ABSORPTION AND METABOLISM OF LIPID

IN THE

YOUNG AND IN THE ADULT LACTATING RUMINANT

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by

J. C. WADSWORTH

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Dairy Research Unit,
Department of Animal Husbandry,
Faculty of Veterinary Science.

University of Sydney,
Sydney.

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ABBREVIATIONS

The following abbreviations are used in this thesis:-

APL	Apiezon L grease
ATP	Adenosine triphosphate
CMC	Critical micellar concentration
CoA	Coenzyme A
DEGS	Diethylene glycol succinate polyester
EGA	Ethylene glycol adipate polyester
EGSP-Z	Organosilicone polyester type
FFA	Free fatty acid
GLC	Gas-liquid chromatography
HDL	High density lipoprotein
ID	Internal diameter
i.u.	International units
LDL	Low density lipoprotein
OD	Outside diameter
TEFA	Total esterified fatty acid
TLC	Thin layer chromatography
4,8,12-TMTD	4,8,12-Trimethyltridecanoic acid
VHDL	Very high density lipoprotein
VLDL	Very low density lipoprotein

The fatty acid designation of Dole, et al. (1959) is used throughout, i.e. number of carbon atoms:number of double bonds, e.g. 18:1 represents octadecenoic acid. Trivial names are used only to designate specific fatty acids, e.g. "linoleic acid" is cis, cis-9,12-octadecadienoic acid, whereas unsaturated fatty acids identified from the results of packed column GLC are designated by the shorthand nomenclature since the position and configuration of the double bonds is rarely indicated by this technique. Methyl branched, saturated fatty acids are indicated by "Br".

CHAPTER 1. INTRODUCTION

HISTORICAL BACKGROUND

Studies of fat absorption may be said to have commenced with the discovery of the mesenteric lacteals and can, therefore, be traced back to the beginnings of Human Anatomy. The lymphatic system was first described in any detail by an Italian physician, Gasparo Aselli, who, in 1622, observed the mesenteric lymphatics of a well-fed dog (Aselli, 1627). Not long after Aselli's death, the flow of lymph from the intestines by way of the receptaculum chyli and the thoracic duct into the external jugular vein was described (Pecquet, 1651; Rudbeck, 1653; Bartholin, 1653), together with the entry of the lymphatic trunks from the lumbar, iliac, hepatic and abdominal regions into the receptaculum chyli (Rudbeck, 1653). It was thought during this period that the products of digestion were carried to the blood by way of the lacteals and thoracic duct (Rudbeck, 1653). Thus, from the very beginning the lymphatic system was recognised as playing an important role in absorption.

The next step forward in the understanding of lipid digestion came with the observation by Claude Bernard in 1856 that the mesenteric lacteals of rabbits fed a fat meal only became lactescent distal to the entrance of the pancreatic duct into the duodenum. This suggested that

pancreatic juice was important in the digestion of fats. Bile was also implicated in fat digestion in the latter half of the nineteenth century (see, for example, Hoppe-Seyler, 1877).

The first coherent theory of fat digestion, the Particulate Theory, was advanced by Munk (1884) and Schäfer (1885). They believed that the bulk of ingested lipid was absorbed without prior hydrolysis as an emulsion and transported across the intestinal epithelium into the lymphatics.

The Particulate Theory was quickly challenged by Pflüger (1900a,b,c) who held that fats were completely hydrolysed in the intestinal lumen. The observation of Henriques and Hansen (1900) that paraffin emulsions were not absorbed cast further doubt on the Particulate Theory. Pflüger thought that the pH of intestinal contents was basic and proposed that liberated fatty acids were absorbed as water soluble soaps. This came to be known as the Lipolytic Theory and was so vigorously championed by Pflüger that it gained wide acceptance. Unfortunately a great deal of personal friction was generated at the same time (see Johnston, 1968). When, later, the pH of intestinal contents was shown to be acidic, the Lipolytic Theory was modified and extended by Verzár and colleagues (see Verzár and McDougall, 1936). These workers demonstrated that bile salts solubilize fatty acids and that inhibitors

of phosphorylation prevent absorption of fat. They concluded that dietary fat was completely hydrolyzed to glycerol and free fatty acid (FFA) in the gut lumen, the fatty acids were absorbed as soluble FFA-bile salt complexes and then re-synthesized into triglyceride via a pathway involving phosphorylation. This latter point was supported by the effect of adrenal hormones on fat absorption.

The Pflüger-Verzár Lipolytic Theory held sway for 40-odd years until challenged by Frazer in 1938. Frazer showed that complete hydrolysis did not occur in the gut lumen, that paraffins were absorbed provided that they were emulsified to particle diameters of 0.5 μ or less, that the only system which could produce a stable emulsion under physiological conditions was the ternary combination FFA-monoglyceride-bile salt and that there were fewer chylomicrons in the circulation of rats after the ingestion of FFA than after the ingestion of triglyceride (Frazer, 1946). This led him to propose the Partition Theory which held that intraluminal hydrolysis was incomplete, that the luminal emulsion was dispersed by the FFA-monoglyceride-bile salt complex and absorbed as charged particles through minute canals in the brush border of the intestinal cell wherein the glycerides were partitioned into the lymphatics and the FFA into the portal vein. Intracellular synthesis or phosphorylation

was not essential and the effect of adrenal hormones on fat absorption was due to their role in controlling electrolyte balance which in turn affected charged particle absorption. Frazer thus used some elements of the Lipolytic Theory but his concept was essentially Particulate in nature.

It had been realized as early as the mid-nineteenth century that bile, pancreatic juice and the lymphatics were involved in fat assimilation but little understanding of what happened to fat during passage across the epithelial cell was gained until the 1950's. This lack of progress was undoubtedly due to the absence of adequate techniques.

With the advent of surgical techniques for the chronic collection of lymph (Bollman, Cain and Grindlay, 1948), the electron microscope, radio-active tracers and improved methods for the isolation of lipids, the basic tenets of Frazer's Partition Theory could be tested. Confirmation of one of Frazer's postulates came rapidly when Zilversmit, Chaikoff and Entenman (1948) showed, using radio-active phosphorus, that phospholipid turnover in the intestinal mucosa was insufficient to permit all the absorbed fat to pass through a phospholipid intermediate.

However, the Partition Theory came under fire from other directions. Electron microscopy demonstrated the continuity of the membrane in the brush border of the

intestinal cell (Granger and Baker, 1949; 1950) suggesting that charged particle absorption could not occur as Frazer had described it. Chaikoff's laboratory showed that 70 - 90% of ingested long-chain fatty acid could be recovered from the thoracic duct lymph of Long-Evans rats (Bloom, et al., 1950). However, short and medium chain fatty acids were partitioned into the portal blood according to chain length (Bloom, Chaikoff and Reinhardt, 1951). It is interesting to note that Frazer's observations on the portal absorption of ingested FFA were carried out in hooded rats. It has since been shown that this phenomenon is peculiar to the hooded variety of rat and does not occur in other strains (Dawson, Gallagher, Saunders and Webb, 1964).

Thus the Partition Theory proved inadequate. Nevertheless it was a theory that, for the first time, could be extensively tested and as such marked the beginning of the modern era in the understanding of fat absorption.

BIOCHEMICAL ASPECTS OF FAT ABSORPTION

The processes involved in fat absorption are well established and have been widely reviewed (Johnston, 1963; Clement, 1964; Senior, 1964; Isselbacher, 1965, 1966, 1967; Dawson, 1967; Johnston, 1968; Hübscher, 1969). Since the major part of this thesis is concerned with the end-product of fat absorption, i.e. lymph lipid, a summary of the events occurring during fat assimilation will now be given, with the emphasis on those processes which may affect the final composition of lymph lipid.

The assimilation of dietary lipid as it occurs in the alimentary tract may be considered in several steps.

Luminal Phase.

Ingested lipid, usually consisting largely of triglyceride, is not water soluble and must be dispersed in water sufficiently to allow it to come into close proximity to the cell membrane for cellular uptake. This is achieved in two stages, which may be termed lipolysis and dispersion, respectively.

Lipolysis. Lipolysis occurs to a limited extent in the stomach. Whether the agent responsible is a true gastric lipase or just regurgitated intestinal contents is still open to question, but the latter is the more probable (see Deul, 1955; Senior, 1964).

Upon entering the duodenum, the food is mixed with

the biliary and pancreatic secretions. It has been shown that if pancreatic juice is diverted from the intestine, very little absorption of triglyceride occurs (Masarei and Simmons, 1966). This is associated with a marked reduction in pancreatic lipase (glycerol ester hydrolase, EC 3.1.1.3) activity of the gut contents and indeed it is apparent that this enzyme is the only lipolytic agent of quantitative significance in triglyceride digestion. This enzyme has a number of interesting features which would appear to uniquely suit its physiological role in fat absorption.

