Adaptation of biliary response to dietary olive oil and sunflower-seed oil in dogs

BY M. C. BALLESTA, M. MAÑAS, E. MARTINEZ-VICTORIA, I. SEIQUER, J. R. HUERTAS AND F. J. MATAIX

Instituto de Nutrición y Technología de Alimentos and Departamento de Fisología, Facultad de Farmacia, Universidad de Granada, Granada, Spain

(*Received 13 June 1990 – Accepted 1 August 1991*)

The effects of adaptation to dietary fat of different degrees of unsaturation (olive oil and sunflower oil) on bile secretion were studied in dogs at rest and after food intake. The animals were prepared with a bidirectional biliary cannula and a duodenal cannula to provide bile return. The two experimental groups were fed on diets containing 150 g fat/kg in the form of either olive oil (O) or sunflower-seed oil (S). The flow-rate under resting conditions and the patterns of response to food were similar in both experimental groups, although postprandial hypersection were significantly greater in volume and more prolonged in group O. No appreciable differences in concentration and output of biliary cholesterol or phospholipids were noted between the two groups. In contrast, the concentration and output of bile acids differed significantly both at rest and after food: concentration and output of bile acids were greater at rest in group S. However, after food intake, these responses were increased only in group O. The results suggest that the type of dietary fat affects biliary response to food, probably through differences in the contribution of the gall bladder in the two experimental groups.

Dietary fat: Biliary secretion: Dog

The arrival of food in the dog digestive tract is known to raise the pressure and rate of emptying of the gall bladder. Emptying peaks at 30 min postprandial and concludes 2 h after food intake (Traynor *et al.* 1984). At the same time, bile acid output rises significantly and remains elevated for 4 h following food intake (Traynor *et al.* 1984).

A number of studies claim that the presence of specific nutrients, i.e. fat, affects bile secretion. Ladas *et al.* (1984) studied the effects of dietary long-chain triacylglycerols (LCT) and medium-chain triacylglycerols (MCT) in humans, and observed that the former more markedly raised the flow-rate of bile. Dietary long-chain triacylglycerols have also been reported to increase the release of cholecystokinin (CCK) (Malagelada *et al.* 1976). The infusion of maize oil into the dog duodenum leads to a dose-dependent, CCK-mediated emptying of the gall bladder (Shiratori *et al.* 1986).

Juste *et al.* (1983) studied how the amount of dietary fat affected bile secretion in the pig and found that the digestibility of fat increased with fat content. Bile flow and bile acid secretion also rose with dietary fat contents ranging between 20 and 100 g/kg, but failed to rise further after feeding with diets containing 200 g fat/kg. A positive correlation was also found between the amount of dietary fat and biliary secretion of cholesterol. The same authors (Juste *et al.* 1985) subsequently investigated diets containing different amounts and types of fat (butter and sunflower oil) and found that cholesterol saturation of the bile was higher in pigs fed on the diet with sunflower oil.

The purpose of the present study in dogs was to determine whether the long-term adaptation to diets with a high fat content but differing in the degree of unsaturation

Table 1. *Composition of the experimental diets**

	Sunflower-oil diet			Olive-oil diet		
	g/kg			g/kg		
	Mean	SE	Energy (%)	Mean	SE	Energy (%)
Protein	218	4·5	19·4	222	3·9	19·4
Fat	159	9·0	31·9	174	4·6	34·4
Carbohydrate	546	11·1	48·7	527	4·9	46·2
Ash	77	2·0	—	77	1·8	—

* The dog chow (Extra® Gabrina Purina), which was the basal diet for the experimental diets, contained (g/kg): dry matter 880, crude protein (nitrogen × 6·25) 210, fat 0, fibre 35, ash 80, sodium chloride 12, calcium 15, phosphorus 9, choline 2 and (mg/kg): iodine 1·3, magnesium 80·0, copper 30·0, iron 400·0, zinc 140·0, cobalt 1·0, retinol equivalent 6·0, vitamin E 13·4, thiamin 10·0, riboflavin 8·0, pyridoxine 10·0, cyanocobalamin equivalent 0·3, menadione 1·0, cholecalciferol equivalent 0·05, nicotinic acid 80·0, folic acid 5·0, pantothenic acid 25·0.

influenced bile flow and the content of cholesterol, bile acids and phospholipids in bile. Olive oil, a dietary source of fat rich in monounsaturated fatty acids, was compared with sunflower oil as a fat source rich in polyunsaturated fatty acids, under resting conditions and after food intake.

