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SYMPOSIUM ON 'LIPID METABOLISM AND ITS CONTROL'

Digestion and absorption of lipids in non-ruminant and ruminant animals: a comparison

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Fat is a major component of the diet of Western man, accounting for about 40% of the energy intake (100–150 g/d). To appreciate the function of lipids in human nutrition it is important to understand the processes of digestion and absorption of lipids. A considerable amount of research has been carried out using simple-stomached animals (e.g. dog, rat) as well as man when practicable (see reviews by Deuel, 1955; Senior, 1966; Hübscher, 1970; Ockner & Isselbacher, 1974). More recently, detailed studies have been made in ruminant animals (see Garton, 1967; Leat & Harrison, 1975) and the present review will examine lipid digestion and absorption from a comparative viewpoint. Most attention will be directed to the absorption of the long-chain fatty acids (FA) ($>C_{14}$), which are absorbed into the lymphatic system, in contrast to short- and medium-chain FA ($<C_{12}$), which are absorbed mainly into the portal veins.

Digestion before the small intestine

Triglyceride (TG) is the major lipid in the diet of most non-ruminant animals. This lipid is insoluble in water, and the major function of the digestive processes is to convert it by hydrolysis into a form which will be potentially soluble in the contents of the intestinal tract. Considerable lipolysis can occur in the stomachs of suckling animals, both non-ruminants and ruminants (Ramsey, Wise & Tove, 1956; Gooden & Lascelles, 1973; Olivecrona, Hernell, Egelrud, Billström, Helander, Samuelson & Fredrikson, 1973), and some lipolysis can occur in the stomach of adult animals, including man (Cohen, Morgan & Hofmann, 1971; Hamosh & Scow, 1973; Hamosh, Klaeveman, Wolf & Scow, 1975). Although secretion of a true gastric lipase cannot be completely excluded, the major site of secretion of this lipase appears to be in the lingual and pharyngeal regions. In adult animals it is difficult to assess the quantitative importance of gastric lipolysis, but

in suckling animals it could be an important initial step in the hydrolysis of milk lipids during the neonatal period when the activity of the pancreatic enzymes may not be optimal.

In the adult ruminant animal the forestomachs (rumen, reticulum and omasum) are well developed and ingested plant food passes first into the reticulo-rumen, where it is subjected to microbial fermentation. Cellulose and carbohydrates are converted into short-chain FA, and proteins are broken down to amino acids and ammonia. Lipids undergo hydrolysis which is followed by hydrogenation. The net result of these processes in the rumen is that the esterified lipids in the diet (e.g. galactolipids, TG, phospholipids) are hydrolysed, and the FA released (mainly 18:2 and 18:3 acids) are progressively hydrogenated to stearic acid (18:0) with the formation of a wide range of geometric and positional isomers (Dawson & Kemp, 1970). These isomers, together with the odd-numbered and branched-chain FA synthesized by the bacteria, give ruminant tissues a characteristic FA pattern. The FA are not soluble in rumen digesta but are attached to the food particles, which may partially explain the lack of absorption of long-chain FA from the rumen.

A major difference between the ruminant and non-ruminant digestion appears at the beginning of the small intestine. Whereas in non-ruminants the digesta lipid is essentially still esterified, as in the diet, in ruminant animals it is mainly in the form of free FA (FFA), which are predominantly saturated.

Digestion in the small intestine

(a) *Non-ruminant animals.* The dietary TG entering the small intestine from the stomach mix with the secretions of bile and pancreatic juice in the duodenum. The bile salts lower the surface tension, allowing emulsification of the lipid droplets with a large increase in the surface area.

Pancreatic lipase, in the presence of co-lipase, acts at the oil–water interface and hydrolyses the TG to FFA and 2-monoglyceride, which, although water-insoluble per se, will dissolve readily in the intestinal contents (pH 6–6·5) when the bile salts exceed their critical micellar concentration (Hofmann & Borgström, 1962, see Fig. 1). Such micelle formation will also allow other water-insoluble compounds like cholesterol and fat-soluble vitamins to be solubilized in the hydrophobic core, and then absorbed. The intricacies of micelle formation will not be discussed further since excellent reviews are available (Hofmann & Small, 1967; Small, 1970). Although earlier theories of fat absorption suggested that absorption of lipids occurred in the particulate form, it now seems that pinocytosis is not of quantitative importance (Rubin, 1966).