Thus pancreatic lipase shows a marked preference for triglyceride and only acts on water-insoluble substrates (Sarda and Desnuelle, 1958). Pancreatic lipase appears to depend for its activity on absorption at an oil-water interface (Desnuelle and Savary, 1963) and consequently its activity is probably dependent on the state of division (i.e., surface area) of the emulsified substrate.

Isolated pancreatic lipase has a pH optimum of 8-9 whereas duodenal contents possess a pH of about 6.0-6.5 (Verzár and McDougall, 1936). It is of relevance, therefore, that bile salts reduce the pH optimum of pancreatic lipase to about 6 (Borgström, 1954) so that, under physiological conditions, the enzyme is activated by bile salts. The reaction catalysed by pancreatic lipase is an

equilibrium between formation and hydrolysis of ester bonds and is, therefore, subject to mass action effects (Borgström, 1964a). Bile salts promote increased ionization of fatty acids which would, therefore, be removed more rapidly from the site of enzyme action due to increased solubility. Thus, when bile salts are present, more fatty acid is ionized at a lower pH than in their absence. This accounts for the acid shift in the pH optimum of the lipase when bile salts are added and for the requirement for bile salts under physiological conditions.

Calcium is necessary for optimum enzyme activity (Entressangles, et al., 1961) and would appear to exert its effect by promoting binding of the lipase molecule to the oil-water interface (Benzonana, 1968).

It has long been known that pancreatic lipase is relatively specific for the 1- and 3- ester bonds of triglycerides, 2-monoglycerides being relatively stable to enzymic hydrolysis (Mattson, Benedict, Martin and Beck, 1952). Approximately one third of triglyceride molecules are completely hydrolysed under physiological conditions (Reiser, Bryson, Carr and Kuiken, 1952; Mattson and Volpenhein, 1962, 1964; Raghavan and Ganguly, 1969). It was thought that this was due to isomerization of 2-monoglycerides to 1-monoglycerides which are susceptible to enzymic hydrolysis. However, Borgström (1964a) has

presented evidence that secondary esters are hydrolysed by pancreatic lipase, albeit at a much lower rate than the primary bonds.

Pancreatic lipase displays distinct substrate specificity, especially with respect to short chain fatty acids, butyrate being released from triglycerides faster than any other fatty acid (Entressangles, et al., 1961). This was recently shown to represent an intermolecular rather than intramolecular specificity, (Sampugna, et al., 1967) so that in triglycerides containing both short and long chain fatty acids, the short and long chain acids are released at a similar rate to each other but more rapidly than the release of fatty acids from triglycerides containing exclusively long chain fatty acids. Thus, for example, pancreatic lipase hydrolyses glyceryl-1-butyrate-2,3-dipalmitate more rapidly than glyceryl trioleate but both butyrate and palmitate are released from the former at the same rate. However, for fatty acids of chain length C_{12} or longer, no specificity could be demonstrated with respect to chain length, degree of unsaturation, or geometrical isomerism of unsaturated fatty acids (Jensen, Sampugna and Pereira, 1964). Nevertheless, the substrate must be in a liquid, rather than crystalline, state, since long chain, saturated fatty acids are only slowly hydrolysed at 37°C when present as high-melting,

trisaturated glycerides (e.g. tristearin) but are readily split when present as lower-melting, mixed glycerides. This may be due to a failure of the enzyme to bind to crystalline fat (Desnuelle and Savary, 1963).

Also present in pancreatic juice is the enzyme phospholipase A (phosphatide acyl hydrolase, EC 3.1.1.4) which hydrolyses the 2-ester bond of phosphatides (Magee, Gallai-Hatchard, Sanders and Thompson, 1962; De Haas, et al., 1963; Belleville and Clément, 1966). Phospholipids (largely phosphatidylcholine) which are present to some extent in the diet and also enter the intestine in considerable quantities in bile (Shrivastava, Redgrave and Simmonds, 1967) are hydrolysed to produce lysophosphatidylcholine, a potent detergent. The significance of the latter during fat absorption in monogastric species is not known but it appears to play an important role in the ruminant and will, therefore, be dealt with later.

Small amounts of cholesterol ester present in the diet are hydrolysed by cholesterol esterase (sterol ester hydrolase, EC 3.1.1.13) which is secreted in pancreatic juice and requires bile salts for its activity (Borja, Vahouny and Treadwell, 1964; Mattson and Volpenhein, 1966).

Dispersion. Reference has already been made to the discovery by Frazer, Schulman and Stewart (1944) that the FFA-monoglyceride-bile salt complex plays an important role in fat absorption. Borgström (1956) suggested that this might be accounted for by the formation of colloidal dispersions (micellar solutions) and it has since been shown that lipid is incorporated into micelles before absorption occurs. This field has been comprehensively reviewed (Hofmann and Borgström, 1962; Borgström, 1962, 1964b; Hofmann, 1966, 1968; Hofmann and Small, 1967).

In order to understand the physico-chemical events involved in micelle formation it will be necessary to discuss the properties of the various lipids involved. Lipids of biological significance may be divided into three groups according to their surface activity (Hofmann and Small, 1967):

1. Insoluble amphipaths which orient at air-water or oil-water interfaces but are insoluble in water. They are always present as a separate phase. Triglycerides, diglycerides, fully protonated fatty acids and cholesterol belong to this class.

2. Swelling amphipaths which are hydrated in water but remain closely associated to form lamellar, cylindrical or cubic molecular aggregates (known as the liquid crystalline state). These compounds, including monoglycerides and phosphatides, are readily dispersed in water to give

"milky" suspensions. However, they possess negligible molecular solubility and are not properly termed micellar dispersions.

3. Soluble amphipaths which possess a finite molecular solubility in water and are highly surface active. Above a certain concentration (critical micellar concentration, CMC) aggregates are formed which are termed micelles. These consist of spherical or rod shaped particles with the hydrophobic portion of the molecules forming a liquid hydrocarbon centre and the ionic or polar part of the molecules projecting into the aqueous phase. The charges on the surface of the micelles prevent coalescence of particles with consequent separation into two phases. Micellar solutions do not scatter light and are, therefore, clear. The molecules of each micelle are in rapid equilibrium with both the molecules in the free solution and with those of other micelles. The components of micellar solutions are, therefore, readily "diffusible" in contrast to the previous two classes of lipid. As the concentration is increased above the CMC, excess amphipath is dispersed in the micellar phase, more micelles of identical composition being formed. Negative free energy is associated with micelle formation which occurs, therefore, spontaneously. Soluble amphipaths include fatty acid soaps, lysophosphatidyl choline, phosphatidic acid and free and conjugated bile salts.

The bile duct carrying bile from the liver and gall bladder enters the duodenum a few inches distal to the pylorus and is closed by the sphincter of Oddi. Each portion of chyme leaving the stomach is mixed with a portion of bile as it passes the opening of the bile duct (Borgström, 1962).

Two lipids of importance for fat absorption are added to the intestinal contents in bile. These are conjugated bile acids (bile salts) and phospholipids (largely phosphatidyl choline). The major bile acids in human bile are cholate, deoxycholate and chenodeoxycholate which are conjugated with glycine and taurine in the approximate ratio of 2:1 (Sjövall, 1959; Dam, Kruse, Jensen and Kallehauge, 1967). It may be noted here that glycine conjugates solubilise more oleic acid than taurine conjugates (Hofmann and Borgström, 1962).

The CMC of a mixture of bile salts resembling that found in intestinal contents in the presence of physiological concentrations of electrolyte and monoglyceride was 1-2 mM (Hofmann, 1963). Since the concentration of bile salts normally present in intestinal contents is 2.5-10 mM (Sjövall, 1959) it is evident that a micellar phase is present in the gut lumen under physiological conditions.

Lipids in the intestinal lumen are distributed between two phases, an oil phase containing triglycerides,

diglycerides and unionized FFA and an aqueous, micellar phase containing bile salts, monoglycerides and partially ionized FFA (Hofmann and Borgström, 1962, 1964; Feldman and Borgström, 1966). It has recently been shown that the solid phase of gut contents may be of importance since the indigestible plant fibre, lignin, binds bile salts (Eastwood and Hamilton, 1968). In addition, protein may compete with the micellar phase for available FFA, since albumen effectively binds FFA in the presence of bile salt micelles (Gallagher and Playoust, 1969).

