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EFFECTS OF A COMBINED OESTROGEN-PROGESTIN PREPARATION ON GASTRIC ACID AND PEPSIN SECRETION, SERUM GASTRIN CONCENTRATION AND BILIARY SECRETION OF BILE ACIDS, PHOSPHOLIPIDS, AND CHOLESTEROL IN THE CAT

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1 Daily ethinyloestradiol (50 μg) and norethisterone acetate (1 mg) treatment (Minovlar) was investigated on gastric acid and pepsin secretion, and fasting serum gastrin concentration in six conscious female cats prepared with chronic gastric fistulae. The effect on biliary secretion of bile acids, phospholipids, and cholesterol was investigated in three conscious female cats prepared with chronic gastric and intestinal fistulae, and cholecystectomy.

2 Treatment for 49 days did not alter the gastric acid or pepsin response to either intravenous pentagastrin infusions or a food stimulus. The fasting serum gastrin concentration remained unaltered throughout the study.

3 Treatment for 18 days did not alter the percentage concentration of cholesterol in the bile, but reduced the percentage concentration of phospholipid. This was mirrored by a rise in the percentage concentration of bile acids in the bile. These trends were quickly reversed on cessation of treatment.

4 There was no sign of cholestasis associated with the treatment. Intestinal flow remained constant throughout the study, there was no lithocholic acid or other abnormal bile acids detectable in any samples, and there was no change in serum aspartate aminotransferase concentration.

5 The results suggest that in female cats, treatment with a combined oestrogen-progestin preparation does not exert any beneficial effects on the aetiology of peptic ulceration through the reduction of acid or pepsin secretion, or the lowering of serum gastrin concentration. The preparation shows a tendency to produce more lithogenic bile, and this may partly explain the greater incidence of gall stones in women on the contraceptive pill.

Introduction

Pregnancy and female sex hormones, including components of the contraceptive pill, exert effects on the hepatobiliary system (Song & Kappas, 1968; Fisher, Price & Yousef, 1976), and on gastric function (Crean, 1963). Epidemiological studies have clearly shown that women treated with oral contraceptives or synthetic oestrogens have an increased incidence of gallstones (Boston Collaborative Drug Surveillance Programme, 1973; 1974), and cholesterol gallstones are found more frequently in premenopausal women than in men (Nilsson, 1966). Clinical studies have shown that ingestion of oral contraceptives by women leads to an increase in the cholesterol saturation index of their bile, and that this index falls when women stop taking oral contraceptives (Bennion, Ginsberg, Garnick & Bennett, 1976). Laboratory work with animals has revealed that ethinyloestradiol, a component of many contraceptive pill preparations, causes cholestasis and other effects in the rat (Gumucio & Valdiviesco, 1971; Davis & Kern, 1976).

In contrast to these potentially pathological effects on the liver and biliary systems, female sex hormones appear to exert a beneficial effect with respect to gastric function. During their reproductive period of life women are much less prone to peptic ulceration than are men, and pregnancy is reported to afford in-

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creased protection against such ulceration (Crean, 1963). Female sex hormones have been reported to reduce gastric acid secretion in man (Parbhoo & Johnston, 1966), cat (Ohja & Wood, 1950; Albinus, Blair, Hirst, Grund, Reed, Sanders & Taylor, 1976) and rat (Amure & Omole, 1970). Endogenous female sex hormones appear to exert an effect on gastric secretion. In many species, including the cat (Hirst, Labib & Reed, 1978), the males secrete more gastric acid than females. Also serum and pyloric antrum gastrin concentrations are higher in male than in female rats, and these gastrin concentrations fluctuate during the oestrous cycle (Amure & Bolarinwa, 1975; Lichtenberger, Nance & Gorski, 1976). Contraceptive steroid preparations may also decrease the incidence of peptic ulceration in young women (Glober, Doll, Fairbairn & Vessey, 1971).

However, a definite relationship between the effects of steroidal contraceptives on hepatobiliary and gastric functions in the same species under the same experimental conditions has not been established. This study was undertaken to investigate the effect of a combined oestrogen-progestin contraceptive preparation on biliary and gastric secretion in female cats.