Both bile and pancreatic juice are required for optimum fat absorption, and in the absence of one or both of these secretions varying extents of fat malabsorption can occur (see Wiseman, 1964). In the absence of pancreatic juice the hydrolysis of TG is impaired, and since TG are virtually insoluble in bile salts there is no means of transporting fat to the mucosal wall: hence the malabsorption of fat in diseases of the pancreas or occlusion of the duct. Malabsorption of fat can also occur in the absence of bile, e.g. in liver disease or obstruction of the bile duct. Bile salts are an

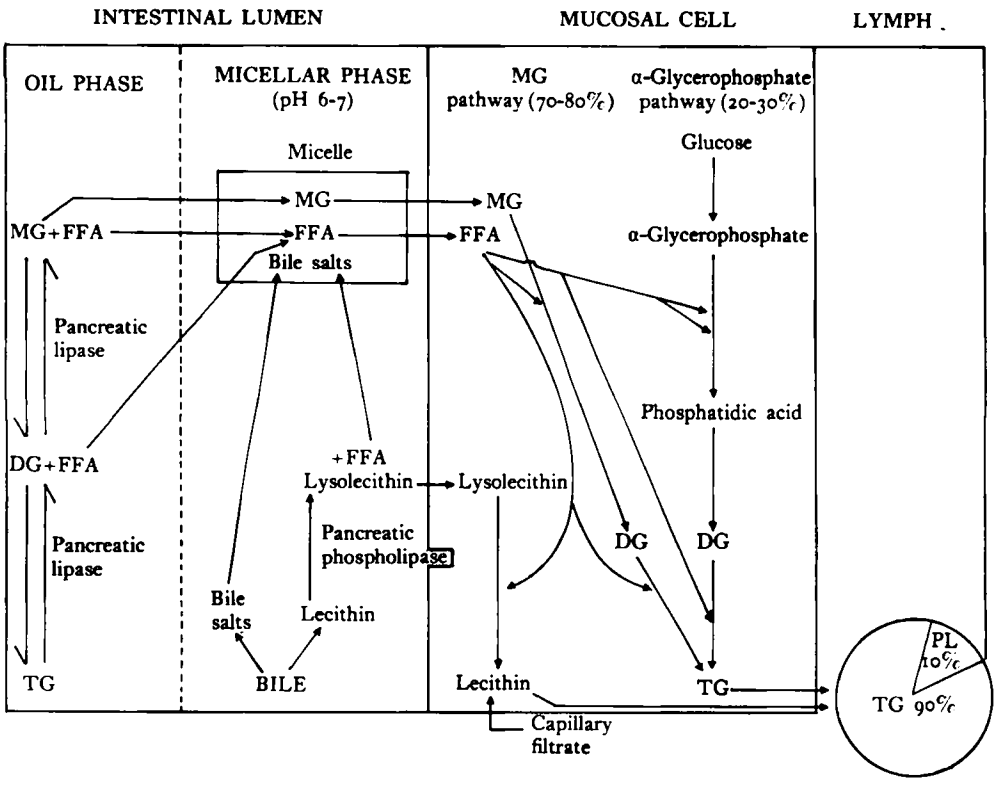

Fig. 1. Simplified scheme for digestion and absorption of lipids in non-ruminant animals. FFA, free fatty acids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; PL, phospholipids.

integral part of the micelle and in their absence monoglyceride and FFA are only poorly soluble in lumen contents. However, bile salts are not indispensible for some fat absorption to occur. Hydrolysis of lipids can occur in their absence and the FA released, particularly the unsaturated FA, have a definite solubility in water, which is, however, markedly increased in the presence of bile salts. In man up to 75% of dietary fat can be absorbed in the absence of bile (Porter, Saunders, Tytgat, Brunser & Rubin, 1971) and the same trend is found in other animals (see Wiseman, 1964). However, the pathway of fat absorption may be different in the absence of bile. In 'bile-fistula' rats less than 25% of the absorbed FA are transported in lymphatics, and the portal system assumes an increased importance (Borgström, 1953; Saunders & Dawson, 1963). The FA absorbed into the portal system are in the free form, and bile salts may be involved in esterification in the mucosal cell.

