Gut, 1972, **13**, 675-681

Non-dietary lipid in the intestinal lumen

P. B. COTTON

From the Gastrointestinal Laboratory, St Thomas' Hospital, London

SUMMARY Lipid in the intestinal lumen is mainly dietary in origin, but there is also an endogenous component from bile, bacteria, and the mucosa (through exudation and cell loss). Perfusion experiments in fasting rats demonstrate that exfoliated cells carry with them into the small intestinal lumen an average of 1·12 mg lipid/30 minutes; lipid classes consisting of phosphatidyl choline (lecithin), triglyceride, cholesterol, cholesterol ester, phosphatidyl ethanolamine, and free fatty acid. Fatty acid also enters the lumen independently of cells by exudation.

Since the rate of lipid exfoliation and exudation considerably exceeds the faecal lipid excretion in fasting rats, efficient reabsorption must normally occur. Calculations based on published data suggest the daily exfoliation of 12 to 30 g lipid into the small intestinal lumen of fasting man. When reabsorption is impaired, especially in states of increased cell turnover, endogenous mucosal lipid may account for a significant proportion of faecal lipid, perhaps sufficient to constitute a state of fat-losing enteropathy.

Faecal lipid does not consist solely of dietary residue. A small faecal lipid excretion persists in animals and man fasting and on fat-free diets: published figures for man are 0·7 g/day (Bouchier, Kellock, and Manousos, 1963) and 1 g/day (Blomstrand, 1955); for dogs 30 mg/kg/day (Sperry, 1927) and 39·4 mg/kg/day (Pessoa, Kim, and Ivy, 1953); and for rats 25 mg/kg/day (Norcia and Lundberg, 1954). Similarly patients have been described whose steatorrhoea consistently exceeded dietary fat intake (Comfort, Wellaeger, Taylor, and Power, 1953). These figures establish the excretion of non-dietary (endogenous) lipid, but are no measure of the amount entering the intestinal lumen, since much of this may normally be reabsorbed. Measurements of fat absorption derived from simple chemical balance studies are consistently lower than those obtained using isotope methods (Blomstrand, 1955), suggesting that non-dietary lipid enters the gut. The same conclusion may be drawn from the lack of any consistent quantitative or qualitative relationship between dietary and faecal lipid. Although increasing dietary lipid may lead to a linear increase in faecal loss (Wollaeger, Comfort, and Osterberg, 1947) this finding is not consistent (Annegers, Boutwell, and Ivy, 1948; Krakower, 1934). The quality of human and animal faecal lipid does not reflect that of the diet (Norcia and Lundberg, 1954; Comfort *et at*, 1953; Annaegers *et al*, 1948; Holmes and Kerr, 1923; Webb, James, and Kellock, 1963). However, this difference cannot be used as evidence for endogenous lipid, since it is largely due to colonic bacterial action. Wiggins, Howell, Kellock. and Stalder (1969) showed that the fatty acid spectrum of ileostomy dejecta lipid closely resembled that of the diet. An endogenous source (Verdino, Blank, and Privett, 1965) must be postulated for lipid found in the lymph draining the intestinal tract of fasting animals: approximately 400-500 mg/kg/day in rats (Kim and Bollman, 1954; Gottenbos and Thomasson, 1963; Baxter, 1966) and 200-400 mg/kg/day in dogs (Rony, Mortimer, and Ivy, 1933; Kim, Bollman, and Grindlay, 1956).

Possible sources of endogenous lipid are (1) bile, (2) bacteria, (3) exudation through the mucosa, and (4) exfoliation of lipid-containing villus cells.

Considerable quantities of biliary lipid enter the intestine: in man 1-2 g/day of cholesterol and 0·5-5 g/day of phospholipid (Doubilet and Fishman, 1961; Balint, Spitzer, and Kyriakides, 1963; Shioda, Wood, and Kinsell, 1967). Baxter (1966) found an average biliary lipid excretion of 3·4 mg/hour in rats shortly after biliary diversion. If bile were the main source of endogenous lipid, its diversion would result in decreased faecal lipid excretion in the fasting state. In dogs the figures rose almost three-fold: from 30 to 85 mg/kg/day (Sperry, 1927), and from

Received for publication 31 July 1972.

39·4 to 142 mg/kg/day (Pessoa *et al*, 1953). Bile diversion led to a fall in intestinal lymph lipid output in the fasting rat (Kim *et al*, 1956; Baxter, 1966).

