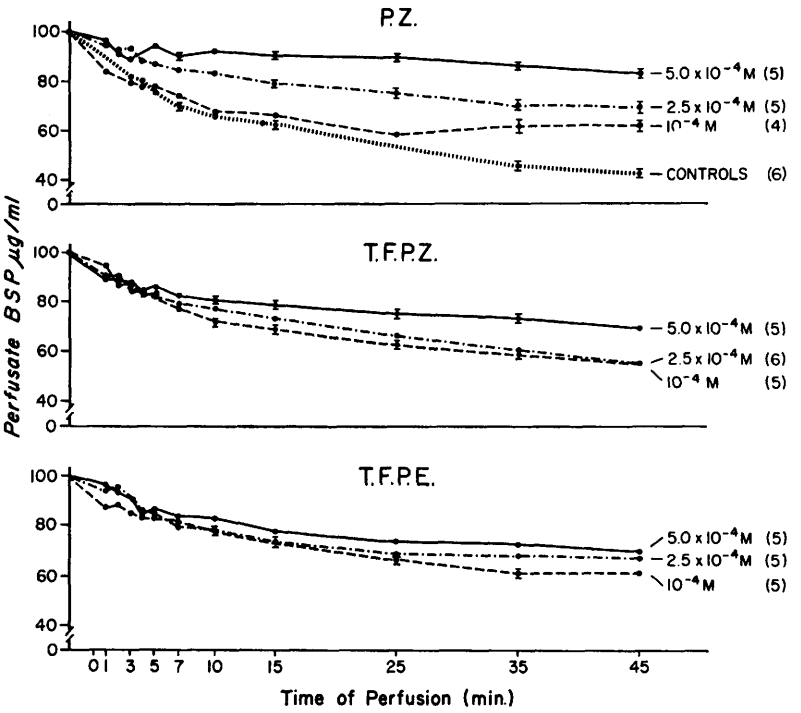

FIG. 1. BSP disappearance from the perfusate of control and drug-treated preparations. Drug concentrations and number of experiments are shown. Bars represent SE.

Biliary concentrations of BSP are shown in Fig. 2. It may be seen that all three drugs, at all concentrations, significantly decreased BSP excretion. At the lower concentraton of drug (10^{-4} *M*) PZ interfered least with excretion (Fig. 2, Table I). At the highest concentration (5×10^{-4} *M*) PZ had the greatest adverse effect (Fig. 2).

Rate of bile flow. A mean rate of 8.8 µl/min (±0.07, SEM) of bile flow was obtained during the initial equilibration period (Fig. 3). The adition of 5.0×10^{-4} *M* of the drugs caused an immediate abrupt decrease in bile flow that did not return to normal until the end of the experiment. Concentrations of 2.5×10^{-4} *M* of all drugs had a similar, but quantitatively lesser effect. At a concentration of 1.0×10^{-4} *M*, only TFPE led to a significant decrease in bile flow, prior to the addition of BSP. (After the addition of BSP, the adverse effect of the dye on bile flow, seemed enhanced by the effects of each of the drugs at all concentrations.)

TABLE I. Adverse Effect of Phenothiazines on Hepatic Excretory Functions.[a]

Drug	Decrease in	
	Rate of bile flow	Biliary BSP excretion
PZ	8 ± 0.9	10.0 ± 1.7
TFPZ	30.1 ± 5.0	21.5 ± 2.0
TFPE	39.2 ± 4.3	14.8 ± 1.8
CPZ	54.0 ± 4.9	41.0 ± 6.0

[a] Expressed as inhibition (%) of control periods. Drugs were compared at 10^{-4} *M* concentration.
[b] SE.

Rate of perfusate flow. The rate of flow was maintained at 58 ± 2 (SE) ml/min in the control period for all livers (Fig. 4). Addition of drugs in a concentration of 5.0×10^{-4} *M* caused an immediate drop in the flow which recovered spontaneously within the following hour. At this concentration, addition of PZ led to a significant decrease in the rate of perfusate flow (to 28 ml/min) that was noticed within 5 min following the treatment. Addition of TFPZ or TFPE was