Methods

Gastric secretion and gastrin studies

Experiments were carried out in six conscious female cats prepared at least six months earlier with chronic cannulated gastric fistulae. The animals were deprived of food for 36 h before experiments but had free access to water. Each animal was investigated approximately twice each week, once with each of the stimuli.

Gastric juice was collected continuously by gravity drainage and divided into 15 min samples. The volume of juice was recorded and 1.0 ml samples were titrated electrometrically to pH 7.0 with 0.1 M NaOH (Radiometer, Copenhagen). The rest of the sample was stored at 4°C overnight and the pepsin activity estimated by a haemoglobin digestion method (Shaw & Wright, 1976). Acid secretion was calculated as μEquiv H^+ kg^{-1} body wt. 15 min^{-1} and pepsin secretion as μg pepsin kg^{-1} body wt. 30 min^{-1}.

Food stimulus: 50 ml of a 1/3 (w/v) homogenized and filtered mixture of a tinned cat food (Whiskas, Pedigree Pet Foods, England) and water, adjusted to pH 7.0, was offered to each animal in a shallow container to facilitate lapping. The food was allowed to remain in the stomach for 15 min, after which the stomach was drained and the gastric secretion collected for the next 45 min. Acid secretion was calculated as the total acid output for this hour of collection. The pepsin output was calculated as the total secreted during the first 30 min of stimulation as the sample volume obtained over the second 30 min was too small to estimate the pepsin concentration.

Pentagastrin stimulus Saline (0.9% w/v NaCl solution) was infused intravenously at a rate of 12 ml/h through an indwelling percutaneous needle (Butterfly 21G, Abbott Labs. Ltd., England) inserted in a cephalic vein. Pentagastrin (Peptavlon, I.C.I. Ltd., England), 1 μg kg^{-1} h^{-1} was added to the saline infusion for 45 min and then the dose of pentagastrin was doubled every 30 min up to a maximum of 16 μg kg^{-1} h^{-1}.

The acid output during the final 15 min of infusion of each dose of pentagastrin was taken as representative of that dose. Pepsin output during the final 30 min of infusion of each dose was determined.

Serum gastrin Blood samples were collected the morning after a 36 h fast, allowed to clot at room temperature, separated, and stored at −20°C. The serum gastrin concentration was estimated by radioimmunoassay as described previously by Blair, Grund, Lund, Piercy, Reed, Sanders, Shale, Shaw & Wilkinson (1977) with the same ^{125}I-gastrin tracer and antibody. The assay had a detection limit of 9 pg/ml serum. The antibody used cross-reacts equally with synthetic human gastrin 17NS (SHG17NS), SHG13NS (5-17), natural HG34NS, natural porcine G17S, synthetic cat gastrin 17NS and is 75 times less cross-reactive with pure natural porcine CCK-PZ. SHG17NS was used as the standard and the results are expressed as pg ≡ SHG17NS per ml serum. The serum gastrin concentrations in all the samples from any one animal were measured in a single assay to eliminate interassay variability.

Body weight The animals were weighed at frequent intervals throughout the study. Serum aspartate aminotransferase (ASP:EC 2.6.1.1.) was estimated before and during treatment as an index of liver function.

Biliary studies

Experiments were carried out in 3 conscious female cats prepared at least 3 months earlier with chronic cannulated gastric and intestinal fistulae. The intestinal fistulae were situated directly opposite the sphincter of Oddi and the animals had undergone a cholecystectomy. Gastric and intestinal juice were both collected by gravity drainage. The animals were starved for 36 h before experiments, but had free access to water. No more than two experiments were carried out each week. After a preliminary period of drainage, gastric and intestinal fluids were collected for 1 h and the intestinal volume was measured.