Little work has been carried out on digestion in the larger non-ruminants, but in the pig digestion is similar to that seen in man (Freeman, Annison, Noakes & Hill, 1967), and there is no reason to believe that other large animals would be different.

(b) *Ruminants.* The major lipid reaching the small intestine of the ruminant

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animal is the FFA fraction, bound as an insoluble complex with the food particles, together with minor amounts of unhydrolysed dietary lipid and microbial lipid. In non-ruminant animals there are two phases in the intestinal lumen: an oil phase and a soluble micellar phase. In ruminant animals there is no oil phase as such but there is still a two-phase system: an insoluble particulate phase and a soluble micellar phase. Digestion in the ruminant small intestine can be envisaged as the transfer of FA from the insoluble, particulate phase to the soluble, micellar phase.

Bile is essential for fat absorption into the lymphatics of sheep (Heath & Morris, 1963; Harrison & Leat, 1972), and bile salts are undoubtedly a major component involved in the solubilization of FA in the lumen of the small intestine (Lough, 1970), and in their absorption into the lymphatics (Harrison & Leat, unpublished results).

From the theoretical viewpoint, since the major part of dietary lipids is hydrolysed in the rumen, it might appear that pancreatic juice would play little or no part in fat absorption in ruminant animals. Further, since negligible amounts of TG reach the small intestine under normal husbandry conditions, there would appear to be no necessity for pancreatic juice to contain a lipase. However there is good, but not yet conclusive, evidence that pancreatic juice is required for optimum fat absorption in adult sheep (Heath & Morris, 1963; Harrison & Leat, 1972). The pancreatic juice of the sheep at least contains a powerful lipase (Arienti, Harrison & Leat, 1974) which is more resistant to acid inactivation than that of the rat, probably because of the extremely acid conditions (pH 2·5–3·5) prevailing in the ruminant duodenum (Harrison & Hill, 1962). The ruminant animal therefore retains the potential to hydrolyse considerable quantities of dietary TG which may escape rumen degradation, e.g. when protected oils are given (Scott, Cook & Mills, 1971; Scott, 1975). However there is a delay in the absorption of TG compared to FFA, probably because the acid conditions in the upper intestine are unfavourable for hydrolysis (Harrison, Leat & Forster, 1974).

The role played by pancreatic juice in lipid absorption in ruminants is not yet clear. One possibility is that pancreatic juice may function through its ability to hydrolyse biliary lecithin to lysolecithin, which could be involved in (a) creating optimum conditions for solubilization of digesta lipids in the lumen, or (b) acting in the mucosal cell as a precursor in the resynthesis of lecithin which may be important for lymph lipid formation (Harrison & Leat, 1972; Leat & Harrison, 1974). Pancreatic juice contains phospholipase A_1 and A_2 activity, which is markedly stimulated by the presence of the secretions of Brunner's glands under the acid conditions found in the sheep jejunum (pH 4–6) (Arienti, Leat & Harrison, 1975). Phospholipase activity is also found in the pancreatic juice of non-ruminants, including man, but its role, if any, in lipid absorption is unknown. The ruminant animal may therefore be a good experimental animal to investigate this aspect of lipid digestion and absorption, since the bile-salt–monoglyceride–FFA micelle which predominates in non-ruminant animals is absent in the ruminant animal, where it is replaced by the bile-salt–lecithin-lysolecithin–FFA micelle (see Fig. 2).