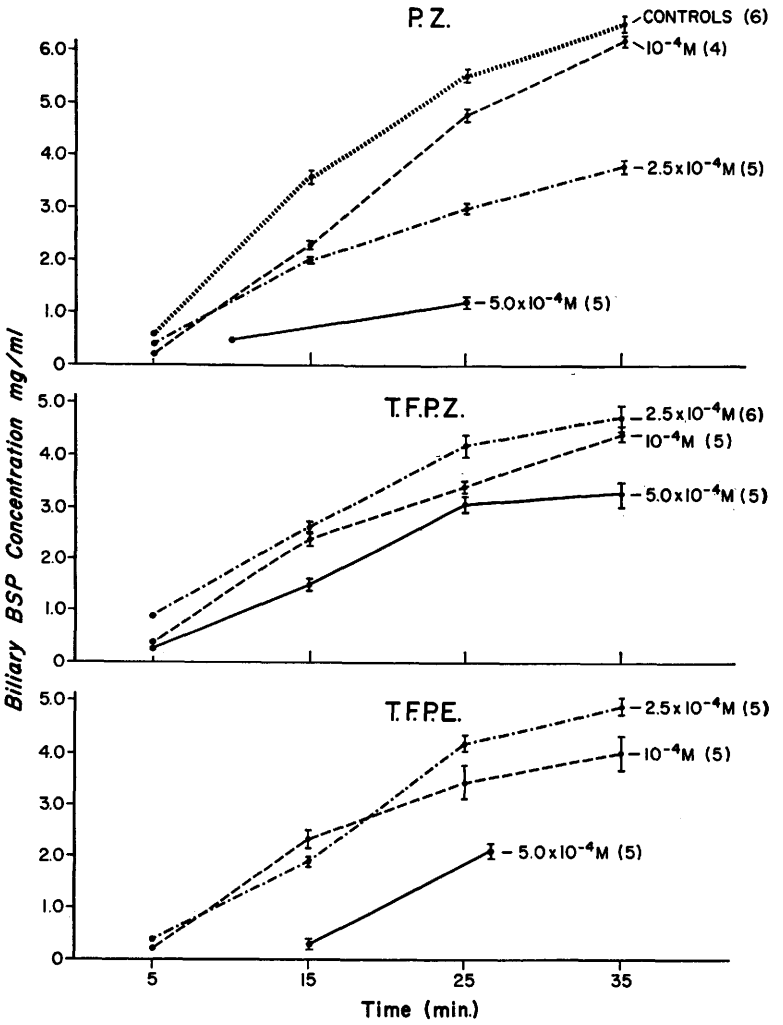

FIG. 2. Effect of phenothiazines on biliary excretion of BSP. Bars represent SE.

followed by similar, but quantitatively lesser effects. At concentrations less than 5.0×10^{-4} *M*, variable effects were observed for all drugs, but no significant differences from controls were found.

Discussion. Perfusion of isolated livers with TFPZ, TFPE and PZ, in all concentrations tested, led to a reduction in bile production, clearance of BSP and the biliary excretion of the dye. In a previous study (7), chlorpromazine (CPZ) was shown to alter adversely the function of isolated livers, at these concentrations of drug. Of the four phenothiazines, PZ seemed to lead to less impairment of the function of the perfused liver at the low concentration of 1×10^{-4} *M*. The paradox of little adverse effect of PZ at a concentration of 10^{-4} *M*, but of an adverse greater than that of the other drugs at higher concentrations (5.0×10^{-4} *M*), may relate to the merging of changes in excretion and in perfusate flow. PZ at high concentrations (5×10^{-4} *M*) caused a significantly more marked and more prolonged reduction in the rate of perfusion flow than did TFPZ or TFPE, an effect that could lead to impairment of BSP uptake and its consequent excretion. At lower concentrations (2.5 and 1×10^{-4}) PZ had no demonstrable effect on perfusate flow, and was found to interfere