Bile is essential for the absorption of triglyceride but not of FFA entering the intestine (Morgan, 1964). It has been found that FFA, dispersed as a fine emulsion, is well absorbed in the absence of a micellar phase and this was attributed to diffusion from monomolecular solution (Simmonds, Redgrave and Willix, 1968). While micellar dispersion is not necessary for absorption of FFA, uptake of this lipid is facilitated by incorporation into micelles (Hoffman, 1969). Monoglycerides are less well absorbed in the absence of micelles (Simmonds, 1969). Thus it would appear that under normal dietary conditions micelle formation is a prerequisite for satisfactory fat absorption.

It has been shown that fatty acids of chain length C_{12}-C_{18} are absorbed equally when in micellar solution (Freeman, Annison, Noakes and Hill, 1967; Gallagher and Playoust, 1969). Thus, any differences in the absorption

of various fatty acids from the gut may be expected to depend on the extent to which they are incorporated into bile salt micelles. Under some circumstances, stearic acid is less well absorbed than palmitic or oleic acids and this has been attributed to poor micellar solubility of stearic acid (Constantin and Savary, 1965). On the other hand, erucic acid, which is highly soluble in mixed micelles, is poorly absorbed (Savary and Constantin, 1966) and elaidic acid (trans 18:1), which might be expected to have lower micellar solubility than oleic acid (cis 18:1; Hofmann and Small, 1967), is better absorbed than oleate (Lavoue and Clément, 1967). Interpretation of experiments on differential micellar solubility of different fatty acids is complicated by the fact that the behaviour of mixtures of fatty acids is not explained by the summation of their separate behaviours (Hofmann, 1966).

Uptake Phase.

Morphology. As pointed out in the Historical Background, controversy over the mode of fat absorption has existed for a considerable time. Early studies with the electron microscope lent no credence to Frazer's postulate that fat was absorbed as charged particles through minute channels in the surface of the intestinal epithelial cell but did reveal the presence of small vesicles immediately below the microvilli (Palay and Karlin, 1959). This observation suggested that lipid was taken up by

pinocytosis.

However, recent reviewers have concluded that pinocytosis cannot account quantitatively for the bulk of lipid uptake (Strauss, 1968; Dobbins, 1969). Thus, during fat absorption, no increase in the frequency of occurrence or size of the pinocytotic vesicles has been demonstrated (Strauss, 1966; Cardell, Badenhausen and Porter, 1967), in contrast to findings during pinocytotic uptake of colostral antibodies by newborn animals (Sibalin and Björkman, 1966). Furthermore, at 0°C, micellar lipid is taken up by intestinal mucosa, but neither esterification (Johnston and Borgström, 1964; Strauss and Ito, 1965) nor intracellular droplet formation (Strauss, 1966) occurs unless the preparations are post-incubated at 37°C. Finally, Cardell, et al. (1967) showed that silver particles suspended in fat emulsions were not taken up by the intestinal mucosa whereas ferritin particles present in the aqueous phase were absorbed by pinocytosis and incorporated into lysozomes. It may be concluded from these studies that lipid enters the intestinal cell by an energy-independent process (probably diffusion) not involving pinocytosis and is subsequently esterified by an energy-requiring process (cf. Hogben, 1966).

Mechanism. The exact mechanism by which lipid penetrates the cell membrane remains uncertain (Hübscher,

1969). Uptake of micellar lipid is Na^+-dependent and a carrier mediated mechanism is possible (Lyon, 1968). Whether micelles are taken up intact or their components absorbed separately remains controversial (see, for example, Gordon and Kern, 1968; Thornton, Vahouny and Treadwell, 1968). Johnston and Borgström (1964) showed that micellar lipids were strongly bound by isolated brush border preparations and could not demonstrate triglyceride synthesis in this fraction. However, Forstner, Riley, Daniels and Isselbacher (1965) detected the enzymes necessary for triglyceride synthesis from FFA and monoglyceride in brush borders, which suggests that these enzymes may be directly involved in the uptake of micellar lipid.

Recently an alternative mechanism was proposed by Munday, Parsons and York (1969). They showed that Ca^{++} stimulates uptake of fatty acids by intestinal mucosa and binding of fatty acids by brush border preparations. This they interpreted as indicating a Ca^{++} dependent process which would result in net uptake of fatty acid in the presence of a Ca^{++} gradient across the brush border. When these observations are coupled with the recent demonstration of carrier-mediated calcium transport by rat duodenum (Walling and Rothman, 1969) it is tempting to suggest that the calcium-binding protein located on the surface of the intestinal epithelial cell (Wasserman,

Corradino and Taylor, 1968) may be involved in transport of fatty acids during fat absorption.

Site of absorption. Fat absorption is virtually complete by the time the digesta leave the jejunum (Booth, 1967) but bile salts are not absorbed until the ileum is reached (Lack and Weiner, 1967). As a result, bile salt concentrations increase down the gut (Dietschy, 1967). Experiments using aminopterin to inhibit cell division have shown that only the more mature epithelial cells of the intestinal villus (those nearest the tip) are capable of absorbing fat (Redgrave and Simmonds, 1967).

Mucosal Phase.

After uptake by the intestinal epithelial cell, FFA and monoglyceride are esterified to produce triglyceride. This esterification is effected by one of two pathways, termed the glycerol-3-phosphate and the monoglyceride pathways, respectively. For excellent reviews of the biochemical events involved see Senior (1964) and Johnston (1968).

Glycerol-3-phosphate pathway. Both pathways share a common initial step (Fig. 1), the activation of long chain FFA by the formation of acyl coenzyme A (CoA) thiolesters under the influence of the enzyme fatty acid:CoA ligase (EC 6.2.1.2; Clark and Hübscher, 1960;

FIGURE 1.

Major steps in the digestion and absorption of long chain fatty acid by the proximal small intestine.

ABBREVIATIONS:

AA	- Amino Acids
CoASH	- Coenzyme A
DG	- Diglyceride
FFA	- Free Fatty Acid
G-3-P	- Glycerol-3-Phosphate
MG	- Monoglyceride
PA	- Phosphatidic Acid
PC	- Phosphatidyl Choline
R.CO.OH	- Fatty Acid
TG	- Triglyceride