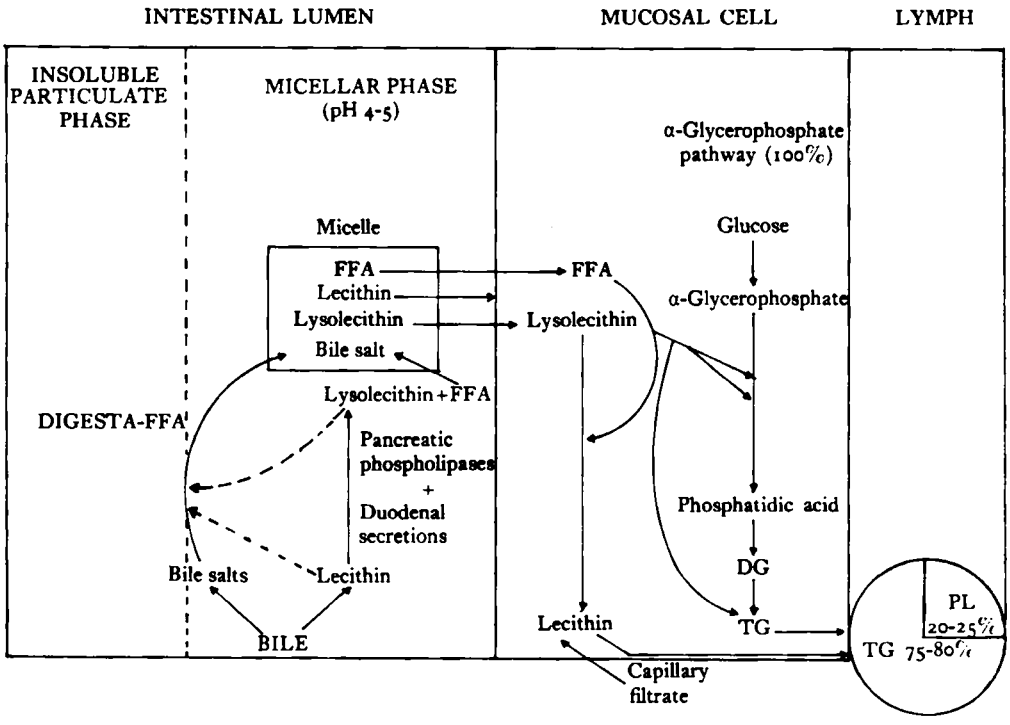

Fig. 2. Simplified scheme for digestion and absorption of lipids in ruminant animals. FFA, free fatty acids; DG, diglycerides; TG, triglycerides; PL, phospholipids.

Another interesting difference in luminal aspects of digestion between non-ruminant and ruminant animals is that whereas absorption in simple-stomached animals takes place from a neutral or slightly acid milieu (pH 6–7), in ruminant animals it occurs under distinctly acid conditions (pH 4–6) (Lennox & Garton, 1968); this extent of acidity in the human intestine would cause fat malabsorption (Go, Poley, Hofmann & Summerskill, 1970). The bile phospholipids and their hydrolysis products may be important in facilitating the solubilization of FA in acid conditions, and the fact that sheep bile contains mainly taurocholic acid (Peric-Golia & Socic, 1968) is also an advantage since this bile salt (pK_a 2·0) is less likely to become insoluble in the acid condition of the sheep intestine than is glycocholate (pK_a 4·7).

Absorption and resynthesis in the mucosal cell

Non-ruminant animals. Present concepts of fat absorption indicate that the mixed micelles of bile-salt–monoglyceride–FFA pass to the mucosal cell and dissociate, allowing the FA and monoglyceride to enter the cell by a process of passive diffusion. The bile salts are then free to form and transport another micelle, but eventually pass to the ileum where they are absorbed. The FA are transported through the cell from the microvillus to the endoplasmic reticulum by a specific binding protein (see Ockner & Isselbacher, 1974). After activation, the FA are

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resynthesized into TG by two pathways: the monoglyceride and the α-glycerophosphate pathways (Fig. 1). Because of the large amounts of monoglycerides absorbed, most of the resynthesis (70–80%) is via monoglyceride (Mattson & Volpenhein, 1964; Kayden, Senior & Mattson, 1967). The mechanism by which the resynthesized TG passes through the cell into the lymphatics is still not well defined. Evidence to date suggests that the resynthesized lipid passes to the Golgi cisternae and is coated with phospholipid, cholesterol and protein. The major protein involved is the apo-β-protein which seems to be of great importance although it is only a minor part of the lipid droplet. In the rare hereditary disease of abetalipoproteinaemia, where the β-protein is virtually absent, there is an inability to form chylomicrons or very-low-density lipoproteins (VLDL).