Colonic bacteria have profound effects on the structure of fatty acids and sterols (Wiggins *et al*, 1969; Tabaqchali and Booth, 1966; Hoet, Hoosens, Evard, Eyssen, and DeSomer, 1963). Although there is no evidence that bacteria secrete lipid, their cell walls contain phospholipid. Gross calculations based on mean faecal weight and bacterial counts (Cantarow and Schepartz, 1962; Sperry, 1929; Fenton, 1960) suggest that bacterial cell walls may contribute around 0·5 g of lipid to the faeces of man on a normal diet. Similar calculations suggest that there is less than 1 mg of bacterial cell wall lipid in the human small intestine, even when abnormally colonized. Bacteria are therefore not a significant source of endogenous lipid in the human small intestine.

Lipid accumulating in isolated intestinal loops and fistulae (Blitstein and Erenthal, 1891; Voit, 1892; Bloor, 1922; Angevine, 1929) must be derived from the mucosa, either by exudation or cell exfoliation. Labelled fatty acids were recoverable from the rat small intestine after their intravenous administration (Burr, McPherson, and Tidwell, 1959) as was labelled cholesterol after intravenous injection of ^{14}C-labelled acetate (Wilson and Reinke, 1968). Reid (1967) and Watkin (1965) noted a small increase in faecal fat excretion in patients given intravenous fat emulsions, but Gordon (1960) could detect no change. Baxter (1966) found no increase in intestinal lymph lipid in fasting dogs rendered hyperlipaemic.

Wiggins *et al* (1969) noted that the fatty acid spectrum of ileostomy dejecta lipid of patients on a very low fat diet closely resembled that of human intestinal mucosa, and suggested that it was derived therefrom. Eckstein (1925) had made a comparable but less sophisticated observation. Sperry (1932) calculated that at least one third of the entire intestinal mucosa would have to be shed daily to account for the faecal excretion of 86 mg lipid/kg which he measured in bile-fistula dogs: he rejected the possibility of such rapid turnover.

We now know that recognizable villus cells are shed at a high rate (Pink, Croft, and Creamer, 1970; Croft, 1970). The small intestinal mucosa contains lipid even in the fasting state: in the dog 8% by weight (Sperry, 1932) and in human duodenal biopsies 4·2% (Saunders, Ways, Parmentier, and Rubin, 1966) and 10·9% (Partin and Schubert, 1970). The exfoliation of cells containing structural and nutritional lipids could be an important source of endogenous lipid in the intestinal lumen. This hypothesis has been tested in rats.

Design of the Study

The entire small intestines of fasting rats were washed through; the resulting mucosal effluent was analysed for lipid and cell content. The exfoliative and exudative components were deduced from this data and the normal lipid content of intestinal villus cells measured in cell suspensions.

ANIMAL EXPERIMENTS

Male albino Wistar rats were reared on Spillers autoclave diet to a weight of 240 to 370 g. For 48 hours before experimentation they were maintained on water only, in false-bottomed cages to prevent coprophagia.

Perfusion experiments were performed under anaesthesia induced by ether and maintained for up to three hours by intraperitoneal pentobarbitone. Polyethylene cannulae were tied in the upper jejunum and distal ileum so as to allow perfusion of the entire small intestine while excluding bile and pancreatic juice. After 20 minutes' rinsing, physiological saline was perfused at rates varying between 105 and 400 ml/30 minutes in different animals. Towards the end of each perfusion period the intestine was gently unkinked to allow clumps of desquamated cells to pass into the collecting bottles, which were acid-washed and contained EDTA. After recording their volumes, perfusion specimens were homogenized at low speed to break up cell clumps; during stirring aliquots were taken for chemical analysis.

Suspensions of intestinal villus cells were obtained from similarly reared and fasted animals. The isolated rinsed intestine was filled with Pronase B grade (Calbiochem, USA) 0·25% in phosphate-buffered saline for 10 to 20 minutes at 37°C. The resulting crude effluent was washed by repeated centrifugation and resuspension. Aliquots were taken for chemical analysis during stirring of the final volume of 75 ml (Cotton, 1972a).

Similar perfusates and cell suspensions were obtained from fed animals.

CHEMICAL METHODS

The cell contents of perfusates and suspensions were measured in terms of deoxyribonucleic acid (DNA) by the method of Croft and Lubran (1965), validated in the intestine by Croft, Loehry, Taylor, and Cole (1968b), and Loehry, Croft, Singh, and Creamer (1969). Measurements were performed in duplicate; mean average deviation of 21 replicate estimations was 6·2%. The addition of standard DNA to perfusates revealed a recovery of 86·7% at a DNA-P concentration of 8 ng atom/ml; recovery fell, however, with increasing dilution, to 60% at 1 ng atom/ml.