MATERIALS AND METHODS

Animals

Eight mongrel dogs of both sexes weighing 15–25 kg were weaned (at 15–20 d of age) and four animals were randomly assigned to each of the two experimental groups, group S (sunflower oil) and group O (olive oil). During the 8-month adaptation period all dogs were housed in individual cages with free access to water. Feeding took place once daily between 09.30 and 12.00 hours to adapt the animals to the experimental regimen and avoid conditioning to a specific feeding time.

Diets

The diets used were prepared from commercial dog chow (Extra R; Gabrina Purina) made up specially for the present study and omitting fat. Sunflower oil (Koipesol R, Koipe S.A.) was added to chow fed to group S, and virgin olive oil (Patrimonio Comunal Olivarero) was added to the chow given to group O animals.

Table 1 summarizes the composition of the two diets which, apart from their different fat sources, were isoenergetic and isonitrogenous and, thus, differed only in fat composition. Table 2 shows the fatty acid composition of the experimental diets.

Surgical preparation

After the 8-month adaptation period the dogs were anaesthetized with an intravenous injection of sodium thiopental (30 mg/kg body-weight). Through a midline laparotomy under strictly aseptic conditions the common bile duct was exposed and dissected, and a bidirectional cannula was positioned in the duct 30 mm distal to the choledoduodenal junction as previously described (Madrid *et al.* 1983). Briefly, the bidirectional cannula consisted of a polyvinyl tube divided at an acute angle at a point 20 mm from the anterior end. This end was narrowed by a rigid ring. A mobile catheter was inserted through the polyvinyl tube and placed at one of two possible positions according to the requirements of the procedure: one position allowed the total collection of bile when the front end of the

Table 2. *Fatty acid composition of experimental diets** (*mol/100 mol total fatty acid content*)

Fatty acid	Sunflower-oil diet	Olive-oil diet
Palmitic (16:0)	9·1	11·7
Stearic (18:0)	3·5	2·4
Oleic (18:1 *n*9)	25·5	60·9
Linoleic (18:2 *n*6)	56·3	15·3
Linolenic (18:3 *n*3)	2·3	0·8

* For details, see Table 1.

mobile catheter was pushed up to fit snugly within the narrowed part of the cannula, whereas in the other position the catheter was withdrawn beyond the bifurcation in the cannula so that when the exteriorized end of the catheter was blocked bile flowed through the sphincter of Oddi into the duodenum.

A second nylon cannula was placed in the proximal duodenum and sutured in position with purse-string sutures. Before starting the experiment all dogs were allowed at least 2 weeks to recover from the operation and the experiments were carried out in the next week.

Experiments

Four experiments per dog (*n* 16) were done with the two diets; bile was collected intermittently, during brief periods only, into preweighed polythene bags as described previously (Madrid *et al.* 1983). Samples of bile from each bag were pooled and stored at −20° until analysis. The remaining bile in the bags was returned to the duodenum via the duodenal cannula to avoid interrupting enterohepatic circulation. When no bile was being collected the bidirectional cannula allowed free passage of bile into the duodenum through the sphincter of Oddi.

Analyses

Phospholipids were determined with a commercial test kit (no. 691844; Boehringer, Mannheim, Germany). Cholesterol levels were measured using a colorimetric test (CHOD-PAP no. 237576; Boehringer, Mannheim, Germany) based on an enzymic reaction according to the technique of Seidel *et al.* (1981). Proteins and pigments were previously removed by the technique of Levin *et al.* (1961). An enzymic method with 3α-hydroxysteroid dehydrogenase (*EC* 1.1.1.50; Sterognost-3-α Pho.; Nyegaard and Co., Oslo, Norway) was used to determine bile acid levels.