Bile acids were determined in 100 μl volumes of intestinal fluid by hydrolysis in Teflon-lined, stainless steel, screw-topped vessels with 6 ml of 15% NaOH in 50% aqueous ethanol for 10 h at 110°C. The hy-

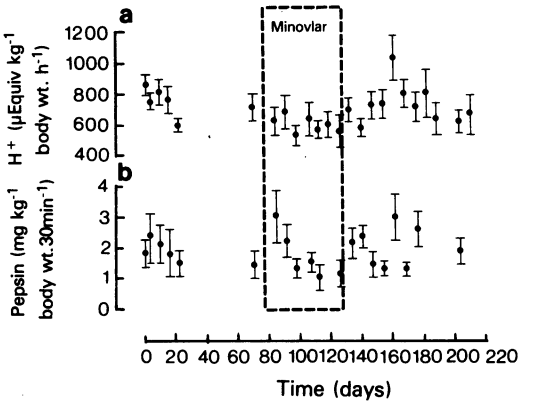

Figure 1 The mean acid (a) and pepsin (b) outputs in six cats in response to a food stimulus before, during and after treatment with Minovlar. Outputs are illustrated as mean; vertical lines show s.e. means.

drolysis medium was acidified and the free bile acids were extracted with ethyl acetate and methylated with freshly prepared diazomethane. The methylated bile acids were converted to trimethylsilyl ethers, and analysed by gas chromatography on 3.1 m × 4 mm glass columns of 1% HiEff 8 BP at 225°C; injection and detector temp., 250°C; carrier gas, N_2 (25 ml/min); flame ionization detection. Bile acids were quantitated by triangulation by reference to the trimethylsilyl ether of a standard solution of methylated cholic acid. No corrections were made for procedural losses. Full details of the method are described elsewhere (Taylor, 1977).

For phospholipid and cholesterol determinations, samples were subjected to the extraction procedure of Folch, Lees & Sloane Stanley (1957) within 1 h of collection. Freezing and thawing of the samples before Folch extraction gave inconsistent and poorly reproducible results. Phospholipid phosphorus was determined by the method of Bartlett (1959), and cholesterol by the method of Zlatkis, Zak & Boyle (1953) as modified by Rosenthal, Pfluke & Buscaglia (1957).

The results are expressed as μmol/ml intestinal fluid for the individual and total bile acids, phospholipid (as phosphorus) and cholesterol, and also as percentage of total bile lipids, *viz.* total bile acids, phospholipid and cholesterol. Lithogenic index was calculated by the method of Thomas & Hofmann (1973) using the equations derived from the data of Admirand & Small (1968), of Hegardt & Dam (1971) and Holzbach, Marsh, Olszewski & Holan (1973) (see Table 3).

Statistical analyses

Results are expressed as means ± 1 s.e. mean (n). Significance of difference was determined by analysis of variance followed by a two-tailed Student's t test. Significance was set at $P < 0.05$.

Experimental design

Minovlar tablets (1 mg norethisterone acetate [4-oestran-17α-ethinyl-17β-acetoxy-3-one] and 50 μg ethinyloestradiol [17α-ethinyl-17β-oestradiol], Schering Chemicals Ltd., England) were administered orally between 15 h 00 min and 16 h 00 min daily throughout the period of treatment. After a control period with no treatment, Minovlar was administered continuously for 49 days in the gastric studies and 18 days in the biliary studies. After completion of the treatment, gastric and biliary secretion studies were continued in order to observe any possible rebound phenomena as well as the time taken for recovery.

Results

No ill-effects were noted in any of the animals on Minovlar treatment. The animals showed no change in body weight.

Gastric secretion studies

There were no significant changes in either gastric acid or pepsin secretion in response to food stimulation (Figure 1) or in response to pentagastrin 1, 2, 4, 8 or 16 μg kg^{-1} h^{-1} (Figures 2 and 3) either during or after treatment with Minovlar. Serum gastrin concentration also remained unchanged during and after Minovlar treatment ranging from a mean concentration of 27 to 83 pg/ml (Figure 4). The third serum gastrin estimation on day 28, before treatment, was significantly higher than all the other estimations (Figure 4). The reason for this anomalous result is unknown. Serum ASP concentration was also not altered by Minovlar treatment in these animals (Table 1).