Lipids were extracted using chloroform-methanol by a double procedure based on that of Folch, Lees, and Sloane-Stanley (1957), and eventually taken up in chloroform (0·5-10 ml). Mean recovery of lipid standards (cholesterol, triglyceride, phosphatidyl choline, phosphatidyl ethanolamine) after addition to perfusates was 93·3% (range 84-110%). There was no evidence of degradation of compound lipids during extraction.

Lipid classes were identified and measured in each specimen by a specially developed method of quantitative thin-layer chromatography (Cotton, 1972b). Silica Gel G plates (20 cm × 20 cm) were prewashed in chloroform-methanol-acetic acid (200: 100:3), divided into 7 mm lanes and activated. Neutral lipids were separated in a two-stage system. The first solvent (benzene 50 : diethyl ether 40 : ethanol 2 : acetic acid 0·2) was run to 12 cm. After drying this was followed by a complete run in the second solvent (hezane 94 : diethyl ether 6). Phospholipids were separated on similar layers using a single solvent (chloroform 65 : methanol 25 : acetic acid 1 : water 4). The plates were dried, sprayed with 50% sulphuric acid and charred on a hot-plate at 200°C.

The density of each chromatographic spot was measured by a Joyce Loebl chromoscan using a reflecting slit beam. Peak heights of scans were compared with those of multiple standards run in parallel on each plate, enabling quantitation (down to 1 μg) of the following individual lipids: cholesterol, cholesterol ester, monoglyceride, triglyceride, free fatty acid, lysolecithin, phosphatidyl choline (lecithin), phosphatidyl ethanolamine, and sphingomyelin. The coefficient of variation of two mixed lipid specimens measured on six different occasions was 7·3% (Cotton, 1972b).

Results

INTESTINAL PERFUSATES

Twenty-eight perfusates (each of 30 minutes) were collected from nine fasting rats. The mean DNA content was 303·9 ng atoms DNA-P (SD ± 98·3). There was no correlation between the DNA content

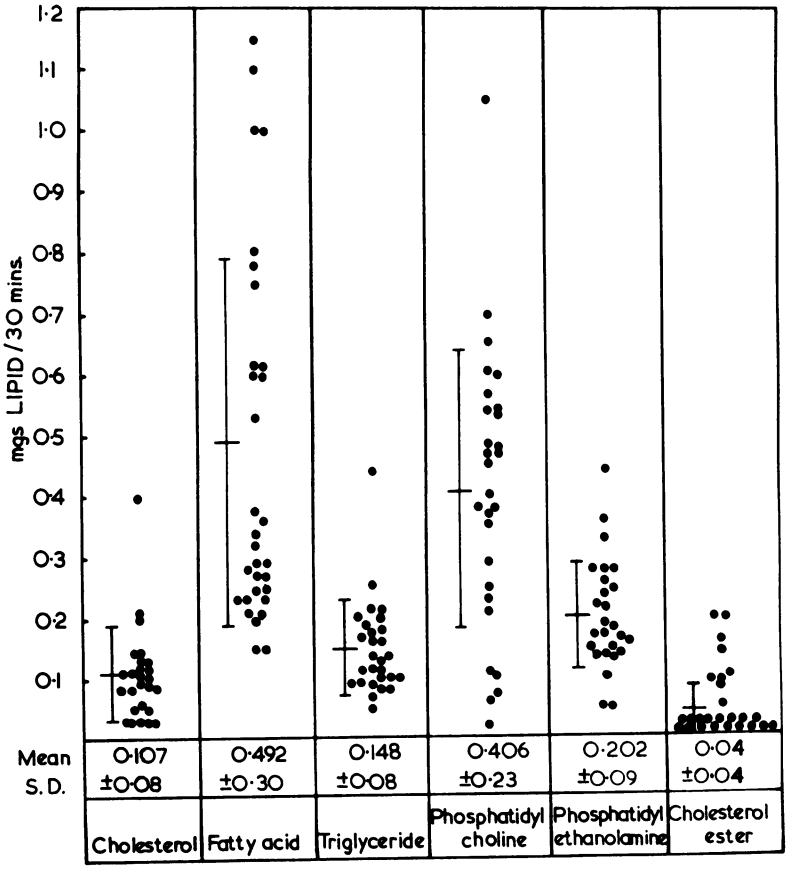

Fig. 1 *Lipid content of intestinal perfusates.*

and rate of perfusion ($r = -0.21$, $P = 0.2$).

The lipid contents are shown in Fig. 1; mean total was 1·40 mg/30 min, with phosphatidyl choline and free fatty acid predominating. Triglyceride, phosphatidyl ethanolamine, and cholesterol also appeared in all perfusates; cholesterol ester was only measurable in 10. Mono- and di-glyceride, sphingomyelin, and lysolecithin were occasionally detected.