The results of all analyses were expressed as mmol/l.

Statistical treatment

Four experiments were carried out on each dog in order to increase the precision of this experiment. Appropriate tests of significance for differences between treatment groups and for changes over time were based on variation between dogs. The following analyses were carried out: variables observed at each sampling were averaged over four experiments on each dog; *t* tests (SPSS/PC *t* test group procedure; Nie *et al.* 1983) were carried out using these means to test for differences between groups at each sampling time.

Changes in each variable between the prefeeding sample and each postfeeding sample were calculated. These differences were also averaged over the four experiments carried out on each dog, and for each group at each sampling time *t* tests were carried out to test

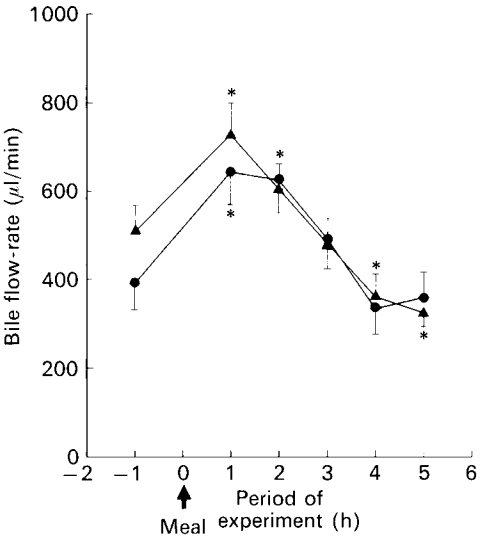

Fig. 1. Bile flow under resting conditions (time − 1) and after food intake (meal) in dogs fed on diets containing sunflower oil (▲) and olive oil (●). Values are means with their standard errors, represented by vertical bars for four experiments/dog. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0{\cdot}05$. For details of diets, see Tables 1 and 2 and for details of procedures, see pp. 176–177.

whether the mean change differed from zero. The differences were considered significant when $P < 0{\cdot}05$.

RESULTS

Food intake

There were no differences in food intake between the groups. All the animals ate the same amount of food (500 g/d) and they had the same energy intake during the experimental period.

Resting secretion (time − 1)

After a 24 h fast there were no significant differences in bile flow between the two experimental groups, although bile flow was slightly higher in the group given sunflower oil as the dietary source of fat (Fig. 1). The concentration (Fig. 2(a)) and output (Fig. 2(b)) of bile acids in resting secretions were significantly greater in group S dogs ($P < 0{\cdot}05$). Although there were no differences in phospholipid concentration between the two groups (Fig. 4(a)), the output (secretion; Fig. 4(b)) was significantly greater in animals fed on the diet containing polyunsaturated fatty acids (group S). The output and concentration of cholesterol (Fig. 3(a, b)) in the bile secreted were similar in both groups.

Response to food (post-prandial period)

The bile flow rose significantly during the first 2 h after food intake (Fig. 1). Subsequently bile flow declined (Fig. 1). After 2 h of hypersecretion bile flow fell sharply below the basal value in group S.

After food intake in group O the concentration of bile acids rose significantly during the first hour and the output of bile acids rose during the first 3 h, whereas in group S no increases were seen during this time-period. In the latter group, in comparison with basal values, the concentration and output of bile acids decreased significantly at 2 and 4 h (Fig. 2(a, b)) respectively after food intake.

