Spontaneous reversal of ethinyl estradiol-induced cholestasis in the rat[1]

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Summary. The spontaneous reversal of ethinyl estradiol-induced cholestasis has been documented 7 days after the last estrogen administration in the rat. This finding supports the hypothesis that estrogens produce only a transient functional failure of the hepatocytic structures responsible for bile secretion.

In rats ethinyl estradiol (EE) induces intrahepatic cholestasis, mainly reducing the bile salt independent fraction of bile (BSIF)[3], and modifies biliary lipid excretion rates[4]. According to Heikel and Lathe[5], the liver has to be stimulated for some days before the inhibitory effect of estrogens on bile secretion can be evoked. The reversibility of EE-induced alterations of bile secretion has been suggested[3], but it is not yet supported by experimental evidence. Moreover the time interval which precedes bile flow normalisation is unknown. We have therefore investigated the effects of 5 days EE treatment on bile secretion 1 and 7 days after the last EE administration in the rat.

Materials and methods. Female Sprague-Dawley rats (Charles River, Italy) weighing about 180 g were housed in wire mesh cages in a well ventilated vivarium with 12 h of light and fed Purina Lab Chow with water ad libitum. EE (17α-ethinyl estradiol, Sigma, St. Louis, MO) dissolved in 2% gum Arabic was administered orally daily by gavage in a dosage of 5 mg/kg b.wt for 5 days. Control animals were handled similarly except that no EE was administered with the vehicle. 1 or 7 days after the last estrogen administration, the EE-treated rats were anesthetized with urethane (1 g/kg b.wt, i.p.): the common bile duct was cannulated with PE-50 polyethylene tubing (Clay Adams, Parsippany, N.J.).

Bile secreted in the 1st 2 h following laparotomy was collected and measured by weight to obtain the bile flow rate. During bile collection, the body temperature was measured by a rectal thermometer and maintained at 36.5-37 °C using a warming lamp. Biliary total bile salts were determined enzymatically by the method of Talalay[6]; cholesterol and phospholipid concentrations were measured according to the methods of Roschlau et al.[7] and Svanborg and Vikrot[8], respectively. The statistical analysis was obtained by Student's t-test; regression lines were computed by the least squares method and compared for differences in slope and intercept as reported by Snedecor and Cochran[9].

Results. As shown in figure 1, EE induced a significant decrease of bile flow and biliary lipid excretion rates in comparison to control values. The reduced bile salt output was consistent with the decrease of the bile salt dependent fraction of bile. 7 days after the last EE administration bile flow, bile salt, cholesterol and phospholipid excretion rates were similar in control and EE-treated rats. In order to assess changes of BSIF, the correlation between bile flow and bile salt secretion was compared (figure 2). The correlation coefficient was significant in all groups of animals with no difference in the slope of the regression lines. By extrapolation to zero of bile salt excretion, a BSIF of 0.61, 0.26 and 0.81 µl/min/g liver was obtained in control and EE-treated animals investigated 1 and 7 days after the last dose of the estrogen, respectively. A significant difference ($p < 0.05$) was found when the BSIF calculated for EE-treated animals was compared with those of controls and EE-treated animals investigated 7 days after the last EE administration.

Discussion. From the results reported, it appears that EE at the given dose decreases bile flow by inhibiting both the bile salt dependent and independent fractions of bile. The reduced biliary output of cholesterol and phospholipids is correlated to the decrease of bile salt secretion

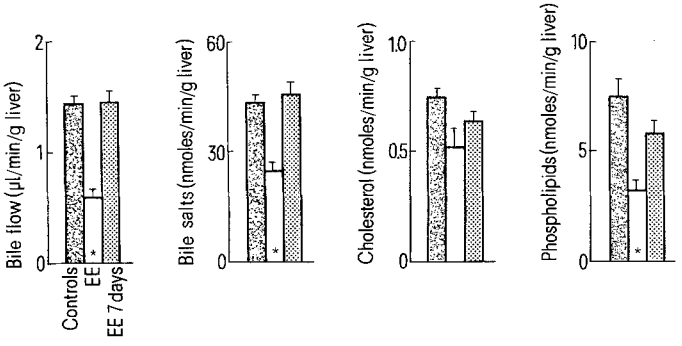

Fig. 1. Effects of ethinyl estradiol administration on bile flow and biliary lipid excretion rates in female rats 1 and 7 days after the last estrogen dose. Values are expressed as mean ± SE for 6 animals. * $p < 0.01$ vs controls and EE-treated rats investigated 7 days after the last estrogen administration.