Ruminants. Virtually no monoglyceride is absorbed from the intestine of sheep reared under normal husbandry conditions, and the major, if not the only pathway of resynthesis is via α-glycerophosphate; but the monoglyceride pathway is still present and potentially active (Cunningham & Leat, 1969). The lecithin of bile does not seem to be absorbed as such but must first be hydrolysed to lysolecithin, which is readily absorbed into the mucosal cell where enzymes are present which reacylate lysolecithin to lecithin (Subbaiah, Sastry & Ganguly, 1969). Although there is a difference in emphasis of pathway of resynthesis there is no evidence that there is any morphological difference in lipid absorption between ruminants and non-ruminants.

Transport of lipids in lymph

The resynthesized lipids are secreted into intestinal lymph (chyle) as small droplets up to 500 nm in diameter, giving the lymph a characteristic lactescence. The appearance of chyle in non-ruminants and ruminants reflects differences in their digestive physiology. In simple-stomached animals, lipid absorption is an intermittent process related to meal-eating, whereas in ruminant animals the chyle has a permanently milky appearance because of the continuous nature of digestion.

The major lipid in thoracic duct lymph is TG, the FA composition of which reflects that of the lumen lipids. In non-ruminants the FA composition of lymph TG will also resemble that of the diet but in ruminants this is not so because of the hydrogenation that has occurred in the rumen, which results in large amounts of stearic acid being absorbed and incorporated into lymph TG. When unsaturated oils are introduced directly into the small intestine of sheep, thus by-passing the hydrogenating effect of the rumen, the unsaturated FA are incorporated unchanged into the lymph TG (Heath, Adams & Morris, 1964; Leat, 1975).

In non-ruminants, TG accounts for 85–90% of lymph lipids, with phospholipid (<10%), cholesterol (2%) and cholesteryl esters (3%) being minor components (Dole & Hamlin, 1962). In ruminants the content of TG is lower (75%) and phospholipids higher (20–25%) (Felinski, Garton, Lough & Phillipson, 1964; Leat & Harrison, 1974), indicating that the lipid droplets in the lymph of ruminants are smaller than in that of non-ruminants. In sheep 75% of lymph lipids are in the VLDL fraction, with maximal concentration in the Sf 150–200 region (G. L. Mills,

W. M. F. Leat & F. A. Harrison, unpublished results). In the cow the percentage of lipid in the VLDL fraction appears to be higher still (>90%), which might explain why chylomicrons are rarely seen in cow plasma (J. L. Linzell, personal communication). Although it was generally believed that fat is transported in the lymph of non-ruminant animals as chylomicrons, recent evidence indicates that the VLDL also play a role in lipid transport from the intestine in simple-stomached animals (Ockner, Hughes & Isselbacher, 1969). The reason for VLDL predominating in ruminant lymph may be a consequence of the relatively low intake of dietary lipid (20–30 g/d in an adult sheep) or of the saturated nature of the FA absorbed (Ockner *et al.* 1969). When unsaturated oils are infused into the duodenum of sheep, there is an increased proportion of lipids in the lymph chylomicron fraction (Harrison *et al.* 1974).

Although the mode of formation of lymph TG is now well defined, the origin and formation of lymph phospholipids is still obscure. Originally it was thought that phospholipids were precursors of TG but the turnover of phospholipids in the mucosal cell appears to be insufficient to account for absorption (Zilversmit, Chaikoff & Entenman, 1948). In both ruminants and non-ruminants a high proportion of lymph phospholipids are of endogenous origin (Whyte, Karmen & Goodman, 1963; Blomstrand, Gurtler & Werner, 1965; Leat & Harrison, 1974). In sheep the flow of bile lecithin is theoretically sufficient to account for most of the lymph phospholipids, but how much is in fact derived from biliary lecithin and how much from other sources, e.g. capillary filtrate, is still unresolved.

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