No appreciable changes from basal values were recorded for biliary cholesterol

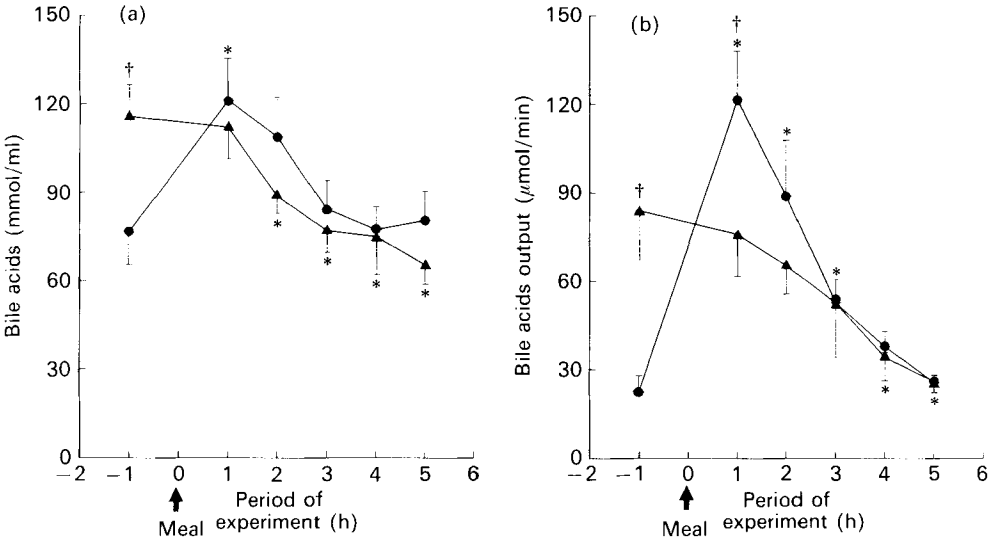

Fig. 2. (a) Bile acid concentration and (b) output of bile acids under resting conditions (time −1) and after food intake (meal) in dogs fed on diets containing sunflower oil (▲) and olive oil (●). Values are means with their standard errors, represented by vertical bars for four experiments/dog. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$. Mean values for the two dietary groups were significantly different: † $P < 0.05$. For details of diets, see Tables 1 and 2 and for details of procedures, see pp. 176–177.

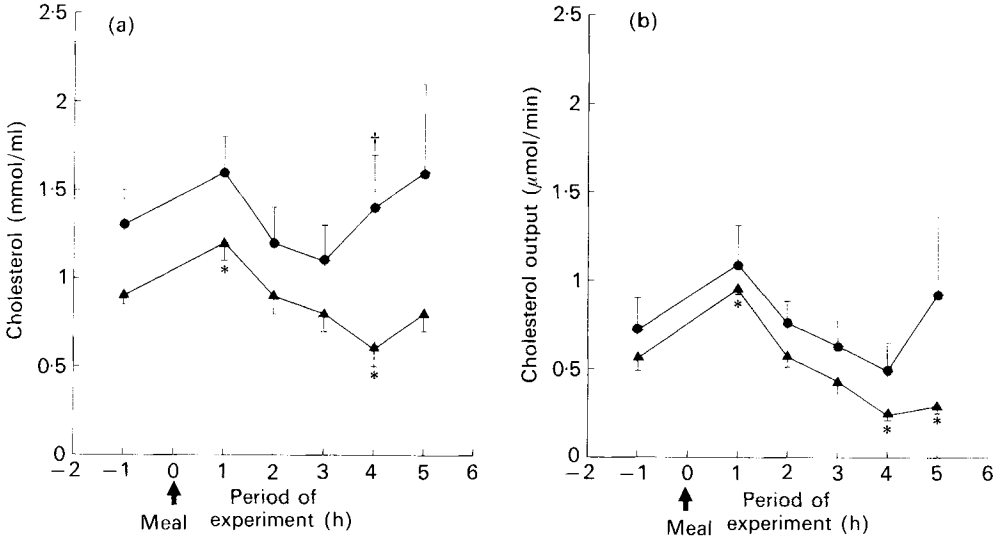

Fig. 3. (a) Cholesterol content and (b) cholesterol output under resting conditions (time −1) and after food intake (meal) in dogs fed on diets containing sunflower oil (▲) and olive oil (●). Values are means with their standard errors, represented by vertical bars for four experiments/dog. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$. Mean values for the two dietary groups were significantly different: † $P < 0.05$. For details of diets, see Tables 1 and 2 and for details of procedures, see pp. 176–177.