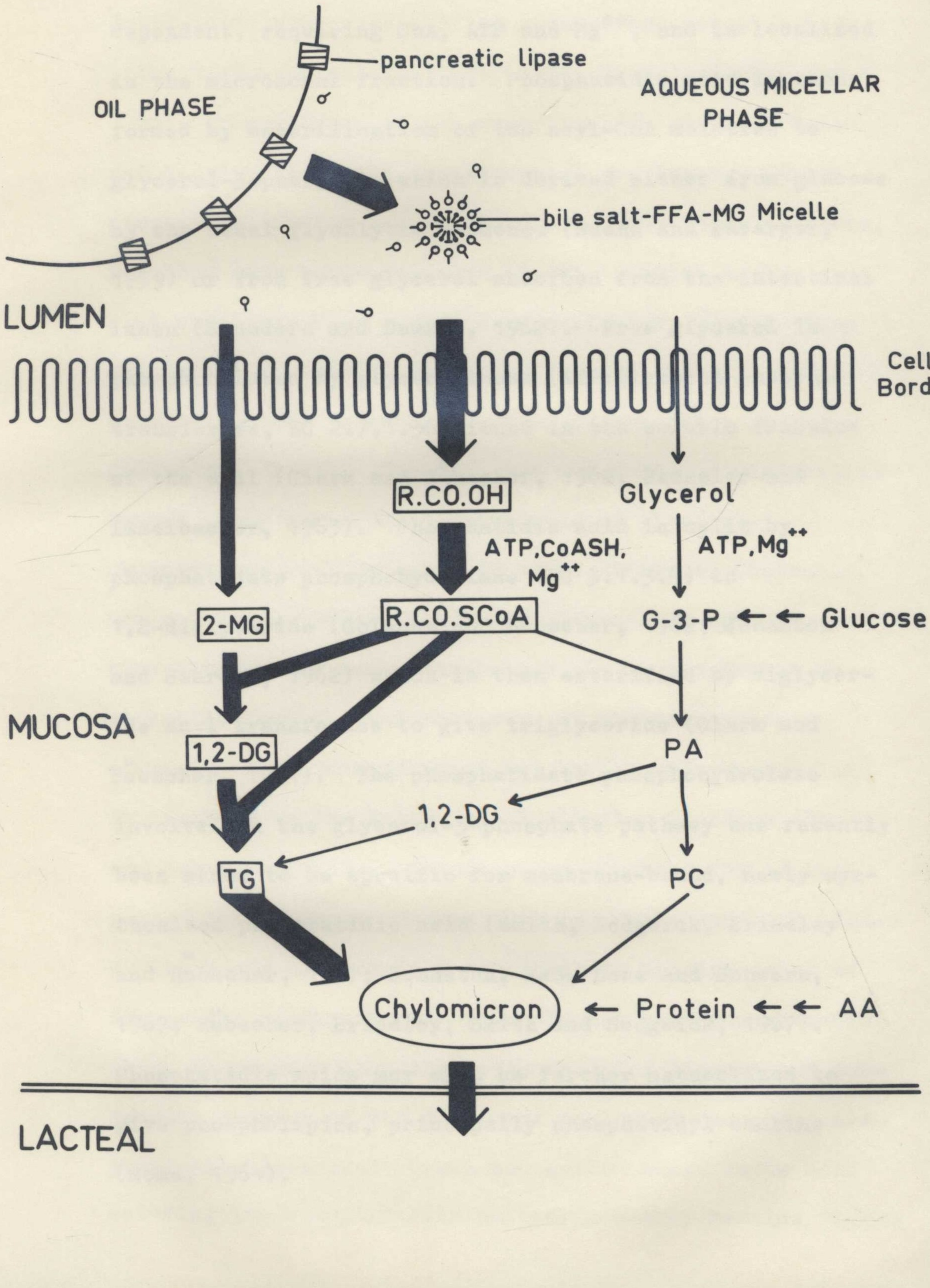

pancreatic lipase
OIL PHASE
AQUEOUS MICELLAR PHASE
bile salt-FFA-MG Micelle
LUMEN
Cell Border
R.CO.OH
Glycerol
ATP,CoASH, Mg++
ATP,Mg++
2-MG
R.CO.SCoA
G-3-P ← ← Glucose
MUCOSA
1,2-DG
PA
1,2-DG
TG
PC
Chylomicron
← Protein ← ← AA
LACTEAL

Figure 1

Senior and Isselbacher, 1960). This reaction is energy dependent, requiring CoA, ATP and Mg^{++}, and is localized in the microsomal fraction. Phosphatidic acid is then formed by esterification of two acyl-CoA moieties to glycerol-3-phosphate which is derived either from glucose by the usual glycolytic sequence (Buchs and Favarger, 1959) or from free glycerol absorbed from the intestinal lumen (Saunders and Dawson, 1962). Free glycerol is phosphorylated by glycerokinose (ATP:glycerol phosphotransferase, EC 2.7.1.30) found in the soluble fraction of the cell (Clark and Hübscher, 1962; Haessler and Isselbacher, 1963). Phosphatidic acid is split by phosphatidate phosphohydrolase (EC 3.1.3.4) to 1,2-diglyceride (Coleman and Hübscher, 1962; Johnston and Bearden, 1962) which is then esterified by diglyceride acyl transferase to give triglyceride (Clark and Hübscher, 1961). The phosphatidate phosphohydrolase involved in the glycerol-3-phosphate pathway has recently been shown to be specific for membrane-bound, newly synthesized phosphatidic acid (Smith, Sedgwick, Brindley and Hübscher, 1967; Johnston, Rao, Lowe and Schwarz, 1967; Hübscher, Brindley, Smith and Sedgwick, 1967). Phosphatidic acids may also be further metabolized to give phospholipids, principally phosphatidyl choline (Noma, 1964).

Monoglyceride pathway. In this pathway, activated fatty acids are re-esterified to absorbed monoglyceride (Fig. 1; Reiser and Williams, 1953; Clark and Hübscher, 1960). The enzymes of the monoglyceride pathway (mono- and di-glyceride acyl transferases) are located in the microsomes (Senior and Isselbacher, 1961, 1962) and form, together with fatty acid:CoA ligase, a multi-enzyme complex which has been purified approximately 70-fold (Rao and Johnston, 1966). The monoglyceride and glycerol-3-phosphate pathways share a common intermediate at the diglyceride level. However, the two pools of diglyceride formed using either 2-monoglyceride or glycerol-3-phosphate as acyl acceptor do not equilibrate (Johnston, Rao and Lowe, 1967). The physical basis of this separation between the two pathways may lie in the recent observation that the intermediates in the monoglyceride sequence remain enzyme bound and that protein-bound CoA is involved in the fatty acid activation reaction (Rao and Johnston, 1967). Brindley and Hübscher (1966) suggested the presence of more than one fatty acid:CoA ligase on the basis of anomalies in substrate specificities of homogenate preparations. It would not seem unreasonable, then, to propose that the fatty acid:CoA ligases and subsequent enzymes of the different acylation sequences are physically separated within the cell. Thus, fatty acid entering the monoglyceride pathway probably remains

completely separate from that entering the glycerol-3-phosphate pathway.

Phospholipids may be synthesized from monoglyceride via lysophosphatidic acid (Paris and Clement, 1965, 1969) and from diglyceride by addition of choline phosphate (Gurr, Brindley and Hübscher, 1965) although the relative importance of these reactions is unknown. The microsomes of intestinal epithelium contain an active monoglyceride lipase (Tidwell and Johnston, 1960; Senior and Isselbacher, 1963) which ensures that in the presence of insufficient fatty acyl CoA, excess monoglyceride does not accumulate (Johnston, 1968).

It appears that, under normal circumstances, the monoglyceride pathway is the predominant mechanism for the re-esterification of absorbed lipid (Noma, 1964; Mattson and Volpenhein, 1964; Kern and Borgström, 1965; Kayden, Senior and Mattson, 1967; Paris and Clement, 1968; Raghavan and Ganguly, 1969) although species differences exist in this respect (Bickerstaffe and Annison, 1969a).

Metabolism of absorbed fatty acid. Long chain fatty acids taken up by the intestinal mucosa may be oxidized to carbon dioxide (Greenberger, Franks and Isselbacher, 1965; Franks, Riley and Isselbacher, 1966). In addition, it has been reported that the carbon chain of fatty acids may be lengthened or shortened by two-carbon units and desaturated by rat gut mucosa (Boucrot and Clément, 1965a)

although Bickerstaffe and Annison (1969b) failed to detect desaturase activity in pig and chicken mucosa. However, the magnitude of these changes is small, with the recovery of unchanged fatty acid usually exceeding 95%.

Intracellular transport and secretion of absorbed lipid. The passage of lipid through the intestinal epithelial cell has been studied with the electron microscope. This subject has been extensively reviewed by Strauss, (1968) and Dobbins (1969). Autoradiographic studies have shown that, after absorption, micellar lipid is distributed diffusely throughout the cytoplasm (Strauss and Ito, 1965). FFA and monoglyceride are then incorporated into triglyceride droplets within the smooth-surfaced endoplasmic reticulum in the apex of the cell (Sjöstrand and Borgström, 1967). These observations are consistent with the microsomal location of the enzymes for fatty acid esterification. Examination of intestinal tissue fixed at intervals after ingestion of a fat meal suggest that, after esterification, lipid droplets in the apex of the cell move via the Golgi complex to the intercellular spaces (Dobbins, 1969). Lipid droplets encountered in the Golgi complex resemble chylomicrons seen in lacteals and it is probable that these droplets are coated with protein in the Golgi complex (Dobbins, 1966). From the intercellular spaces, secreted

chylomicrons make their way across the basement membrane underlying the epithelium and enter the lacteals, predominantly through intercellular junctions (Casley-Smith, 1962).

INCORPORATION OF DIETARY FATTY ACID INTO LYMPH LIPIDS

Short vs. Long Chain Fatty Acid.

As previously mentioned, fatty acids of chain length less than C_8 are transported entirely via the portal vein (Hyun, Vahouny and Treadwell, 1967), C_8-C_{12} fatty acids are partitioned according to chain length into the blood and lymph and fatty acids containing more than 12 carbon atoms are transported almost entirely as chylomicron triglyceride (Bloom, et al., 1951). This differentiation in the absorption of short and long chain fatty acids is probably due to a combination of several factors, including the appreciable water solubility of short chain fatty acids, the marked preference for long chain fatty acid displayed by fatty acid:CoA ligase resulting in poor esterification of short chain acids within the mucosa (Brindley and Hübscher, 1966) and the presence of a microsomal lipase in the intestinal cell which specifically hydrolyses esters of short chain fatty acids (Playoust and Isselbacher, 1964).

Thus, short chain fatty acids are probably released from the intestinal cell in the free state. It would be expected from their water solubility that short chain fatty acids would enter vascular and lymphatic capillaries with equal ease. The high ratio of blood to lymph flow through the intestinal tissue would then result in the bulk of these fatty acids being transported in the bloodstream (Dawson, et al., 1964).

Long chain fatty acids, on the other hand, are largely esterified and incorporated into chylomicrons which probably cannot penetrate the basement membrane surrounding the vascular capillaries but enter the lacteals, which have no basement membrane (Vodovar, Flanzy and François, 1967).

