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.

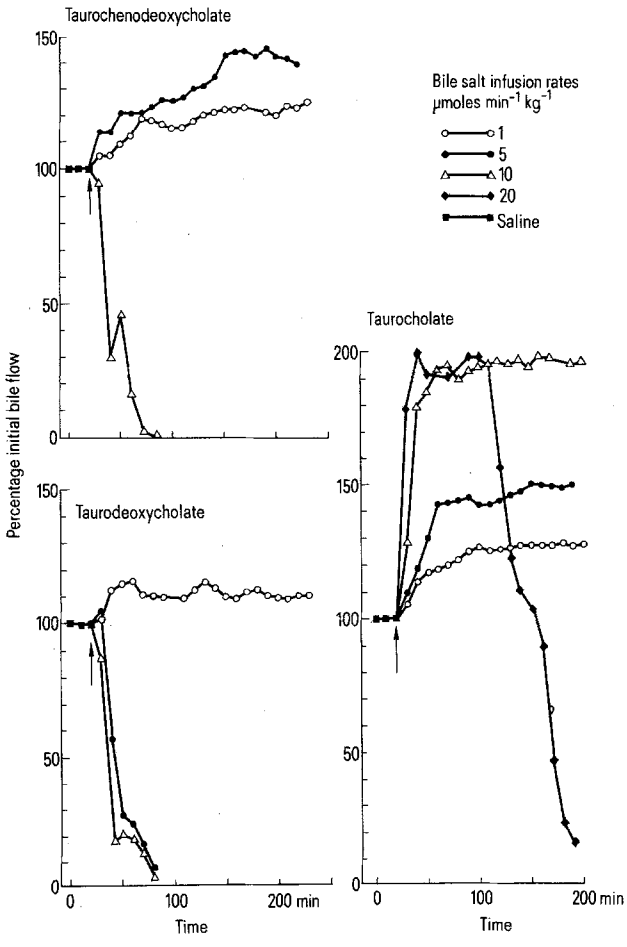

Fig. 1. Bile flow in rats during an initial 30-min infusion of saline, followed (arrow) by infusion of bile salt at 1, 5, 10 or 20 μmoles min^{-1} kg^{-1}. A continuous infusion of saline at 49 μl min^{-1} did not change the initial basal bile flow and, for clarity, this is not included on the figure.

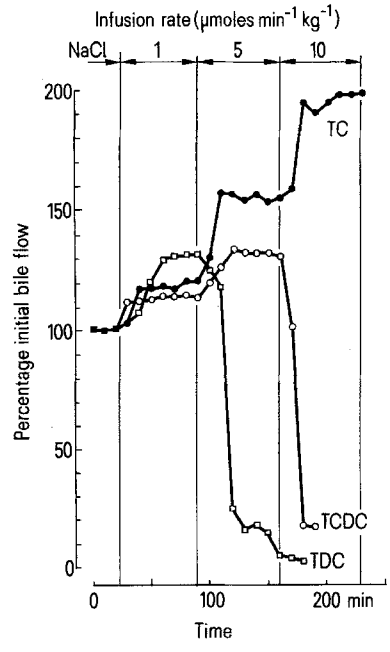

Fig. 2. The effect on bile flow of incremental bile salt infusion rates (1, 5, 10 μmoles min^{-1} kg^{-1}). The volume of solution infused was held constant at 54 μl min^{-1}. Panels represent the period of infusion at each rate.

Similar results were obtained when the infusion rate of the bile salt was increased in a step-wise fashion (figure 2). Increments of bile flow of a similar order of magnitude were observed with TC infusion. The onset of the cholestatic response to TCDC and TDC occurred immediately after switching the infusion rate to that which produced cholestasis in the previous experiment.

Discussion. It appears that the cholestatic effect observed in the present work is more dependent upon the rate of infusion of bile salt than on the absolute amount administered over a period of time. For example, it was possible to infuse 1 mmole kg^{-1} of TCDC at 5 μmoles min^{-1} kg^{-1} and observe only the choleretic effects; however, less than 0.1 mmoles kg^{-1} TCDC infused at 10 μmoles min^{-1} kg^{-1} produced an immediate drop in bile flow (figure 1). This effect was also true for TDC and TC and is most clearly seen in the experiment where rats received a single bile salt at differing infusion rates (figure 2).

Bile salts are actively transported into bile, and it is tempting to speculate that the decreases in bile flow were the result of the transport maxima (T_m) of the various bile salts being exceeded, thereby allowing toxic levels of the bile salts to be accumulated in the liver. Such a mechanism is feasible, for in rats the V_{max} of hepatic uptake of TC (1.2–2.0 μmoles min^{-1} g^{-1} liver)[13,14] is greater than the excretory T_m into bile (0.22–0.35 μmoles $min^{-1}g^{-1}$ liver)[15-17], hence hepatocyte levels of TC may become markedly elevated during infusions which exceed the T_m of TC. We were unable to find T_m estimates of TDC and TCDC for the rat in the literature; indeed, determination of T_m's for the more toxic dihydroxy bile salts would be difficult because the infusion rates required to produce T_m conditions also cause marked cholestasis and haemolysis. However, based on the present study, we would estimate the excretory T_m of TDC and TCDC into bile to be of the order of 1–5 and 5–10 μmoles min^{-1} kg^{-1} b.wt, respectively, and the T_m for TC to be approximately 10–20 μmoles min^{-1} kg^{-1} which agrees quite well with the published T_m for TC (11–13.5 μmoles min^{-1} kg^{-1})[15-17]. Although marked haemolysis was produced during cholestatic infusions of bile salts, the decrease in bile flow observed in these experiments was not a result of excessive haemoglobin load on the liver since infusions of distilled water, at rates which produced greater haemolysis than the bile salts, had no effect on bile flow.