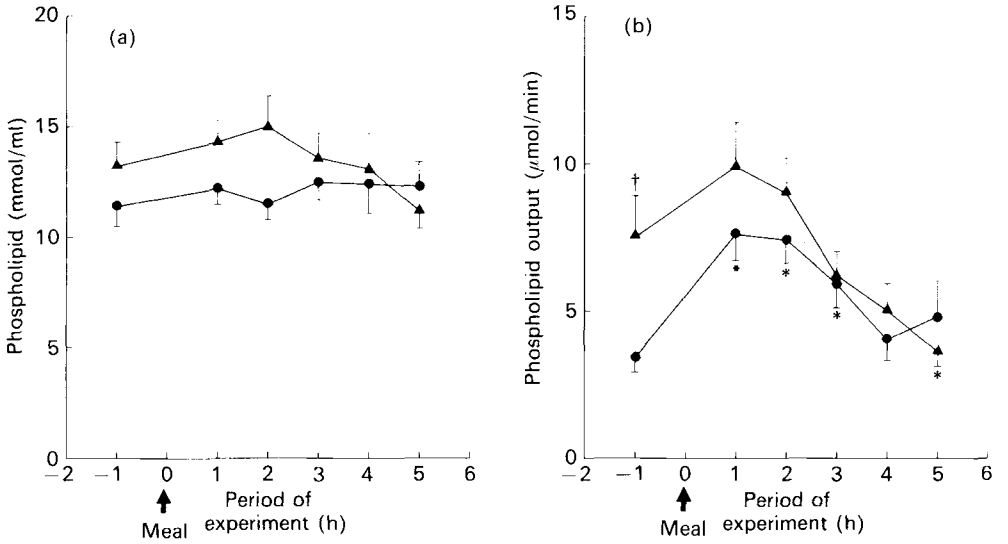

Fig. 4. (a) Phospholipid content and (b) phospholipid output under resting conditions (time −1) and after food intake (meal) in dogs fed on diets containing sunflower oil (▲) and olive oil (●). Values are means with their standard errors, represented by vertical bars for four experiments/dog. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$. Mean values for the two dietary groups were significantly different: † $P < 0.05$. For details of diets, see Tables 1 and 2 and for details of procedures, see pp. 176–177.

concentration or output in group O animals during the postprandial period (Fig. 3(a, b)). In contrast, in group S both variables increased from baseline values during the first hour after eating and fell to below baseline values at 4 h (Fig. 3(a, b)).

Phospholipid concentrations during the postprandial period were unchanged relative to baseline values for both experimental groups, and there were no significant differences between groups (Fig. 4(a)).

The phospholipid output increased significantly after feeding in group O, whereas in group S the phospholipid output declined throughout the postprandial period and became significantly lower at 5 h after eating.

DISCUSSION

Under resting conditions the differences between the two experimental groups in biliary flow and particularly in the concentration and output of bile acids should be attributed to changes in motor patterns of activity in the gall bladder and sphincter of Oddi during interdigestive periods. Traynor *et al.* (1984) attributed the variations observed in the amount and composition of bile (biliary acids and bilirubin) secreted into the duodenum during these periods to modifications in motor activity, especially during phase II of the motor migratory complexes (MMC) when rhythmic contractions of the gallbladder have been observed along with changes in motility of the sphincter of Oddi. Such motor patterns can be partly traced to the rise in motilin recorded during phase II of the MMC (Suzuki *et al.* 1981; Takahashi *et al.* 1982). Moreover, other peptides, including pancreatic polypeptide (Everson *et al.* 1982; Lawson *et al.* 1983), neurotensin (Fujimura *et al.* 1984; Walker *et al.* 1985), CCK (Scott *et al.* 1985) and peptide YY (Aponte *et al.* 1985; Pappas *et al.* 1985), also affect the motility of the extrahepatic biliary tree and the relevant region

of the gastrointestinal tract as well as influencing plasma levels of motilin. Plasma levels of certain humoral agents may also be differentially affected by the type of dietary fat, or more specifically by the major fatty acid present in the diet. Although we cannot offer any definite explanation for these differences they may nevertheless be related to the different patterns of periodic contraction of the gall bladder during interdigestive periods. This in turn would affect choleresis and bile acid content in the bile secreted into the duodenum, thus leading to an increase in the enterohepatic circulation of these bile salts.