There was no significant correlation between perfusion rates and the perfusate content of any of the measured lipid classes except free fatty acid (Fig. 2). By contrast, free fatty acid was the only lipid whose perfusate content was not related to the DNA content (Fig. 3). Data for cholesterol ester were insufficient.

Thus it can be concluded that free fatty acid enters the lumen independently, while all the other measured lipids do so in or with exfoliated cells.

VILLUS CELL SUSPENSIONS

The lipid contents of cell suspensions from seven fasting rats are shown in Fig. 4, expressed in terms of the mean perfusate DNA content (303·9 ng atoms DNA-P). Histological examination of the source mucosae suggested that most of the cells in the suspensions were derived from the distal half of the villi.

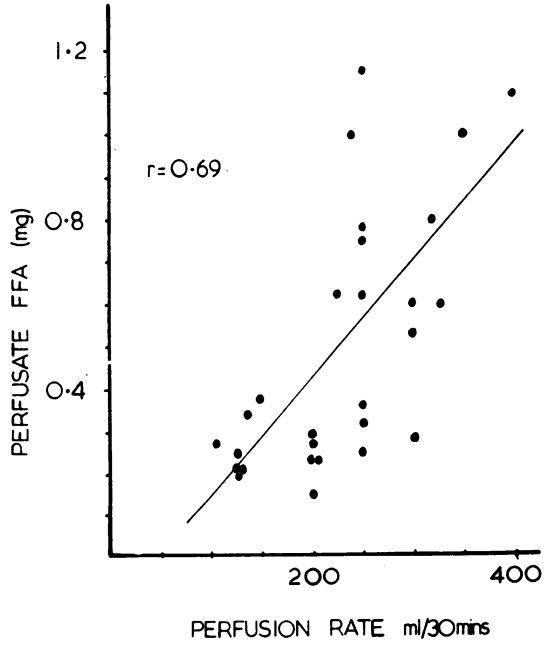

Fig. 2 *Intestinal perfusates. Free fatty acid (FFA) content related to perfusion rate.*

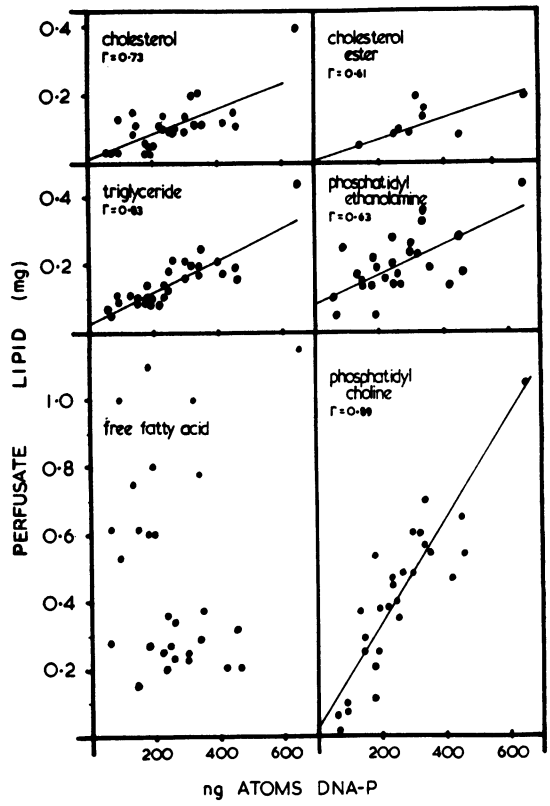

Fig. 3 *Intestinal perfusates. Lipid contents related to DNA contents.*

When the mean lipid content of the cell suspensions is compared with that of the perfusates (for the same DNA content), it can be seen (Fig. 4) that there was no significant difference for cholesterol, cholesterol ester, phosphatidyl choline, phosphatidyl ethanolamine, and triglyceride. It can therefore be stated that these lipids left the mucosa in desquamated cells, a total of 0·91 mg lipid/30 min. Desquamated cells also contain some free fatty acid (0·21 mg/303·9 ng atoms DNA, from Fig. 4). The total mean figure for exfoliated lipid is therefore 1·12 mg/30 min in fasting animals.

There is more free fatty acid in the perfusates than can be accounted for by cell loss alone (Fig. 4), and there is no evidence that it has been released by degradation of compound lipids. Some free fatty acid therefore exudes directly through the mucosa; the amount varies directly with the rate of perfusion, from nil to about 1 mg/30 min.