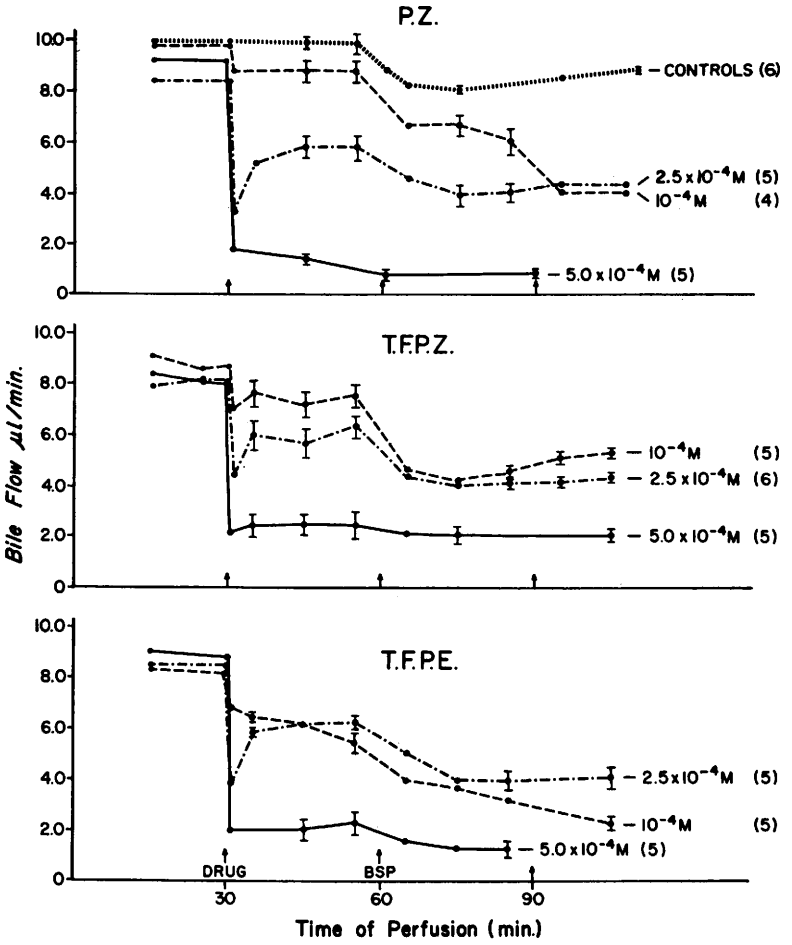

FIG. 3. Effect of phenothiazines on the rate of bile flow produced by perfused livers. Number of experiments is shown in brackets. Bars represent SE.

with hepatic function less than the other two phenothiazines.

Comparison of the results of the present study with those previously reported for CPZ is shown in Table I. At a concentration of 10^{-4} *M*, PZ inhibits hepatic excretory function only slightly, while CPZ, TFPZ and TFPE all appear to have a significantly greater adverse effect. These observations are consistent with those obtained with an *in vitro* system employing Chang cells (4) and rabbit liver slices (1), in which the adverse effects of phenothiazine were enhanced by a trifluomethyl or chlorine atom at the R_2 position of the molecule. Attempts have been made *in vitro* to correlate the known "surface activity" of phenothiazines to their clinical ("tranquilizing") potential (9); and a presumed relationship between molecular structure of these compounds and their ability to alter cellular membrane permeability has been inferred (4). Our data indicate that the four phenothiazines tested (CPZ, TFPZ, TFPE and PZ) also interfere with the excretory ability of the *ex vivo* perfused rat liver either by an effect similar to that on membranes of isolated cells or by some other interference with bile secretion.

The relevance of these observations to the development of manifest hepatic injury in humans remains to be determined. Although PZ appears less likely to produce hepatic injury in patients than does CPZ, there are no useful values comparing the incidence of

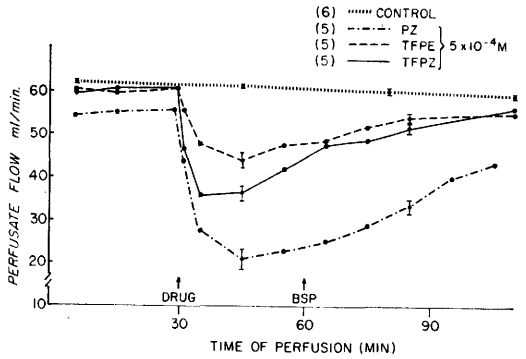

FIG. 4. Rate of perfusate flow in livers treated with 5×10^{-4} *M* of phenothiazines. Bars represent SE. Number of experiments is shown in brackets.

adverse effects of the various phenothiazines on the liver. Nevertheless, the parallel differences among several phenothiazines with regard to the adverse effects on *in vitro* models employing Chang cells, rabbit liver slices and the perfused liver, support the validity of these *in vitro* models as measures of the potential for producing hepatic injury. These observations are also consistent with the hypothesis that phenothiazine-induced jaundice may result from the combined effects of hypersensitivity to the drug and drug-induced hepatic injury.

Summary. Phenothiazine derivatives (promazine, trifluprom azine, and trifluoperazine) were tested for their effect on the function of the isolated, perfused rat liver. All three drugs at concentrations of 10^{-4} *M* or higher led to significant inhibition of bile output, clearance of sulphobromophthalein (BSP) and the biliary excretion of the dye; although, at the lowest concentration (10^{-4} *M*) perfusion with promazine (PZ) seemed to lead to lesser impairment of hepatic excretory function than did the other two phenothiazines. The flow rate of perfusate was altered by all drugs at higher concentrations (5×10^{-4} *M*); the most significant and lasting effect in this respect was that of PZ.

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