Biliary secretion studies

There was no sign of cholestasis associated with the Minovlar treatment, the mean intestinal flows being 2.5 ± 0.4(12), 2.3 ± 0.4(15) and 3.0 ± 0.6(6) ml/h before, during and after treatment respectively (Figure 5). The mean values for the concentrations of the individual and total bile acids are given in Table 2. Cholic acid was the predominant bile acid in almost all the samples, with smaller amounts of deoxycholic and chenodeoxycholic acid. Smaller peaks corresponding to the minor bile acids found in cat gall-bladder bile by Taylor (1977) were seen in some bile samples, but these amounted to less than 2 per cent of the total bile acids and are not included

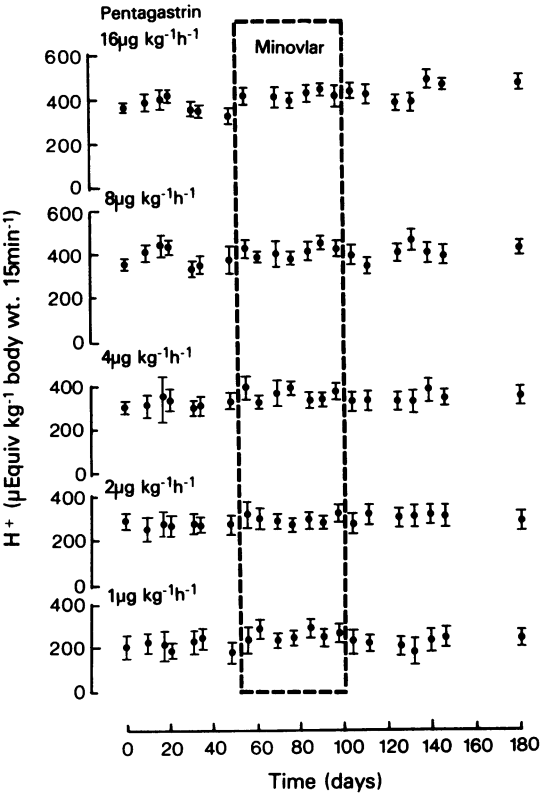

Figure 2 The mean acid output in response to intravenous infusion of pentagastrin 1, 2, 4, 8 and 16 μg $kg^{-1}\,h^{-1}$ in the same six cats before, during and after Minovlar treatment. Pentagastrin 1 μg $kg^{-1}\,h^{-1}$ was infused for 45 min and the dose doubled every 30 min. The acid secretion during the last 15 min of infusion of each dose of pentagastrin is illustrated in the graph as the mean output; vertical lines show s.e. means.

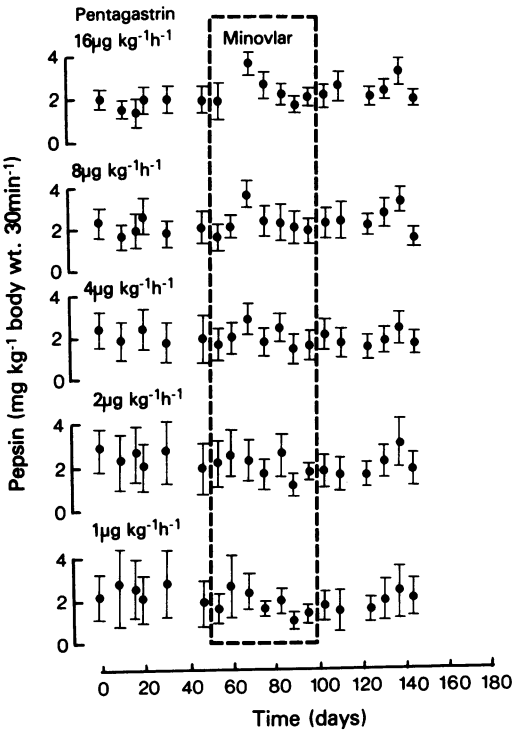

Figure 3 The mean pepsin output in response to intravenous infusion of pentagastrin 1, 2, 4, 8 and 16 μg $kg^{-1}\,h^{-1}$ in the same six cats before, during and after Minovlar treatment. The experiments are the same as those illustrated in Figure 2.