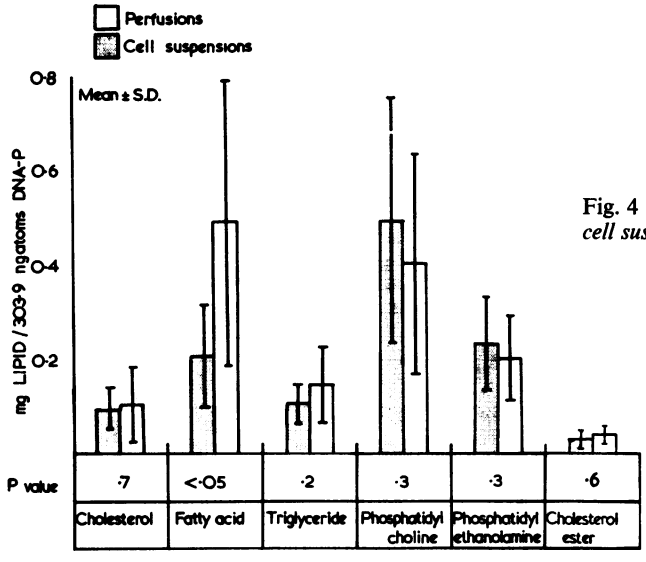

Fig. 4 *Mean lipid contents of intestinal perfusates and cell suspensions related to the same DNA content.*

Discussion

In fasting rats the mean figure for lipid exfoliation (that is, excluding free fatty acid exudation) was 1·12 mg/30 min. Fast perfusion rates were chosen to minimize possible reabsorption; any conclusion rests on the belief, for which there is good evidence, that the technique was not unduly traumatic, and collected only physiological cell loss. The rate of DNA loss showed no tendency to rise in individual animals, and there was no correlation with perfusion rate. Such a correlation was, however, found by Loehry (1969) and Da Costa (1970), using comparable perfusion techniques. These authors did not unkink the intestine during perfusions, and their results may simply represent more efficient harvesting of cells at higher flow rates. This is consistent with the fact that their highest rates of DNA loss approximate to the mean rate in the present work. By calculation, this mean rate can be shown to represent approximately $4{\cdot}5 \times 10^7$ cells/hour, which is remarkably close to the figure for cell production rate derived from the data of Clarke (1970a, b) who used a totally different method. In addition, the DNA content of the entire mucosa of the rat small intestine was measured during this work, at approximately 30 000 ng atoms DNA-P; a loss rate of 303·9 ng atoms/30 min represents a physiological turnover time of around two days. Perfusates contained no blood, and mucosal histology was normal to light microscopy even after prolonged experiments.

These data refer to starved animals, whose mucosal lipid content and cell production rate are minimized (Clarke, 1970c). The mucosa contains more lipid after a lipid-containing meal (Saunders *et al*, 1966; McManus and Isselbacher, 1970), and cell loss is likely to be increased. Nine perfusates were carried out in three rats, starting two hours after placing 60 mg lipid emulsion in the stomach; mean perfusate lipid content was 1·68 mg/30 min, a significant increase occurring in all of the lipid classes present in the fed emulsion (Cotton, 1971).

These figures have been concerned solely with the small intestine since its rates of cell turnover and loss are vastly greater than those of the stomach or colon (Creamer, 1971).

The significance of free fatty acid exudation is difficult to assess. Correlation with perfusion rate may simply reflect better recovery, ie, a balance between exudation and rapid reabsorption, since the perfusate concentration of free fatty acid remains remarkably constant. The mucosa is permeable to most water-soluble molecules, the blood-to-lumen clearance being greatest for those of smallest molecular weight (Loehry, Axon, Hilton, Hider, and Creamer, 1970). Free fatty acid has the smallest molecule of the lipids measured in this present work (although only marginally smaller than cholesterol). Lipids normally exist in protein complexes, and the free fatty acid-albumin complex is by far the smallest of the lipoproteins. However, free fatty acid exudation cannot be considered to be a purely passenger phenomenon, since the rate of albumin loss is insufficient (Da Costa, Croft, and Creamer, 1970).