Klaassen[8] showed that there was no apparent correlation between the detergency of a bile salt in terms of its ability to form micelles and its choleretic potency. However, our results suggest that detergency may be an important factor in the cholestatic response. Bile salts are known to cause cell lysis, and the order of potency in producing haemolysis[18] and their ability to inhibit enzymes of liver plasma membranes[19] is the same order as their cholestatic potency (DC > CDC > C). In addition, the relative cholestatic potencies of TDC, TCDC and TC are similar to their relative destructive properties for the lipoproteins associated with cytochrome P_{450}[20,21]. This last observation is the focal point for the hypothesis on the aetiology of intrahepatic cholestasis proposed by Schaffner and Popper[22], although it is not known how, or even if, such an effect could result in cholestasis[4].

In conclusion, our results show that bile salt overload can lead directly to cholestasis in the rat. Whether or not this effect is important in initiating cholestasis in man is uncertain. It does seem likely, however, that reduction in the capacity of the liver to excrete bile salts, resulting in their

intrahepatic accumulation, would be deleterious in a cholestatic or pre-cholestatic condition. In this respect, the capacity of the liver to hydroxylate mono- and di-hydroxy bile salts to the less toxic tri-hydroxy forms is a useful protective mechanism. The fact this metabolic pathway is more active in the rat than in man suggests that toxic effects of dihydroxy and monohydroxy bile salts could be expected at a low order of concentration in man unless this is balanced by a greater capacity to excrete the accumulated bile salts.

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Localization of substance P-like immunoreactivity in *Hydra*

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Summary. Substance P-like immunoreactivity was found in *Hydra attenuata* mainly but not exclusively in the nerve and interstitial cells, localized in the cytoplasm and on the cell surface membranes.

We have recently reported that substance P (SP) strongly stimulated hydra head regeneration[2], and the present immunological investigation was undertaken as a first attempt to establish if under normal conditions SP is detectable in this animal. SP is a small peptide mainly present in nerve[3] and intestinal cells[4] of all animals in which it has been looked for, but its presence in hydra has not yet been reported.

Materials and methods. Hydra attenuata were mass cultured in hydra medium[5] and fed once a day with *Artemia salina nauplia.* 3 sets of animals were examined. The 1st set was quick frozen in liquid nitrogen and sectioned at 8 µm with a cryo-cut microtome. The 2nd set was fixed in 4% formaldehyde in hydra medium for 3 h at 4 °C, dehydrated and embedded in paraffin wax. The 3rd set was freeze-dried, fixed with formaldehyde vapour at 60 °C and embedded in paraffin wax. To reveal the presence of SP, an indirect immunofluorescence technique[6] was employed. The sections were incubated 30 min at 37 °C in a moist chamber with anti-SP rabbit serum as 1st layer. The linkage of I^{125} labelled SP with this serum was 42% at 1:10000 serum dilution. The sections were rinsed 3×5 min with phosphate-buffered saline and reincubated with fluorescein-conjugated sheep anti-rabbit globulins (Gibco) as 2nd layer. In control experiments, the 1st layer stage was performed 1. with normal rabbit serum, 2. with anti-SP serum absorbed with newt liver powder, 3. with anti-SP serum absorbed with synthetic SP (Sigma); or 4. only the 2nd layer was applied. After rinsing 3×5 min with phosphate-buffered saline, the sections were mounted in buffered glycerin and examined in a Zeiss fluorescence microscope equipped with an HBO W lamp, a Zeiss 427902 exciting filter and a Zeiss 427903 stop filter. Photomicrographs were taken on Kodak Tri-X-Pan or Ektachrome 200 films. Counterstaining of the same sections was carried out either with hematoxylin-eosin or with silver stain. The best results were obtained with the cryostat sections.

Results and discussion. A bright green specific fluorescence appeared mainly in the ectodermis (figure, A, B, D). This SP-like immunoreactivity was present on the surface membrane and in the cytoplasm but not in the nuclei of nerve cells, interstitial cells, some large stem cells (figure I, B) and on the surface membrane of cnidoblasts. Some large gastrodermal cells also exhibited the specific fluorescence (figure, C). Most of the gastrodermis was filled with faint yellow fluorescent dots, possibly corresponding to fatty elements. The mesoglea, the central part of the cnidoblasts as well as most gastrodermal and some ectodermal cells, were devoid of fluorescence. With normal rabbit serum as 1st layer, or use of the 2nd layer alone, only a faint unspecific yellow fluorescence of the gastrodermis could be seen. Absorption of the antiserum with liver powder did not change the distribution of the specific green fluorescence, while use of the SP absorbed antiserum completely abolished it.

These results show that SP is present in *Hydra attenuata,* and moreover the SP-like immunoreactivity is not restricted to nerve cells but, to judge from the intensity of the fluorescence, SP could be abundant. The walls of most of the ectodermal cells appear rich in SP-like immunoreactivity, and this observation may be related to the previous report by Lentz et al.[7] that, where a nerve cell extension is contiguous with an ectodermal cell, it often contains neurosecretory granules situated adjacent to the plasma membrane. When isolated, these neurosecretory granules are able to induce supernumerary heads on regenerating gastric pieces of hydra[8]. Moreover they are known to contain