in the calculation of total bile acids. No peak corresponding to lithocholic acid was observed in any of the samples. There was no consistent or significant change in individual or total bile acids before and during treatment, when the results were expressed as μmol/ml of bile. However, when the total bile acids were expressed as a percentage of the total lipids in the sample (see Figure 6), the mean value before treatment (55.2 ± 4.9%) was significantly lower than the value during treatment (67.3 ± 1.9%). Table 2 shows the mean concentrations (μmol/ml) of phospholipid and cholesterol in the samples from the three cats. There were no significant differences in the concentrations of these lipids before and during treatment. When these results were expressed as percentage of total biliary lipids, the cholesterol values before treatment (12.2 ± 0.9%) were not significantly different from those during treatment (12.7 ± 1.3%). On the other hand, the pretreatment value for phospholipids (27.6 ± 1.5%) was significantly higher than the value during treatment (18.5 ± 1.5%). These values were calculated, using Student's *t* test, from the 12 values obtained before treatment, and the 15 values obtained during treatment.

It is apparent from Figure 6 that there is an inverse relationship between the percentages of total bile acids and phospholipids. This is statistically significant ($r = -0.96$). There was no significant correlation ($r = -0.51$) between the total bile acids and cholesterol values, nor between those of phospholipid and cholesterol ($r = 0.33$). When treatment was stopped there was a fall in the percentage of total bile acids (71.3 ± 2.9% to 64.7 ± 2.2%) which was not significant, whereas the percentage rise in phospholipids after stopping treatment (17.5 ± 0.9% to 25.1 ± 0.7%) was significant. There was no significant difference between the cholesterol values (11.1 ± 2.1% and 10.2 ± 2.0%).

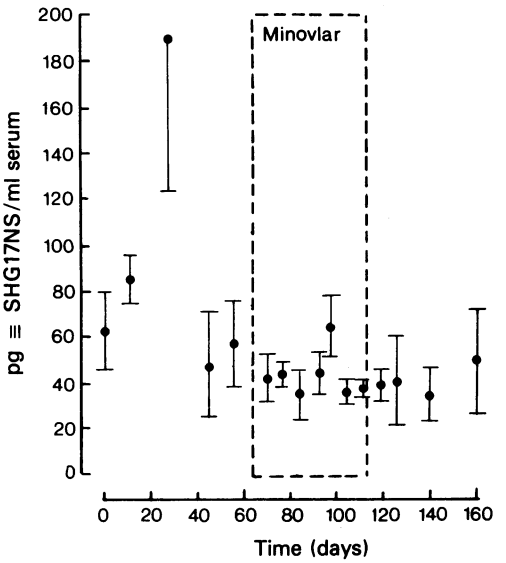

Figure 4 Fasting serum gastrin concentration in the same six animals before, during and after Minovlar treatment. Serum gastrin concentrations are illustrated as mean in pg ≡ SHG17NS/ml serum; vertical lines show s.e. means.

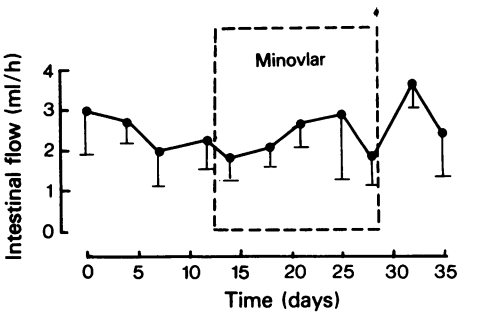

Figure 5 Mean intestinal flow rates in three cats before, during and after Minovlar treatment. Results are illustrated as mean flows; vertical lines show s.e. means.

Discussion

Gastric studies

There is much epidemiological and experimental evidence that exogenous and endogenous female sex hormones have a beneficial effect on the aetiology of peptic ulceration, and that this effect may be mediated by reduction of gastric acid secretion (Ojha & Wood, 1950; Crean, 1963; Parbhoo & Johnston, 1966; Amure & Omole, 1970; Landor & Wild, 1970; Omole, 1972; Albinus *et al.*, 1976). However, there are many reports where no or inconclusive effects of female sex hormones have been shown (Parbhoo & Johnston, 1966; Doll, Langman & Shawdon, 1968; Kauffmann & Spiro, 1968; Landor & Wild, 1970). Since most studies have been concerned only with oestrogenic compounds we felt that the effects of a combined oestrogen-progestin preparation on gastric secretion should be investigated.