These considerations are reflected in the fatty acid composition of lymph lipids from animals fed fats containing a wide range of fatty acids (Bhalareo, Inoue and Kummerow, 1963).

Saturated vs. Unsaturated Fatty Acid.

Balance studies. With the advent of the classical balance techniques for studying absorption (Boussingault, 1839) it became possible to compare absorption of different fats. Studies of this nature have been reviewed (Deuel, 1955; Thieulin, 1968; Holt and Clark, 1969).

It was quickly recognised that some fats were less well absorbed than others. Langworthy and Holmes (1915)

proposed that the efficiency of absorption depended on melting point, fats melting above 50°C being poorly absorbed. However, Holt, et al., (1935), in an encyclopaedic work, discounted melting point as being the primary factor influencing fat digestibility. They contended that fat digestibility was determined by fatty acid composition and presented evidence for selective malabsorption from mixed fats of saturated, long chain fatty acids. Their results were supported by the less extensive study of Mattil and Higgins (1945) who tested purified 18:0 and 18:1 in various triglyceride combinations and concluded that 18:0 was poorly absorbed, no matter how it was fed. This concept was challenged by Mattson (1959) who showed in an elegant experiment that fats of similar saturated fatty acid content could possess different digestibilities, but that digestibility was correlated with the content of trisaturated triglyceride. All that could be concluded from these studies was that fats with high melting points and high contents of long chain, saturated fatty acids tended to be poorly absorbed.

Recent work in this field has focussed attention on the role of fatty acid composition in determining fat digestibility. The content of individual fatty acids in the diet and excreta have been determined and digestibility coefficients calculated for each. These studies have been

unanimous in concluding that stearic and palmitic acids are poorly absorbed whereas shorter-chain and unsaturated fatty acids are well absorbed (see, for example, Norcia and Lundberg, 1954; Renner and Hill, 1961a,b; Young and Garrett, 1963; Flanzy, Rérat and François, 1968; Radostits and Bell, 1968; Tomarelli, Meyer, Weaber and Bernhardt, 1968; Filer, Mattson and Foman, 1969; Hamilton and McDonald, 1969). The interpretation of these results is complicated by the fact that hydrogenation of unsaturated fatty acid takes place in the large intestine (Hoet, et al., 1963; Ward, Scott and Dawson, 1964; Bottino, 1967; Carlson and Bayley, 1968; Wiggins, Howell, Kellock and Stalder, 1969). It would seem, therefore, that some of the conclusions drawn from such studies are invalid.

Results obtained from balance studies have been variable and conflicting probably due to differing experimental conditions. In this connection, factors such as the occurrence of coprophagy in some species (Tadayyon and Lutwak, 1969a) and the level of minerals, particularly calcium and magnesium, included in the diet (Holt, et al., 1935; Tadayyon and Lutwak, 1969b) may influence digestibility values obtained for fat. Furthermore, methods used to determine lipid in faeces differ considerably between laboratories. Classical ether extraction does

not extract all lipids and also removes non-lipid material (Carroll, 1958). A considerable proportion of the fatty acids in faeces are present as soaps which are only partially removed by solvent extraction unless the faeces are first acidified (Carroll, 1958; Carroll and Richards, 1958; Clarke and Roberts, 1967). However, the fact remains that palmitic and stearic acids and their glycerides, when fed as the sole source of fat in the diet, are poorly absorbed (Scribante and Favarger, 1954; Carroll, 1958; Carroll and Richards, 1958; Young and Garrett, 1963).

It was realised early that the defect in absorption of long chain, saturated triglyceride was poor hydrolysis in the intestine (Mattil and Higgins, 1945; Carroll and Richards, 1958), although this could not explain the malabsorption of the free acids. It has subsequently been suggested that long chain, saturated fatty acids and their derivatives are less well incorporated into micellar dispersions than their unsaturated homologues (Savary, 1966; Holt and Clark, 1969) although evidence on this point is sparse. In this regard, it is interesting that the monoglycerides of palmitic and stearic acid seem to be better absorbed than the free acids (Scribante and Favarger, 1954; Renner and Hill, 1961a; Tomarelli, et al., 1968; Filer, et al., 1969) and that unsaturated fatty acids appear to exert a "solvent" effect upon the

saturated fatty acids (Young and Garrett, 1963; Savary, 1966).

Thus, discrimination against palmitic and stearic acid may occur at the level of lipolysis and dispersion under certain circumstances. Long chain, saturated fatty acid is, nevertheless, well absorbed when present together with unsaturated fatty acids in mixed glycerides, as occurs under most dietary conditions (Mattson, 1959; Young and Garrett, 1963).

Lymph collection studies. Monogastric animals usually display a distinct pattern of absorption when fed a fat meal. The lipid concentration in lymph rises quickly after feeding and then falls off exponentially. However, reports of the time taken to attain maximum concentration of lipid in lymph have varied between 2 hr. (Morgan, 1966) and 16 hr. (Coots, 1964a,b) after feeding. Similarly, reports of the time required for essentially complete absorption of a fat meal have varied between 9 hr. (Morgan, 1966; Morgan and Borgström, 1969) and greater than 24 hr. (Boucrot and Clément, 1965a, 1968; Coots, 1964a,b). This variability is probably due to differences in factors such as the quantity and type of fat fed, and the physiological status of the experimental animals.

A number of reports have compared the fatty acid composition of lymph triglyceride or total fat with that

of the diet. Of these, some have concluded that there is little resemblance between dietary and lymph lipid (Verdino, Blank and Privett, 1965; Lavoue and Clément, 1967) whereas others have shown marked resemblance between the two lipids (Bragdon and Karmen, 1960; Bhalareo, et al., 1963). Those authors who found little resemblance between dietary and lymph lipid attributed this to dilution of absorbed fatty acids with endogenous lipid. In this connection, the proportion of endogenous fatty acid present in lymph triglyceride has been variously reported as less than 20% (Mattson and Volpenhein, 1962, 1964; Constantin and Savary, 1965) or 40% or more (Karmen, Whyte and Goodman, 1963; Boucrot and Clément, 1965b; Verdino, et al., 1965). In the latter studies, the dose of fat fed would appear to have been less than in the former experiments. Rampone and Lino (1966) found that the resemblance between dietary and lymph lipid depended on the concentration of fat in the lymph. When the lipid content of the lymph was high, the fatty acids resembled those in the diet but the resemblance became less as the lipid concentration decreased. It has since been shown that the proportion of endogenous fatty acid found in lymph lipid depends on the amount of fat fed and the stage of absorption at which the sample is collected (Savary and Constantin, 1967; Boucrot and Clément, 1968). It may, therefore, be concluded that the output of endogenous

fatty acid in lymph lipid changes much less, if at all, than that of exogenous fatty acid during fat absorption. Provided that sufficient fat is fed and lymph samples are taken during the period of maximum absorption, the fatty acid composition of lymph triglyceride will be representative of that absorbed from the intestine, endogenous fatty acids contributing no more than 20% of the total fatty acid.

Experiments utilising radioactively labelled fats (Blomstrand, Dahlback and Linder, 1959; Whyte, Karmen and Goodman, 1963; Coots, 1964a,b) have shown that absorbed fatty acids are incorporated largely into lymph triglyceride (95% or greater) with small amounts being incorporated into lymph phospholipid (2-4%) and cholesterol ester (0-3%). However, incorporation into the different lipid fractions of lymph displays marked specificity with respect to different fatty acids.

The first reports of preferential incorporation of different fatty acids into lymph lipids came from Goodman's laboratory (Karmen, et al., 1963; Whyte, et al., 1963). In experiments in which rats were fed several fats containing tracer quantities of radioactively labelled fatty acids (16:0, 18:0, 18:1 and 18:2), it was found that, except for a slight discrimination against 18:0, the formation of lymph triglyceride displayed no preference for particular fatty acids. On the other hand, lymph

cholesterol ester preferentially incorporated 18:1 and lymph phosphatidyl choline showed a marked preference for 18:0 and a lesser preference for 18:2 with a relative discrimination against 18:1.

The lymph used for extraction in these experiments represented 24 hour collections so that variations in the pattern of incorporation over the absorptive period could not be determined. Blomstrand, Gürtler and Werner (1964) conducted a similar experiment in a human patient using 16:0, 18:1 and 18:2 and, in addition to confirming the results of Karmen, et al.(1963), were able to demonstrate that there was no change in the relative incorporation of these fatty acids throughout the absorptive period.