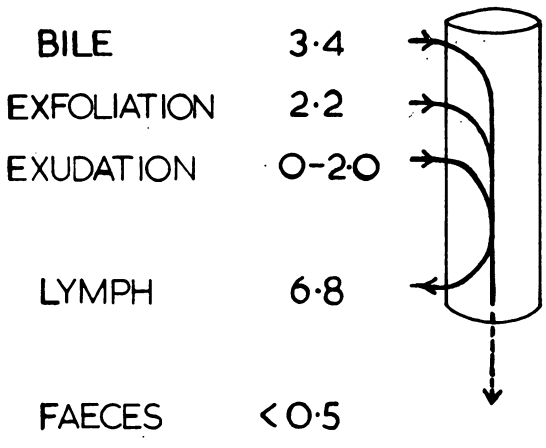

Fig. 5 *Endogenous lipid in the small intestine of the fasting rat (mg/hour) (see text).*

The total villus compartment of these fasting rats contained approximately 20 mg free fatty acid, and the fatty acid spectrum of the mucosal neutral lipids (as measured by gas-lipid chromatography) closely resembled that of the perfusates (Cotton, 1971). Exuded free fatty acid is thus probably derived from the mucosal pool, which may be replenished from plasma, lymph, or local synthesis. The mechanics and significance of lipid transfer are being further studied.

Using the data derived in this work it is now possible to comment on the economy of endogenous lipid in the small intestine of the fasting rat. In Fig. 5 the data for bile and lymph lipid are derived from Baxter (1966). Since approximately 6 mg lipid enters the small intestine each hour, and the faecal loss (including bacterial lipid) is less than 0·5 mg, endogenous lipid must normally be reabsorbed efficiently, presumably into the intestinal lymph. This process is considerably less efficient when bile is diverted; lymph lipid reabsorption falls to approximately 2 mg/hour or less (Kim and Bollman, 1954; Baxter, 1960) and the faecal loss increases. Experiments with radioactively labelled villus cell suspensions, placed in the lumen, have demonstrated that cellular lipid is reabsorbed (Cotton, 1971).

Baxter (1966) minimized the exfoliative contribution, since he found the total mucosal lipid pool of the small intestine (300 g rats on a low fat diet) to be only 15 mg; assuming normal turnover rates, exfoliation could not exceed 0·5 mg/hour. Baxter's figure for total small intestinal lipid is seriously at variance with data from the present work. The mucosal surface was scraped off the small intestine of eight fasted rats (weighting 262-315 g); mean total lipid content was 46·7 mg (range 36·4-94·2). From histology and DNA data it was clear that the specimens did not represent the total villus cell compartment (Cotton, 1972a). From the data of McManus and Isselbacher (1970), a comparable mean figure of 81·75 mg can be derived (in fasted 200 g rats). Sperry (1932) published a figure of 274 mg/kg for fasting dogs.

There are no direct measurements of lipid exfoliation or exudation in man. From human intestinal perfusion experiments, Croft (1970) calculated a loss of 6×10^{10} cells/day, with a total weight of 287 g. If these cells are representative of the duodenal mucosa, which contains 4·2% (Saunders *et al*, 1966) or 10·9% (Partin and Schubert, 1970) lipid weight in the fasting state, we can speculate on the daily exfoliation of 12 to 30 g of lipid. Since faecal lipid excretion (including bacterial lipid) in the fasting state is less than 1 g/day, the concept of massive lipid exfoliation presupposes efficient reabsorption. When reabsorption is impaired, especially in states of high cell loss such as the coeliac syndrome (Croft, Loehry, and Creamer, 1968), steatorrhoea may be partly endogenous, a state of fat-losing enteropathy. This is the only possible explanation in patients whose faecal fat loss exceeds dietary intake.

In the coeliac syndrome, net intestinal loss has been established for protein, calcium (Melvin, Hepner, Bondier, Neale, and Joplin, 1970), and iron (Singh, 1970). Rowntree (1930) found coeliac children to be in negative balance for vitamin A. It is possible that endogenous loss may be a significant factor in fat-soluble vitamin deficiency in such patients, and probable that this is an exfoliative phenomenon.

It is a pleasure to thank Dr Brian Creamer for his advice and encouragement, St Thomas' Hospital Endowment Fund for support, and Miss Judy Cole for technical assistance. This work forms part of a Cambridge MD thesis (1971).

References

Angevine, R. W. (1929). Fat excretion. VI. Excretion by Thiry-Vella fistulas. *J. biol. Chem.*, 82, 559-565.

Annegers, J. H., Boutwell, J. H., and Ivy, A. C. (1948). The effect of dietary fat on fecal fat excretion and subjective symptoms in man. *Gastroenterology*, 10, 486-495.

Balint, J. A., Spitser, H. L., and Kyriakides, E. C. (1963). The lipids of bile and their role in fat absorption. *Clin. Res.*, 11, 32.

Baxter, J. H. (1966). Origins and characteristics of endogenous lipid in the thoracic duct lymph in rat. *J. Lipid Res.*, 7, 158-165.

Blitstein, M., and Erenthal, W. (1891). Neue Versuche sur Physiologie des Darmkanals. *Pflügers Arch. ges. Physiol.*, 74-99.