A preliminary report from this laboratory indicated that daily Minovlar treatment reduced the pepsin response to pentagastrin, although, in contrast, the pepsin response to a food stimulus was raised. The acid output in response to food stimulation was decreased, but that to pentagastrin stimulation was unaltered. Fasting serum gastrin concentration fell throughout the study (Albinus *et al.*, 1976). The results from the current more detailed and controlled study do not substantiate these earlier findings (Figures 1–4). However, the previous studies were carried out on four male and two female cats. Male cats have been shown to secrete greater amounts of acid than females (Ojha & Wood, 1950; Hirst *et al.*, 1978), and this difference is not entirely explained by differences in body weight (Hirst *et al.*, 1978). Therefore, it is possible that the gastric acid reduction seen in the previous study in response to exogenous female sex hormones is masked in the present study by the presence of the endogenous female sex hormones. This is supported by the reports that ovariectomy causes an increase in acid secretion in the cat (Ojha & Wood, 1950) and the rat (Omole, 1972). A similar argument might be used to explain the lack of change in serum gastrin concentration (Figure 4) compared with that seen in the earlier study (Albinus *et al.*, 1976). Male rats have higher serum gastrin concentration than females and

Table 1 Serum aspartate aminotransferase before and during treatment with Minovlar

Days of treatment	*Aspartate aminotransferase (mean ± s.e. mean) u/l*
Nil	14.3 ± 1.9 (6)
21	11.5 ± 1.3 (6)
48	12.7 ± 0.8 (6)

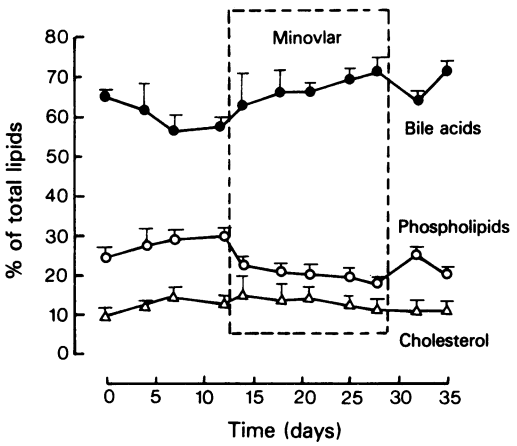

Figure 6 Concentration of bile acids (●), phospholipids (○) and cholesterol (△) in duodenal fluid expressed as a percentage of the total bile lipids before. during and after Minovlar treatment. Results are illustrated as mean percentages in three cats; vertical lines show s.e. means.

ovariectomy abolishes this difference. Oestradiol treatment of ovariectomized rats reduces the serum gastrin concentration towards that of normal females (Lichtenberger *et al.*, 1976). Similar sex differences have been noted in the gastrin concentration of the antral mucosa (Amure & Bolarinwa, 1975; Lichtenberger *et al.*, 1976).

It is clear from the present study that the combined oestrogen-progestin contraceptive pill used had no significant effect on gastric function in the female cats when administered at doses around 20 times that used in the human female. However, it should be noted that the amount of ethinyloestradiol given to the animals is much less than the doses of oestrogenic compounds used by other workers (Ojha & Wood, 1950; Amure & Omole, 1970; Landor & Wild, 1970), some of which are above the usual pharmacological range.

The possible mechanism whereby steroidal contraceptives might exert a beneficial effect on the aetiology of peptic ulceration cannot be answered from this study. It appears that any beneficial effect is not due to a direct action on gastric acid, pepsin or gastrin secretion. It is possible that the protective effects might be due to the stimulation of mucus production (Parbhoo & Johnston, 1966; Albinus *et al.*, 1976) or increased epithelial cell turnover (Crean & Rumsey, 1971) but further investigations will be necessary to provide evidence for these views.