Under resting conditions the higher phospholipid output in the bile of dogs given sunflower oil as the source of dietary fat seems to be related to the greater secretion of bile acids which occurs in this group during this period (Figs. 2(b), 4(b)). This concurs with the results of Mazer & Carey (1984) who described similar behaviour of these two bile components in several species including the dog.

Polyunsaturated fatty acids have been shown to raise biliary secretion of cholesterol (Watanabe *et al.* 1962; Dam *et al.* 1967), a finding which may be related to their presumed hypocholesterolaemic effect. Under the present experimental conditions no difference was recorded between the diet rich in polyunsaturated fatty acids and that rich in monounsaturated fatty acids with respect to biliary cholesterol content.

Patterns of biliary response to food intake in both experimental groups suggest a greater involvement of the gall bladder in animals fed on the diet rich in olive oil, as shown most clearly by the marked rise in bile acid concentration and output after eating. This increase in bile acids may be responsible for the prolonged bile flow in this group, compared with basal values, because a greater pool of bile acids enters the enterohepatic circulation thus raising the efficiency of the enterohepatic circulation of these anions. The type of dietary fat, therefore, affects vesicular emptying in response to food, a logical finding in the light of the fact that oleic acid, the major fatty acid in olive oil, is one of the most potent stimulators of CCK release known to date (Modlin *et al.* 1979; Konturek *et al.* 1986) while the hormone CCK is in turn a highly effective stimulator of vesicular contraction (Mutt & Jorpes 1971; Lawson *et al.* 1983; Scott *et al.* 1985).

The authors thank the Delegate for Provincial Promotion of the Provincial Council of Jaén, Mr Isidoro Reverte, and the President of the Provincial Council of Jaén, Mr Cristóbal López Carvajal, for their support and encouragement throughout the study. They also thank Ms Karen Shashok for translating the original manuscript into English, and Ms Elisa Alcover for her excellent secretarial assistance. This study was supported by the Provincial Council of Jaén (Spain).

REFERENCES

Aponte, G. W., Fink, A. S., Meyer, J. H., Takemoto, K. & Taylor, I. L. (1985). Regional distribution and release of peptide YY (PYY) with fatty acids of different chain length. *American Journal of Physiology* **249**, G745–G750.

Dam, H., Kruse, I., Jensen, M. K. & Kalemange, H. E. (1967). Studies on human bile. II. Influence of two different fats on the composition of human bile. *Scandinavian Journal of Clinical and Laboratory Investigation* **19**, 367–378.

Everson, G. T., McKinley, C., Lawson, M., Johnson, M. & Kern, F. Jr (1982). Gallbladder function in the human female: effect of the ovulatory cycle, pregnancy and contraceptive steroids. *Gastroenterology* **82**, 711–719.

Fujimura, M., Sakamoto, T. & Khalil, T. (1984). Physiological role of neurotensin in gallbladder contraction in the dog. *Surgery Forum* **35**, 192–194.

Juste, C., Corring, T. & Demarne, Y. (1983). Response of bile flow, biliary lipids and bile acid pool in the pig to qualitative variations in dietary fats. *Journal of Nutrition* **113**, 1691–1701.

Juste, C., Corring, T. & Demarne, Y. (1985). Effect of a lipid diet on cholesterol saturation in the bile of pigs. *Reproduction, Nutrition, Développement* **25**, 815–821.

Konturek, S. J., Tasller, J., Bilski, J., Jong, A. J., Jansen, J. B. M. J. & Lamers, C. B. (1986). Physiological role and localization of CCK release in dogs. *American Journal of Physiology* **250**, G391–G397.