Blomstrand, R. (1955). A study on the intestinal absorption of fat in normal adults and in non-tropical sprue with carbon-labelled oleic acid and palmitic acid. *Acta med. scand.*, 152, 129-138.

Bloor, W. R. Quoted by Hill, E., and Bloor, W. R. (1922). Fat excretion. *J. biol. Chem.*, 53, 171-178.

Bouchier, I. A. D., Kellock, T. D., and Manousos, O. (1963). The origin of faecal fat in subjects without steatorrhoea. In *Proceedings of the 2nd World Congress on Gastroenterology, Munich*, **2**, 659-661.

Burr, W. W., Jr., McPherson, J. C., and Tidwell, H. C. (1960). Secretion of labeled blood lipids into the intestine. *J. Nutr.*, **70**, 171-175.

Cantarow, A., and Schepartz, B. (1962). *Biochemistry*, 3rd ed. Saunders, Philadelphia.

Clarke, R. M. (1970a). Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat. *J. Anat. (Lond.)*, **107**, 519-529.

Clarke, R. M. (1970b). A new method of measuring the rate of shedding of epithelial cells from the intestinal villus of the rat. *Gut*, **11**, 1015.

Clarke, R. M. (1970c). The effect of starvation upon mucosal architecture and cell production rate in the small intestine of the rat. *J. Anat. (Lond.)*, **107**, 384.

Comfort, M. W., Wellaeger, E. E., Taylor, A. B., and Power, M. H. (1953). Non-tropical sprue; observations on absorption and metabolism. *Gastroenterology*, **23**, 155-178.

Cotton, P. B. (1971). The contribution of the mucosa to non-dietary lipid in the intestinal lumen. M.D. Thesis, Cambridge.

Cotton, P. B. (1972a). Lipid content of rat small intestinal mucosa and sera, in the fasting and fed state. *Digestion*. (In press)

Cotton, P. B. (1972b). Quantitative thin layer chromatography of lipids using densitometry. (In preparation).

Creamer, B. (1971). Loss from the small intestine. *J. roy. Coll. Phycns Lond.*, **5**, 323-332.

Croft, D. N. (1970). Body iron loss and cell loss from epithelia. *Proc. roy. Soc. Med.*, **63**, 1221-1224.

Croft, D. N., Loehry, C. A., and Creamer, B. (1968a). Small-bowel cell-loss and weight-loss in the coeliac syndrome. *Lancet*, **2**, 68-70.

Croft, D. N., Loehry, C. A., Taylor, J. F. N., and Cole, J. (1968b). DNA and cell loss from normal small-intestinal mucosa. *Lancet*, **2**, 70-73.

Croft, D. N., and Lubran, M. (1965). The estimation of deoxyribonucleic acid in the presence of sialic acid. Application to analysis of human gastric washings. *Biochem. J.*, **95**, 612-620.

Da Costa, L. R. (1970). M.D. Thesis, London.

Da Costa, L. R., Croft, D. N., and Creamer, B. (1971). Protein loss and cell loss from the small-intestinal mucosa. *Gut*, **12**, 179-183.

Doubilet, H., and Fishman, L. (1961). Human biliary-pancreatic secretion. *Amer. J. Gastroent*, **35**, 499-512.

Eckstein, H. C. (1925). The fatty acids in the subcutaneous fat of man. *J. biol. Chem.*, **64**, 797-806.

Fenton, P. F. (1960). In *Medical Physiology and Biophysics*, 18th ed. Saunders, Philadelphia.

Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissue. *J. biol. Chem.*, **226**, 497-509.

Gordon, H. (1960). Studies on the origin of fecal fat. *J. clin. Invest.*, **39**, 992.

Gottenbos, J. J., and Thomasson, H. J. (1963). The fatty acid composition of thoracic lymph fat of rats fed single triglycerides. In *Biochemical Problems of Lipids*, edited by A. C. Frazer, pp. 272-279. Elsevier, Amsterdam.

Hoet, P. P., Hoosens, J. V., Evard, E., Eyssen, H., and DeSomer, P. (1963). Intestinal bacteria and faecal fat. In *Biochemical Problems of Lipids*, edited by A. C. Frazer.

Holmes, A. D., and Kerr, R. H. (1923). Notes on the ether extract of feces. *J. biol. Chem.*, **58**, 377-381.

Kim, K. S., and Bollman, J. L. (1954). Absorption of fat in the absence of both bile and pancreatic juice. *Arch. Surg.*, **69**, 247-254.

Kim, K. S., Bollman, J. L., and Grindlay, J. H. (1956). Metabolism of endogenous lipid of the intestine. *Amer. J. Physiol.*, **184**, 445-448.

