which, when given separately, do not produce complete neuromuscular blockage (figures 1,b and 3,c), rapidly provoked complete block of the transmission of nervous stimuli (figure 2,c).

It has been published[5] that high concentrations of ascorbic acid block the transmission of nervous impulses on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat, and that this kind of block was not reversed by neostigmine or calcium.

Our results indicate that ascorbic acid interacts with muscle relaxants and neostigmine, and that the block produced has the same characters as that which is produced by prostaglandins[6,7] (figure 3). The prostaglandins $A_1$, $A_2$, $E_1$, $E_2$ and $B_1$ block the transmission of nervous impulses on the isolated neuromuscular preparation of phrenic nerve-hemidiaphragm of the rat. This effect has an indirect mode of action, which is due to the liberation of big amounts of the transmitter by ascorbic acid (figure 1,c). According to these observations, it is possible to explain the results produced by the simultaneous administration of ascorbic acid, in concentrations smaller than that producing complete neuromuscular blockage, and neostigmine (figure 2,a). On the other hand, antagonism was found between ascorbic acid and d-tubocurarine, to the production of neuromuscular blockage (figure 2,b). This is because the amount of acetylcholine released by the nervous stimuli in the presence of ascorbic acid is large, and competes with the d-tubocurarine, producing a biological competition. Finally, the synergism found between ascorbic acid and succinylcholine (figure 2,c), suggests that these substances are working actively producing neuromuscular blockage by the same mechanism.

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## Diurnal rhythm of ethanol metabolism in the rat[1]

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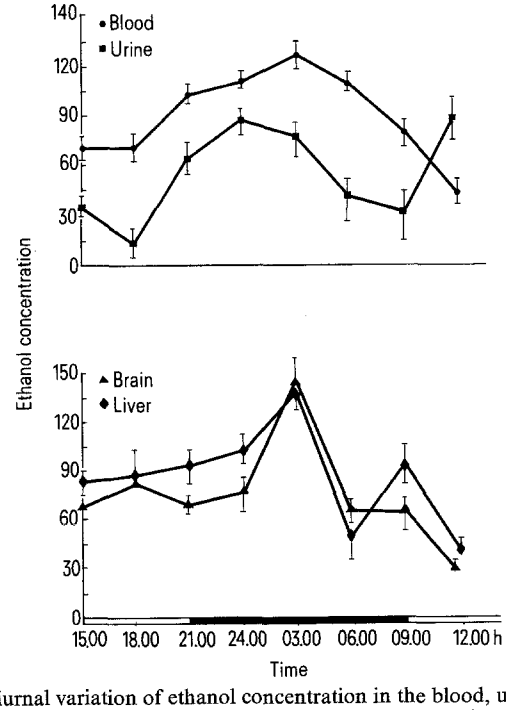
*Summary.* Male Sprague-Dawley rats injected with 2.0 g/kg of ethanol and analyzed 1 h later at 8 specific times of the day showed diurnal rhythms for alcohol concentrations in the blood, urine, brain and liver tissues. The circadian fluctuation noted for the concentrations of blood and tissue ethanol might indicate a diurnal variation in the enzymatic metabolism of ethanol.

Many studies have demonstrated that the effect of a drug, chemical or poison has, is circadian-phase dependent. Ethanol toxicity was found to be circadian-phase dependent[2,3]. The circadian variations in drug response can be attributed to several factors including mechanisms associated with changes in target enzymes, variation in the drug absorption rate and fluctuation in the rate of excretion. In an attempt to exploit the circadian behavior of ethanol effect this work was undertaking to measure the variation of ethanol levels in rats treated with a fixed ethanol dose.

*Methods.* Male Sprague-Dawley rats weighing approximately 150 g each were used in these experiments. The animals were adapted to a 12 h light/12 h dark cycle for a minimum period of 3 weeks prior to treatment. The temperature was maintained at 23±1 °C, and the animals were fed and watered ad libitum. The test animals were injected with 2.0 g/kg of ethanol at 8 different times of the day, 1 h prior to sacrificing. Immediately after sacrificing, the blood was collected in heparinized tubes and was centrifuged. Brain and liver samples were dissected out and stored at −20 °C until analyzed. The brain and liver portions were homogenized in distilled water equivalent to 8 times the tissue weight. Deproteinization of tissue samples was accomplished by 6.25% trichloroacetic acid. Ethanol levels were determined using an enzymatic procedure[4].

The analysis of data obtained was based on the establishment of a regression line for standards and samples. The readings were then converted to mg% ethanol using the regression equation from the standard line. The regression equation used ($\hat{y} = -21.1 + 204.3x$) has a 0.98 coefficient of determination. The data was further subjected to analysis




Diurnal variation of ethanol concentration in the blood, urine, liver and brain. Each point represent the average of 8 animals ±SEM. The shaded area of the graph represents the dark phase. Ethanol concentration is expressed as mg/100 ml of body fluids or as mg/100 g of tissue studied.

of variance and differences in means were detected by a modified Duncan multiple range test.