Biliary studies

Female sex hormones have widespread biochemical and physiological effects on the hepatobiliary system (Song & Kappas, 1968). In the rat ethinyloestradiol

Table 2 Individual and total bile acids (μmol/ml), phospholipid (μmol/ml) and cholesterol (μmol/ml) in duodenal bile of three female cats before, during and after treatment with Minovlar

	Days before treatment				*Days of treatment*					*Days after treatment*	
	12	*8*	*5*	*0*	*3*	*7*	*10*	*14*	*17*	*4*	*7*
Cholic acid											
mean	8.83	6.57	5.33	4.87	7.06	8.33	5.40	9.30	6.80	8.47	9.10
s.e. mean	2.81	2.03	0.68	0.26	2.43	2.56	1.53	2.99	0.92	1.77	1.80
Deoxycholic acid											
mean	1.83	3.50	2.20	1.93	2.40	2.43	3.80	4.10	2.40	2.07	4.03
s.e. mean	0.32	0.74	0.44	0.42	0.25	0.66	0.12	2.49	0.17	0.18	0.47
Chenodeoxycholic acid											
mean	0.37	0.80	0.83	1.00	1.03	0.53	0.67	3.10	0.80	0.57	1.03
s.e. mean	0.18	0.06	0.03	0.17	0.09	0.19	0.09	2.56	0.10	0.07	0.32
Total bile acids											
mean	11.03	10.87	8.37	7.80	10.50	11.38	9.90	16.50	10.00	11.10	14.17
s.e. mean	3.14	1.59	1.08	0.80	2.09	3.39	1.46	7.90	1.00	1.73	2.44
Phospholipid											
mean	4.37	4.90	4.40	4.00	3.43	3.33	3.00	3.40	2.47	4.40	3.33
s.e. mean	1.72	1.40	0.91	0.35	0.27	0.27	0.52	1.30	0.30	0.87	0.55
Cholesterol											
mean	1.49	2.10	2.15	1.74	2.08	1.84	1.92	3.07	1.56	1.82	2.08
s.e. mean	0.18	0.50	0.39	0.30	0.38	0.24	0.16	1.83	0.31	0.53	0.38

Results are means ±s.e. means (n = 3).

causes cholestasis (Forker, 1969; Davis & Kern, 1976; Kern, Eriksson, Curstedt & Sjövall, 1977), and at least part of this diminution in bile secretion is due to the inhibitory effects of oestrogens on the bile salt independent bile flow (Gumucio & Valdiviesco, 1971). In the present investigation there was no evidence of a significant dimunition of bile secretion, since the amount of intestinal fluid collected over 1 h was 2.5 ± 0.4 ml (12) before, and 2.3 ± 0.4 ml (15) during treatment (Figure 5). However, we cannot equate these flow rates solely with biliary secretion as the intestinal fluid collected was a mixture of bile with pancreatic and duodenal secretions. However, the absence of lithocholic and other abnormal bile acids from the intestinal samples, and the constant values for serum ASP (Table 1) support the view that intrahepatic cholestasis did not occur in these cats.

Oestrogens have marked effects on biliary lipid composition in man and animals. The most significant effect of oestrogens and contraceptive steroids is a tendency for the bile to become supersaturated with cholesterol, thus increasing the possibility of cholesterol gallstone formation. This cholesterol supersaturation is usually expressed by the lithogenic index, derived from the relative proportions of cholesterol, phospholipid and bile acids in the bile. Thus Bennion *et al.* (1976) showed the lithogenic index of human female bile was increased during, and decreased after oral contraceptive treatment. Furthermore, Pertsemlidis, Panveliwalla & Ahrens (1974) reported an increase in biliary cholesterol concentration in women using oestrogen-progestin preparations. This was accompanied by a decrease in bile acid concentration. These data are compatible with the epidemiological studies which show that contraceptive pill users have a higher incidence of gallstones than non-users (Boston Drug Surveillance Programme, 1973). In contrast to these effects in humans, ethinyloestradiol causes a decrease in the synthesis and biliary secretion of cholesterol in the rat (see Davis, Showalter & Kern, 1977) and a decrease in biliary cholesterol concentration in baboons (Morrissey, Panveliwalla, McSherry, Dietrick, Niemann & Gupta, 1977). The cholesterol concentration in the baboon bile was further reduced by the addition of norethisterone to the oestrogen, although the progestin itself had no effect (Morrissey *et al.*, 1977). We found no change in the cholesterol concentration of duodenal fluid during Minovlar treatment in the cat (Table 2). The saturation index for the cat biles before and during treatment was not significantly different when calculated with the equation of Admirand & Small (1968), but was significantly raised when calculated using the equations of Hegardt & Dam (1971) and Holzbach *et al.* (1973) (Table 3). Nevertheless, it is obvious that ethinyloestradiol, with or without a synthetic progestin, produces different effects in different species, the cat behaving more like the human than does either the baboon or rat.