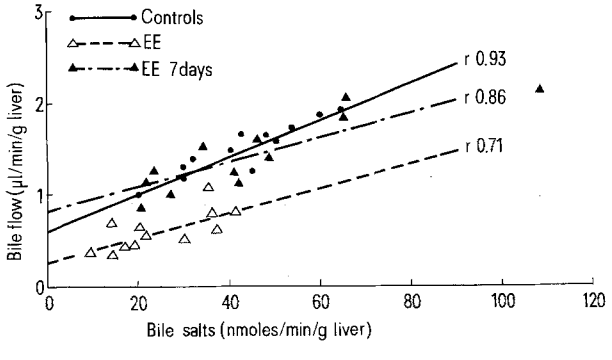

Fig. 2. Relationship between bile flow and bile salt excretion in control rats and in rats treated with ethinyl estradiol investigated 1 and 7 days after the last estrogen administration. Each point represents a 1 h bile sample during 2 h bile collection period. Regression equations, calculated by the method of least squares, were: $y = 0.61 + 0.020\,x$ for controls; $y = 0.26 + 0.014\,x$ and $y = 0.81 + 0.013\,x$ for rats investigated 1 and 7 days after the last ethinyl estradiol dose, respectively.

which promotes biliary lipid excretion by formation of micellar complexes[4].

The present data indicate the complete reversal of EE-induced cholestasis and related biliary lipid abnormalities 7 days after the last dose of the estrogen, and confirm the ability of this hormone to affect only transiently the mechanisms of bile formation. To the substances, i.e. phenobarbital[10], S-adenosyl-L-methionine[11], Triton WR-1339[12], which by different mechanisms inhibit the effects of EE on bile secretion, we can add the possibility that a lag period of only some days reverses EE-induced bile flow impairment. This finding is consistent with clinical reports in humans which demonstrate that recurrent cholestasis of pregnancy resolves spontaneously approximately 2 weeks after delivery when endogenous gonadal and placental derived estrogens decrease[13]. Our data support the hypothesis[14] that EE-induced cholestasis depends by a transient failure of the canalicular pumps responsible for bile salt and sodium active transports with no irreversible damage to the hepatocytic structures involved in bile formation.

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Naltrexone influence on hibernation

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Summary. In the garden dormouse, opiate receptor blockade by naltrexone decreased the score for sleeping behaviour during hibernation at 24.00 h, indicative of a possible involvement of endorphins in the control of hibernation.

In a preliminary open study, the influence of opiate receptor blockade on hibernation was investigated in the garden dormouse (Gartenschläfer; *Eliomys quercinus*). This investigation was prompted by the observation that various changes in body functions (such as sensitivity to pain, respiratory function and circulation) which occur during hibernation, may also be caused by endogenous ligands of opiate receptors, endorphins (for review[2,3]).

During November 1978, 2 groups of animals (body weight 80–110 g; 4 animals in each group, housed individually at a normal dark-light-rhythm at 21 °C) were injected i.p. 4 times a day at 06.00 h, 12.00 h, 18.00 h and 24.00 h with the opiate antagonist naltrexone (25 mg/kg) or saline, volume 0.1 ml. The relatively high dosage of naltrexone was chosen to get a reliable occupancy of opiate receptors throughout each 6-h interval. 2 days after having started the injection schedule the animals were housed in darkness at 4 °C and deprived of food. The first 3 days of hibernation conditions (adaptation period) and the following 5 experimental days were evaluated separately. Altogether, the injection schedule was run for 10 days. Each animal was scored 5 min prior to and 10 min after each injection. Hibernation was rated as follows: Animals which were wide awake (eyes open, fast escape movements) received score 0, those which were sleepy (eyes open, but no escape movements or eyes closed, slow rigid movements) received score 1, and those which were sleeping (typical hibernation posture, as shown in figure 1) received score 2.

Injections were stopped after 10 days; all animals were again housed at a normal dark-light-rhythm at 21 °C, and

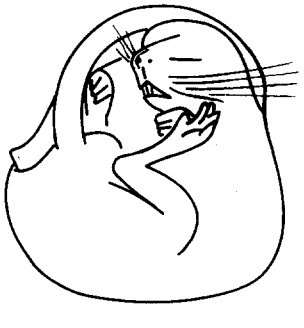

Fig. 1. Typical hibernation posture of the garden dormouse.

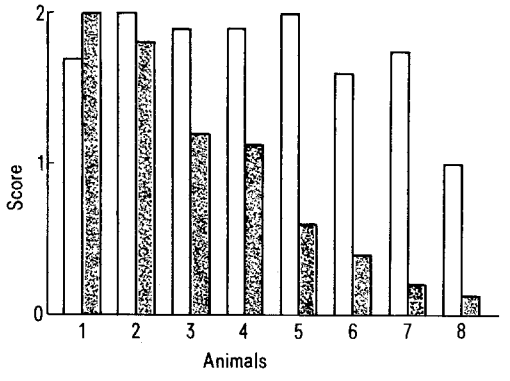

Fig. 2. Score for sleeping behaviour at 24.00 h. Mean values from 5 experimental days, starting at the end of the period of adaptation to hibernation conditions. Animals are ordered according to the increasing magnitude of effect. ☐ Saline treatment, ▨ naltrexone treatment.