Ladas, S. D., Isaacs, P. E. T., Murphy, G. M. & Slader, G. E. (1984). Comparison of the effects of medium and long-chain triglyceride-containing liquid meals on gallbladder and small intestine function in normal men. *Gut* **25**, 405–411.

Lawson, M., Everson, G. T., Klingsensmith, W. & Kern, F. Jr (1983). Coordination of gastric and gallbladder emptying after ingestion of a regular meal. *Gastroenterology* **85**, 866–870.

Levin, S. J., Johnson, C. J. & Boyle, A. J. (1961). Spectrophotometric determination of several bile acids as conjugates. Extraction with ethyl-acetate. *Annals of Chemistry* **33**, 1407.

Madrid, J. A., Mañas, M., Salido, G. M., Martinez-Victoria Muñoz, E. & Mataix, F. J. (1983). Use of bidirectional cannula to study the biliary secretion in conscious dogs. *Laboratory Animals* **17**, 307–310.

Malagelada, J. R., Dimango, E. P., Summerskill, W. H. J. & Go, W. L. W. (1976). Regulation of pancreatic and gallbladder functions by intraluminal fatty acids and bile acids in man. *Journal of Clinical Investigation* **58**, 493–499.

Mazer, A. N. & Carey, M. C. (1984). Mathematical model of biliary lipid secretion: a quantitative analysis of physiological and biochemical data from man and other species. *Journal of Lipid Research* **25**, 932–952.

Modlin, I. M., Hansky, J., Singer, M. & Walsh, J. H. (1979). Evidence that the cholinergic enteropancreatic reflex may be independent of CCK release. *Surgery USA* **86**, 352–361.

Mutt, V. & Jorpes, E. (1971). Hormonal peptides of the upper intestine. *Biochemical Journal* **125**, 57P-58P.

Nie, N. H., Hull, C. H., Jenkins, J. G., Streinbrenner, K. & Bent, D. H. (editors) (1983). *SPSS: Statistical Package for the Social Sciences*. New York: McGraw-Hill.

Pappas, T. N., Debas, H. T., Goto, Y. & Taylor, I. L. (1985). PYY inhibits meal stimulated pancreatic and gastric secretion. *American Journal of Physiology* **248**, G118-G123.

Scott, R. B., Eidit, P. B. & Shaffer, E. A. (1985). Regulation of fasting canine duodenal bile acid delivery by sphincter of Oddi and gallbladder. *American Journal of Physiology* **249**, G622–G633.

Seidel, J., Schulumberger, H., Klose, S., Ziegenhorn, J. & Wahlefeld, A. W. (1981). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Journal of Clinical Chemistry and Biochemistry* **19**, 838.

Shiratori, K., Watanabe, S., Chey, W. Y., Lee, K. Y. & Chang, T. M. (1986). Endogenous CCK drives gallbladder emptying in dogs. *American Journal of Physiology* **251**, G553–G558.

Suzuki, T., Takahashi, I. & Itoh, Z. (1981). Motilin and gallbladder. New dimension in gastrointestinal physiology. *Peptides* **2**, 229–233.

Takahashi, I., Suzuki, T., Aizawa, I. & Itoh, Z. (1982). Comparison of gallbladder contractions induced by motilin and cholecystokinin in dogs. *Gastroenterology* **87**, 1154–1159.

Traynor, O. J., Dozois, R. R. & Dimagio, E. P. (1984). Canine interdigestive and postprandial gallbladder motility and emptying. *American Journal of Physiology* **246**, G426–G432.

Walker, J. P., Khalil, T., Weiner, I., Fagan, C. J., Townsend, C. M., Greeley, G. H. Jr & Thompson, J. C. (1985). The role of neurotensin in human gallbladder motility. *Annals of Surgery* **201**, 678–683.

Watanabe, N., Gimbell, N. S. & Johnston, C. G. (1962). Effect of polyunsaturated and saturated fatty acids on the cholesterol holding capacity of human bile. *Archives of Surgery* **85**, 136–141.

Printed in Great Britain