As shown in Table 3, the phospholipid/bile acid (PL/BA) ratio decreased during Minovlar treatment, whereas the cholesterol/bile acid (CH/BA) ratio did not change. In contrast, the CH/PL ratio increased during treatment. The increase in the CH/PL ratio and the decrease in the PL/BA ratio is consistent with the concept that female sex hormones exert their lith-

Table 3 Cholesterol saturation index, and phospholipid (PL), bile acid (BA) and cholesterol (CH) ratios of bile samples before and during treatment with Minovlar (calculated from data in Figure 6)

	Days before treatment				*Days of treatment*				
	12	*8*	*5*	*0*	*3*	*7*	*10*	*14*	*17*
Cholesterol saturation index									
From equation of Admirand & Small (1968)	0.97	1.13	1.51	1.33	1.43	1.25	1.32	1.17	1.15
Mean (s.e. mean)		1.24 (0.12)				1.26 (0.05)			
From equation of Hegardt & Dam (1971) and Holzbach *et al.* (1973)	1.34	1.48	1.76	1.53	2.03	1.84	1.91	1.84	1.89
Mean (s.e. mean)		1.54 (0.09)				1.90 (0.03)*			
Lipid ratios									
PL/BA	0.38	0.44	0.51	0.52	0.35	0.32	0.30	0.28	0.25
Mean (s.e. mean)		0.46 (0.006)				0.30 (0.04)*			
CH/BA	0.15	0.19	0.26	0.22	0.23	0.19	0.21	0.17	0.16
Mean (s.e. mean)		0.21 (0.02)				0.19 (0.01)			
CH/PL	0.40	0.43	0.50	0.43	0.66	0.60	0.68	0.61	0.64
Mean (s.e. mean)		0.44 (0.02)				0.64 (0.01)*			

* Value during treatment significantly different from pretreatment value.

ogenic effect by causing a decrease in the concentration of phospholipid (Figure 6) relative to that of cholesterol, i.e. an increase in the lithogenic index (see above). However, this cannot be the only explanation for the increase in lithogenic index seen during contraceptive steroidal treatment, as Pertsemlidis *et al.* (1974) have shown there is an absolute increase of cholesterol concentration in women.

The data illustrated in Figure 6 strongly suggest that in the cat there is an inverse relationship between bile acid and phospholipid concentrations in the bile. To our knowledge, such a close negative correlation between biliary phospholipids and bile acids has not been reported previously. This information taken with increase in the CH/PL ratio without a corresponding change in the CH/BA ratio, suggests that the steroidal preparation given to the cats caused a decrease in phospholipid content of the bile and this was compensated for by an increased production of bile acids. We realise that other interpretations of our data are possible.

The doses of steroidal contraceptives used in this study (approx. 25 $\mu g\ kg^{-1}\ day^{-1}$ ethinyloestradiol) are large when compared to those used in humans. However, our results are more likely to be relevant to human studies than those in the rat where 40–200 times more (1 to 5 $mg\ kg^{-1}\ day^{-1}$ ethinyloestradiol i.p.) steroid was used (Forker, 1969; Davis & Kern, 1976; Kern *et al.*, 1977). Furthermore, it is possible that steroids may produce changes in biliary secretion in one direction when used in physiological/pharmacological concentrations, whilst opposite changes may be induced by toxicological concentrations.

In conclusion, an oral contraceptive in doses approximately 20 times those used by women does not have large effects on gastric or biliary secretions in the cat. Whether the small effects on biliary secretion noted here would be of any significance in the epidemiology of gallstone formation, or would become more pronounced with longer periods of treatment, remains to be established.

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