That exogenous 18:0 is less well incorporated into lymph triglyceride than the other long chain fatty acids has been confirmed (Boucrot and Clément, 1965b). However, Coots (1964a) showed that incorporation of ingested 18:0 into the total lipids of lymph was similar to that for 16:0 and 18:1 (i.e., about 97%). It would appear, therefore, that the discrimination against 18:0 in the formation of lymph triglyceride is due to competition with phospholipid synthesis for the available substrate. Indeed, it has been shown that up to 20% of absorbed 18:0 may be incorporated into lymph phospholipid compared with only 2-4% for the other fatty acids (Borgström, 1952;

Blomstrand, et al., 1959; Boucrot and Clément, 1965a; Coots, 1964a).

At this point it is logical to seek the basis of this preferential incorporation of fatty acids into lymph lipids at the enzymic level. Little evidence is available on this point. In vitro experiments in which reaction rates for individual fatty acids have been compared under noncompetitive conditions have given results which do not correspond with the specificities observed in vivo (Ailhaud, Samuel, Lazdunski and Desnuelle, 1964; Hansen, 1965a; Brindley and Hübscher, 1966; Bickerstaffe and Annison, 1969a). Nevertheless, from experiments in which both 16:0 and 18:1 were included in the incubation medium, Johnston and Rao (1965) concluded that the monoglyceride and diglyceride acyl transferases of the monoglyceride pathway are non-specific with regard to the fatty acid utilised. Fatty acid:CoA ligase, however, did exhibit relative discrimination between the two fatty acids. As previously mentioned, Brindley and Hübscher (1966) suggested the presence in the intestinal mucosa of several fatty acid:CoA ligases possessing different substrate specificities, although these enzymes were not isolated. It may be, therefore, that selectivity in fatty acid utilisation by the three acylation sequences (glycerol-3-phosphate pathway leading predominantly to phospholipid, monoglyceride pathway leading to

triglyceride and cholesterol esterification) resides in a separate fatty acid:CoA ligase associated with each one.

In addition to the above, it has been reported that elaidic acid (trans 18:1) and the various geometrical isomers of linoleic acid are absorbed to the same extent and are distributed amongst the lymph lipids in a fashion similar to palmitic and oleic acids (Coots, 1964a,b; Lavoue and Clément, 1967). Hydroxylated fatty acids (ricinoleic acid), on the other hand, are absorbed more slowly and probably less efficiently than unhydroxylated acids (Risser, Kummerow and Perkins, 1966). Erucic acid (22:1) is also poorly incorporated into lymph lipids (Savary and Constantin, 1966).

LIPOPROTEINS AND THE SECRETION OF ENDOGENOUS LIPID BY THE INTESTINE

Chemistry.

General. The lipoproteins of blood plasma and lymph are usually classified into five major classes (Vandenheuvel, 1962; Janado, Martin and Cook, 1966; Skipski, et al., 1967) and various sub-classes are often recognised (Wurm and Straus, 1968). A summary of the properties of the different lipoproteins is given in Table 1.

TABLE 1. CLASSES OF LIPOPROTEINS AND THEIR PROPERTIES

References: Dole and Hamlin, 1962; Vandenheuvel, 1962; Skipski, et al., 1967; Janado, et al., 1966; Cox and Tanford, 1968; Alaupovic, et al., 1966; Kalab and Martin, 1968; Lindgren, Jensen, Wills and Freeman, 1969; Bierman, et al., 1966.

TABLE 1

Lipoprotein Class	Electrophoretic Mobility	Density	Sf	Molecular Weight	Lipid (Wt. %)	Protein (Wt. %)
Chylomicrons	alb.	< 0.93	> 400	$>400 \times 10^6$	98-99	1-2
VLDL	α_2 (preβ)	< 1.006	20-400	17×10^6	90-92	8-10
LDL	β	1.006-1.063	0-20	$2-3 \times 10^6$	80	20
HDL	α_1	1.063-1.21	---	0.25×10^6	50	50
VHDL	α_1 & alb.	> 1.21	---	---	1	99

There is often some confusion between VLDL and LDL since the term "β-lipoprotein" is used by different authors to include either or both. It should also be pointed out that the electrophoretic mobility of chylomicrons quoted in Table 1 refers to electrophoresis on media other than paper, since, due to binding of the lipid, chylomicrons remain near the origin on paper electrophoresis (Dole and Hamlin, 1962).

Chylomicrons carry largely triglyceride (85-90% of total lipid) and contain only 1-2% by weight of protein (Dole and Hamlin, 1962). Decreasing proportions of triglyceride and increasing levels of phospholipid are associated with the higher density lipoproteins until the VHDL are reached where the lipid consists largely of phospholipid and albumen-bound FFA (Skipski, et al., 1967; Alaupovic, et al., 1966). This compositional distribution has been explained by assuming that lipoproteins consist of a spherical lipid (largely triglyceride) core surrounded by a monomolecular layer, containing the phospholipid and protein (Vandenheuvel, 1962; Zilversmit, 1967; Fraser, 1970). The higher density lipoproteins do not conform to this model (Vandenheuvel, 1962).

Protein moiety. The inter-relationships between the protein moieties of lipoproteins are of interest and have been extensively studied in recent years. There are at

least three major protein species found associated with lipoproteins (Rodbell, 1958). These consist of the major protein associated with LDL and containing N-terminal glutamic acid, the protein of HDL containing N-terminal aspartic acid and that associated with VLDL and chylomicrons and which contains N-terminal serine.

It has been shown that dog and human chylomicrons contain three proteins, designated A, B and C (Rodbell and Fredrickson, 1959). The A protein is identical with that of HDL in respect to electrophoretic mobility, solubility in veronal buffer, N-terminal amino acid (aspartic acid) and peptide map. The B protein is probably identical with the protein which is characteristic of LDL, the C protein being only a minor component.

The identity of chylomicron A and HDL proteins has been challenged on the basis of differences in amino acid composition but these may have been due to the presence of components other than the A protein (Wathen and Levy, 1966; Levy, Lynch, McGee and Mehl, 1967) or to the use of polyanion fractionation techniques (see Dole and Hamlin, 1962).

VLDL contains both the A and B proteins (Gustafson, Alaupovic and Furman, 1966; Levy, Lees and Fredrickson, 1966; Camejo, 1967; Windmueller and Levy, 1968; Ockner, Block and Isselbacher, 1968). In addition, Gustafson, et al. (1966) separated a third protein moiety from VLDL.

The third protein has since been resolved into at least two components having N-terminal serine and threonine residues, respectively, and which were not found in LDL or HDL (Brown, Levy and Fredrickson, 1969).

Metabolism.

The evidence currently available suggests that the major protein of HDL and VHDL (the A protein) is an "apoprotein" which acts as a lipid carrier in the circulation, forming lower density lipoproteins by the addition of lipid (Eder, Roheim and Switzer, 1964). Support for this view comes from the observation that lipoprotein lipase, which is involved in the uptake of chylomicrons by peripheral tissues, is capable of converting chylomicrons to higher density lipoproteins (Lindgren, Freeman, Nichols and Gofman, 1956) and from the rapid transfer of labelled protein from chylomicrons to HDL upon entry of chylomicrons into the bloodstream (Rodbell, Fredrickson and Ono, 1959). This view has proved to be over-simplified and has been modified in various ways (Vandenheuvel, 1962; Trams and Brown, 1966; Buckley, Delahunty and Rubinstein, 1968).

Chylomicrons are synthesized by the intestinal mucosa and are secreted into lymph (Dole and Hamlin, 1962). Zilversmit has recently reviewed his own work on the structure of chylomicrons (Zilversmit, 1967) and concluded that they consist of a triglyceride droplet surrounded by an

adsorbed film of phospholipid and protein. All of the cholesterol ester and part of the free cholesterol may be found dissolved in the triglyceride core, with the remainder of the free cholesterol present in the lipoprotein envelope. (cf. Huang and Kuksis, 1967). The median diameter of rat chylomicrons was found to be 0.15-0.2 μ (Zilversmit, Sisco and Yokoyama, 1966). Upon entering the circulation they undergo some small changes in composition due to exchange reactions with other serum lipoproteins (Bierman and Strandness, 1966) and are taken up, in the first instance, largely by liver and adipose tissue (Havel and Goldfien, 1961; Nestel, Havel and Bezman, 1962; Olivecrona, 1962) and, in the lactating animal, by the mammary gland (Lascelles, Hardwick, Linzell and Mepham, 1964; McBride and Korn, 1964). A considerable amount of lipid taken up by the liver as chylomicrons is thought to be returned to the blood as VLDL and LDL (Kay and Entenman, 1961; Robinson, 1964) although this has been questioned (Mayes, 1969).

The liver is responsible for the bulk of the synthesis of serum lipoproteins, other than chylomicrons (Borgström and Olivecrona, 1961; Roheim, Switzer, Girard and Eder, 1966; Windmueller and Levy, 1967). Evidence has been presented that the synthesis of the protein moiety occurs in the ribosomal fraction from liver but not in liver mitochondria or kidney ribosomes (Bungenberg

de Jong and Marsh, 1968). Newly synthesized VLDL accumulates in the Golgi apparatus of the liver cell before secretion (Mahley, Hamilton and Lequire, 1969).