Krakower, A. (1934). Fecal fat and its relation to fat in the diet. *Amer. J. Physiol.*, **107**, 49-54.

Loehry, C. A. (1969). M.D. Thesis, Cambridge.

Loehry, C. A., Axon, A. T. R., Hilton, P. J., Hider, R. C., and Creamer, B. (1970). Permeability of the small intestine to substances of different molecular weight. *Gut*, **11**, 466-470.

Loehry, C. A., Croft, D. N., Singh, A. K., and Creamer, B. (1969). Cell turnover in the rat small intestinal mucosa: an appraisal of cell loss. *Gut*, **10**, 13-18.

McManus, J. P. A., and Isselbacher, K. J. (1970). Effect of fasting versus feeding on the rat small intestine. *Gastroenterology*, **59**, 214-221.

Melvin, K. E. W., Hepner, G. W., Bordier, P., Neale, G., and Joplin, G. F. (1970). Calcium metabolism and bone pathology in adult coeliac disease. *Quart. J. Med.*, **39**, 83-113.

Norcia, L. N., and Lundberg, W. O. (1954). Fat excretion. The influence of dietary fat on fecal fat excretion. *J. Nutr.*, **54**, 491-508.

Partin, J. C., and Schubert, W. K. (1969). Small intestinal mucosa in cholesterol ester storage disease. *Gastroenterology*, **57**, 542-558.

Pessoa, V. C., Kim, K. S., and Ivy, A. C. (1953). Fat absorption in absence of bile and pancreatic juice. *Amer. J. Physiol.*, **174**, 209-218.

Pink, I. J., Croft, D. N., and Creamer, B. (1970). Cell loss from small intestinal mucosa: a morphological study. *Gut*, **11**, 217-222.

Reid, D. J. (1967). Intravenous fat therapy. *Brit. J. Surg.*, **54**, 198-204.

Rony, H. R., Mortimer, B., and Ivy, A. C. (1933). The source of lipids found in the thoracic duct lymph in fasting. *J. biol. Chem.*, **102**, 161-170.

Rowntree, J. I. (1930). A study of the absorption and retention of vitamin A in young children. *J. Nutr.*, **3**, 265-287.

Saunders, D. R., Ways, P. O., Parmentier, C. M., and Rubin, C. N. (1966). Studies on the lipid composition of the human small bowel mucosa. *J. clin. Invest.*, **45**, 1516-1525.

Shioda, R., Wood, P. D. S., and Kinsell, L. W. (1967). Human biliary output of lipids in relation to diet. *Fed. Proc.*, **26**, 2.

Singh, A. K. (1970). Excretion of endogenous non-haem iron by the gastrointestinal tract in human subjects and its influence on the size of body store of iron with particular reference to coeliac disease. *Brit. J. Haemat.*, **18**, 597-609.

Sperry, W. M. (1927). Lipid excretion. IV. *J. biol. Chem.*, **71**, 351-378.

Sperry, W. M. (1929). Lipid excretion. V. *J. biol. Chem.*, **81**, 299-319.

Sperry, W. M. (1932). Lipid excretion. VIII. *J. biol. Chem.*, **96**, 759-768.

Tabaqchali, S., and Booth, C. C. (1966). Jejunal bacteriology and bile-salt metabolism in patients with intestinal malabsorption. *Lancet*, **2**, 12-15.

Verdino, B., Blank, M. L., and Privett, O. S. (1965). Endogenous lipid composition of the intestinal lymph of rats raised on fat-free, lard or corn-oil diets. *J. Lipid Res.*, **6**, 356-362.

Voit, F. (1892). Beiträge zur Frage der Secretion und Resorption im Dünndarm. *Z. Biol.*, **29**, 325-397.

Watkin, D. M. (1965). Fecal excretion of lipids before, during and after hyperalimentation with fat administered intravenously. *Amer. J. clin. Nutr.*, **16**, 213-223.

Webb, J. P. W., James, A. T., and Kellock, T. D. (1963). The influence of diet on the quality of faecal fat in patients with and without steatorrhoea. *Gut*, **4**, 37-41.

Wiggins, H. S., Howell, K. E., Kellock, T. D., and Stalder, J. (1969). The origin of faecal fat. *Gut*, **10**, 400-403.

Wilson, J. D., and Reinke, R. T. (1968). Transfer of locally synthesised cholesterol from intestinal wall to intestinal lymph. *J. Lipid Res.*, **9**, 85-92.

Wollaeger, E. E., Comfort, M. W., and Osterberg, A. E. (1947). Total solids, fat and nitrogen in the feces. III. *Gastroenterology*, **9**, 272-283.