*Results.* As it shows in the figure a diurnal rhythm is noted for ethanol levels for both blood plasma and urine. While the diurnal patterns are somewhat similar in that the peak levels occurred during the dark period, the acrophase for blood was 03.00 h and 24.00 h for urine. A trough was noted for both fluids during the light phase at 12.00 h for the blood and 18.00 h for the urine. These differences between throughs and peaks and the differences for acrophases between the 2 samples were highly significant ($p < 0.01$).

The figure also shows the circadian variation of ethanol level for both brain and liver. The acrophases and troughs occurred at identical times, 03.00 and 12.00 respectively. In both cases, the difference between troughs and peak were significant ($p < 0.01$). The figure shows the similar pattern between brain and liver. Both peaks and troughs occurred at similar times with very little differences in the total concentration at specific times of day. It also appears that, at 12.00 h all levels decline with exception of urine.

*Discussion.* The data presented here indicates that there is a diurnal rhythm in either the distribution, absorption and/or metabolism of ethanol as indicated by varying levels in the blood, urine, liver and brain tissues. For all tissues observed, higher levels during the dark phase when the animals were most active and this may indicate a diurnal pattern in the transport of ethanol or diurnal rhythm in the enzymatic activity of alcohol dehydrogenases. The fact that at 12.00 h all measured levels of ethanol decline except for the urine and the difference in the acrophases of blood and urine might indicate possible circadian variation in the rate of ethanol excretion. It was concluded from this work that the circadian fluctuation in ethanol toxicity which has been reported previously[2,3], might be due to fluctuation in the ethanol blood levels.

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## Choleretic and cholestatic effects of infused bile salts in the rat

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*Summary.* In rats, at low infusion rates taurocholate (TC), taurochenodeoxycholate (TCDC) and taurodeoxycholate (TCD) each produced an increase in bile flow of 20–50%. However, at high infusion rates (5–20 μmoles $min^{-1}$ $kg^{-1}$) the cholestatic effects of the bile salts were revealed and the relative toxicity of the bile salts was seen to be TDC > TCDC > TC.

Substantial retention of bile salts can occur in liver disease, particularly cholestatic liver disease[2], and there has been speculation about the role that bile salts might play in initiating or aggravating cholestatic liver disease[3,4]. Although it is clear that there is a fraction of bile flow which is independent of bile salt secretion[5,6], the active transport of bile salts across the bile canalicular membrane is a major factor in the regulation of bile flow[7]. An increase in bile flow (choleresis) is the usual response to the infusion of bile salts, but this depends to some extent upon the chemical nature of the bile salt[8]. Dehydrocholate and taurocholate are both efficient choleretic agents, whereas the monohydroxy bile salt, taurolithocholate, produces a dose-dependent inhibition of bile flow (cholestasis) which may be prevented by infusion of micelle forming primary bile salts[9,10]. In addition, a sex- and dose-related cholestatic response to the dihydroxy bile salt, chenodeoxycholate, has been demonstrated in the isolated perfused rat liver[11], and it has recently been shown that high doses of taurocholate can inhibit bile formation in isolated perfused rat livers[12]. We therefore reasoned that the relative choleretic and cholestatic properties of other bile salts administered in vivo may also be dose-dependent. In the present communication we report on the differential choleretic and cholestatic effects in the rat of taurine conjugates of the major di- and tri-hydroxy bile salts found in mammalian livers, namely, chenodeoxycholate (TCDC), deoxycholate (TDC) and cholate (TC).

*Materials and methods.* Male Wistar rats (300–350 g) that had been allowed food and water ad libitum were anaesthetized with sodium pentobarbital (50 mg $kg^{-1}$, i-p.) and their rectal temperature maintained at 37 °C with the aid of heating pads. A femoral vein and the common bile duct were cannulated with SV-45 polyvinyl and PE-10 polyethylene tubing, respectively, and bile flow measured gravimetrically over 10-min collection intervals. A basal bile flow was determined in each rat, while infusing saline, 30 min before the infusion of bile salt. Sodium salts of TC (Koch-Light Labs), TCDC and TDC (Sigma Chemical Co.) were dissolved in saline at concentrations which allowed the desired amount of bile salt to be delivered.

The experiments were divided into 2 groups: a) a continuous infusion of bile salt at either 1, 5, 10 or 20 μmoles $min^{-1}$ $kg^{-1}$ for up to 240 min (Sage infusion pump set to deliver 49 μl $min^{-1}$); b) 60-min sequential infusions of bile salt at 1, 5 and then 10 μmoles $min^{-1}$ $kg^{-1}$ (Palmer slow injection apparatus set to deliver 54 μl $min^{-1}$). 2–3 animals were used for each infusion rate and anaesthesia was maintained throughout the experiments with supplementary doses of pentobarbital.

*Results.* Changes in bile flow in representative rats used for each bile salt and at each infusion rate are shown in figures 1 and 2. In experiments where infusion at a constant rate for 240 min was employed (figure 1), TC caused the bile flow to rise to a dose-dependent plateau. However, at the highest infusion rate (20 μmoles $min^{-1}$ $kg^{-1}$) the choleretic plateau response was followed by a rapid decrease in bile flow after approximately 90 min of infusion. TDC and TCDC also caused choleresis at infusion rates of 1 and 1–5 μmoles $min^{-1}$ $kg^{-1}$, respectively, but with higher infusion rates an immediate cholestatic response was observed.