However, it has only lately been recognised that the intestine also produces β-lipoprotein (VLDL). In view of the important contribution this lipoprotein makes to the endogenous lipid in lymph from the intestine, an account of the evidence for its intestinal synthesis will now be given.

Lipoprotein Secretion by the Intestine.

General. It was recognised as early as 1932 by Rony, Mortimer and Ivy that thoracic duct lymph from non-fat fed animals contains endogenous lipid in excess of that derived from the capillary filtrate. It has now been unequivocably demonstrated by several studies that the intestine secretes endogenous lipid into the lymph which drains it and that this endogenous lipid is largely VLDL.

Coxon and Robinson (1962) cannulated hepatic and intestinal ducts in fasted or glucose fed dogs and found that the concentration of esterified fatty acid in intestinal lymph exceeded that in blood plasma whereas that in hepatic lymph did not. Baxter (1966) reported that the lipid outputs from thoracic and intestinal lymphatic ducts of rats fed a fat-free diet were similar. He also collected thoracic duct lymph after diversion of the intestinal

lymph and found that the lipid output was greatly reduced. By separating the lymph lipoproteins into VLDL and higher density ($d > 1.006$) fractions Baxter (1966) was able to show that the $d > 1.006$ lipoproteins were always present in lower concentration in lymph than in plasma whereas VLDL in the intestinal lymph was present in much higher concentration than in plasma. By contrast, the major lipoprotein of extra-intestinal lymph was the $d > 1.006$ lipoprotein. Approximately 80% of the lipid of intestinal lymph was present in VLDL and 70% of this lipid was triglyceride.

Windmueller and Levy (1968) adopted the approach of suppressing hepatic lipoprotein secretion by feeding orotic acid to rats maintained on a fat-free diet. Under these circumstances the plasma "β-lipoprotein" concentration is reduced to negligible levels while the "α-lipoprotein" level in lymph is maintained. They confirmed that the "α-lipoprotein" concentration of intestinal lymph is lower than that of blood plasma whereas that of "β-lipoprotein" is higher. "β-lipoprotein" was identified as VLDL and "α-lipoprotein" as HDL. The VLDL fraction contained 95% of the lymph total fatty acids, in agreement with Baxter's (1966) observations. Interestingly, Windmueller and Levy (1968) calculated that approximately 10% of circulating plasma triglyceride is derived from the VLDL of intestinal lymph.

Origin of the protein moiety. Rodbell, et al. (1959) fed labelled amino acids to dogs and followed incorporation of radioactivity into the proteins of lymph chylomicrons and lymph and plasma HDL. They concluded from specific activities that plasma HDL was not the major source of either lymph HDL or chylomicron A protein. Intestinal mucosal cells were then incubated in vitro with labelled amino acids and chylomicrons were isolated from the medium (Rodbell, et al., 1959). It was shown that mucosal cells incorporate amino acids into both the A and B proteins of chylomicrons. This suggested that the intestinal mucosa synthesizes both chylomicron and HDL proteins de novo.

Hatch, Aso, Hagopian and Rubenstein (1966) showed that radioactive amino acids absorbed by the gut of fat-fed rats were incorporated into the lipoproteins isolated from intestinal mucosa, lymph and blood serum. It was shown that amino acids were incorporated into all lipoprotein fractions but the highest specific activities were found in chylomicron-like particles from the mucosa and in lymph and serum chylomicrons. Amino acids were also incorporated into the VLDL fraction. Inhibitors of protein synthesis reduced or abolished this incorporation. Electrophoresis of the soluble proteins of the mucosal cell, the chylomicron-like particles isolated from the mucosa and lymph chylomicrons revealed two protein com-

ponents which were common to all three (Hatch, et al., 1966). This led the authors to suggest that during fat absorption, the more abundant soluble proteins are adsorbed to the surface of fat droplets which are then secreted as chylomicrons. It seems reasonable to suggest that one of the electrophoretic components observed by Hatch, et al. (1966) in soluble proteins from the intestinal mucosa may be identical with the A protein studied by Rodbell, et al. (1959). Antigenic analysis of these proteins using antisera with A reactivity could settle this question.

It may be added here that observations on chylomicron formation when inhibitors of protein synthesis are administered and in the diseased state, abetalipoproteinaemia, have led to the suggestion that the synthesis of the B protein by the intestine is essential for the transport of fat across the intestinal mucosa (Isselbacher, 1965). However, Redgrave and Zilversmit (1969) showed that the results of experiments involving inhibitors of protein synthesis could be accounted for by physiological disturbances such as inhibition of gastric emptying and, therefore, questioned the necessity of protein synthesis for fat absorption.

^{3}H-Leucine administered intravenously to rats fed a fat-free diet is incorporated into lymph VLDL and to only a small extent into the higher density lipoproteins

of lymph (Windmueller and Levy, 1968). Specific activities of protein in the plasma lipoproteins were lower than in the lymph lipoproteins, thus demonstrating that the small intestine synthesises the protein moiety of VLDL de novo.

Origin of endogenous lipid. The origin of the endogenous lipid in the lymph of non-fat fed rats has been studied in recent years. Baxter (1966) collected bile from rats maintained on a fat-free diet and estimated the output of biliary lipid. He also collected thoracic duct lymph from rats in which the bile had been diverted from the small intestine. However, diversion of bile caused a drop in lymph flow with consequent clotting trouble and the data was not presented as reliable. Nevertheless, Baxter estimated that biliary fatty acid could account for approximately half of the lymphatic output of VLDL.

Clear evidence that the endogenous lipid in lymph from fasted rats is derived largely from absorption of biliary lipid has been obtained by the Western Australian group (Morgan, 1964, 1966; Shrivastava, et al., 1967). These workers observed that the stable output of esterified fatty acid in the thoracic duct lymph of normal, fasted rats is about 20μEq./hr. whereas in the bile fistula rat this output varies but is usually less than

10μEq./hr. (Morgan, 1964, 1966). Indeed, in the last of these studies it was shown that the output of esterified fatty acid in thoracic duct lymph decreased by 80% when a bile fistula was produced (Shrivastava, et al., 1967). This drop in lipid output was not due to the absence of bile salts but could be more than accounted for by the absence of bile lipid. Gas chromatography showed that before bile drainage was established, the fatty acid composition of fasting lymph lipids was similar to that of bile but after biliary drainage the fatty acid pattern closely resembled that of plasma lipid.

ASSIMILATION OF LIPID IN ADULT RUMINANTS

The subject of digestion and absorption of lipids by ruminants has been exhaustively reviewed (Garton, 1959, 1960a, 1961, 1963, 1964, 1965, 1967, 1969; Lough and Garton, 1968). No attempt will be made, therefore, to review the literature on this subject in depth. Rather the events occurring in the stomachs and intestines of ruminants during fat assimilation will be summarised with emphasis on recent findings.

Nature of Dietary Lipid.

Of the ruminant species, the best studied have been sheep and cattle, for obvious economic reasons. In Australia, sheep and cattle graze pasture and normally

receive only low levels of supplementation with conserved fodders and concentrates. Pasture plants usually do not contain more than 6% by weight of the dry matter as total lipid. This lipid consists largely of galacto-glyceryl esters of long chain fatty acids together with smaller amounts of phospholipids and neutral lipids and very little FFA (Garton, 1960a). The fatty acids of pasture species usually contain linolenic acid as the major constituent (Garton, 1960b; Czerkawski, 1967a; Van der Veen and Olcott, 1967; Gray, Rumsby and Hawke, 1967). The fatty acid composition of plant lipids has been reported to depend on the stage of growth, the proportion of linolenic acid decreasing in older plants (Oksanen and Thafvelin, 1965; Klopfenstein and Shigley, 1967), but this has been attributed to level of photo-synthetic activity rather than age, per se (Gray, et al., 1967).

The fatty acid composition of hay resembles that of the fresh sward but losses of unsaturated acids may be incurred, especially if preparation of the hay is carried out under wet conditions (Oksanen and Thafvelin, 1965; Czerkawski, 1967a; Van der Veen and Olcott, 1967). The fatty acid composition of lipids in concentrates, on the other hand, depends on the source, e.g. linseed meal contains linolenic acid as the major component (Kudzal-Savoie, Raymond, Kuzdzal and Petit, 1966) whereas corn meal

contains linoleic acid as the major component (Miller, Varnell and Rice, 1967).

Nature of Rumen Lipid.

Rumen lipids consist largely of FFA (about 70%) together with small amounts of neutral lipid and phospholipid (Bath and Hill, 1967; Lennox, Lough and Garton, 1968). A number of fatty acids are present in ruminal digesta which are not present or present only at lower levels in the diet. These include branched chain and odd carbon number fatty acids together with geometrical and positional isomers of unsaturated fatty acids. To illustrate this point, some data obtained by various authors on the fatty acid composition of lipid from the ruminal digesta of cattle is given in Table 2. It may be seen that there are quite low levels of C_{18} unsaturated fatty acids and high levels of stearic acid in rumen lipid compared with dietary lipid.

Digestion in the Rumen.

The observed differences between dietary and rumen lipid prompted intensive investigations of digestive processes which occur within the rumen. It has been shown that the high proportion of saturated fatty acids in rumen lipid arises from hydrogenation of unsaturated fatty acids by the rumen micro-organisms. The positional and geometrical isomers of unsaturated fatty acids are intermediates in this process. Furthermore, odd carbon number and branched chain fatty acids are synthesized from short

TABLE 2. FATTY ACID COMPOSITION OF BOVINE RUMEN LIPID

(a) Number of carbon atoms:number of double bonds
Br = branched chain

(b) Katz and Keeney (1966)

(c) Robertson and Hawke (1964)

(d) Macleod and Perry (1966)

(e) Roberts (1966)

TABLE 2

Fatty Acid (a)	Fraction of Rumen Contents			Rumen Contents from Animals Fed:			
	Total (b)	Super-natant (c)	Particulate Matter (c)	Soybean Oil Meal (d)	Whole Soybean (d)	Hay (e)	Grain (Barley) (e)
12:0	--	2.1	1.3	0.8	0.8	--	--
13:Br	--	0.7	1.0	--	--	--	--
13:0	--	1.3	0.4	--	--	--	--
14:Br	0.6	0.4	1.7	--	--	--	--
14:0	1.8	2.9	4.8	4.5	3.2	29.0	7.7
14:1	--	--	--	2.9	2.4	2.5	2.9
15:Br	2.7	0.8	7.8	11.9	8.7	1.8	3.3
15:0	2.3	0.6	5.4				
16:Br	0.9	--	--	--	--		
16:0	30.4	24.8	30.3	23.4	21.9	16.0	25.7
16:1	--	0.2	2.5	3.0	2.2	0.4	0.8
17:Br	--	0.6	2.7	--	--	--	--
17:0	2.0	1.0	2.7	--	--	--	--
18:Br	--	--	--	--	--	--	--
18:0	42.6	33.7	16.7	26.1	42.8	15.0	39.2
18:1 cis	1.7	11.8	11.3	11.6	13.1	4.4	8.4
18:1 trans	5.3						
18:2 cis	2.5	5.9	5.8	5.0	4.2	2.1	3.0
18:2 trans	0.9						
18:3 cis	5.9	13.0	5.6	0.8	0.7	5.0	1.8
18:3 trans	0.4						

chain precursors by the rumen bacteria.

Some aspects of these processes will now be dealt with.

Hydrogenation. Two groups of workers have recently examined in detail the pathways of hydrogenation of the various unsaturated fatty acids by rumen micro-organisms. In a series of studies, R. M. C. Dawson and colleagues incubated strained rumen fluid with radioactively labelled fatty acids and isolated intermediates by thin layer (TLC) and gas-liquid (GLC) chromatography (Ward, et al., 1964; Wilde and Dawson, 1966; Kemp and Dawson, 1968). Tove's group incubated a Butyrivibrio fibrisolvens culture isolated from the rumen with labelled fatty acids and isolated intermediates by GLC (Polan, McNeill and Tove, 1964; Kepler, Hirons, McNeill and Tove, 1966; Kepler and Tove, 1967). A summary of their findings is presented in Fig. 2. It should be pointed out that only the major pathway is presented in each case. Various other intermediates lie on subsidiary pathways and appear as minor components in rumen lipids.

The cis-trans-cis conjugated 18:3 which forms the first intermediate in the hydrogenation of linolenic acid (cis-cis-cis-9,12,15-18:3) has been isolated and identified (Kepler and Tove, 1967; Kemp and Dawson, 1968). The non-conjugated diene, however, may be cis-trans or trans-cis in configuration and the positions

FIGURE 2.

Principal pathways of hydrogenation of linoleic and linolenic acids in the rumen.

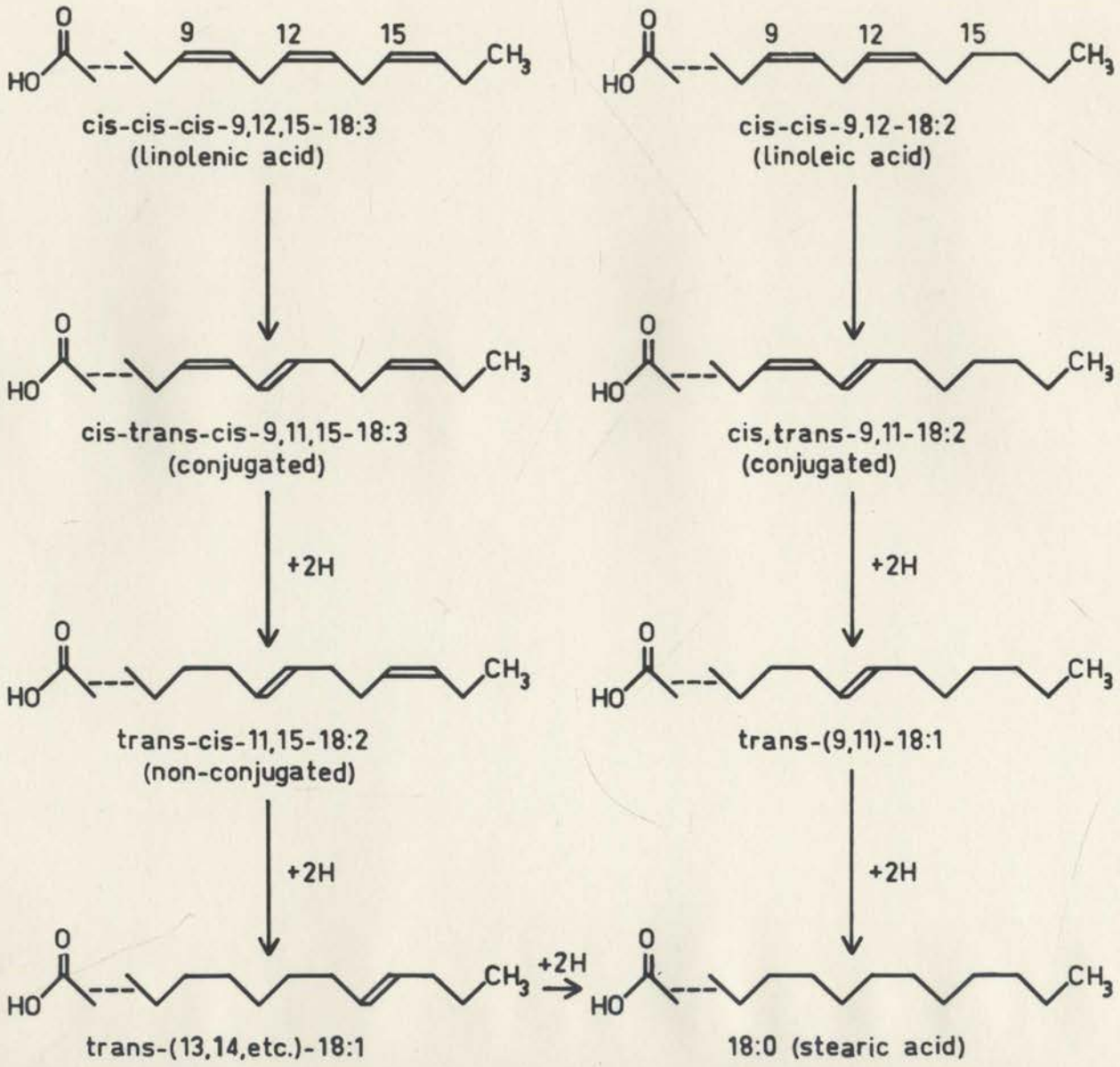

cis-cis-cis-9,12,15-18:3
(linolenic acid)
cis-cis-9,12-18:2
(linoleic acid)
cis-trans-cis-9,11,15-18:3
(conjugated)
cis,trans-9,11-18:2
(conjugated)
+2H
+2H
trans-cis-11,15-18:2
(non-conjugated)
trans-(9,11)-18:1
+2H
+2H
+2H
trans-(13,14,etc.)-18:1
18:0 (stearic acid)

Figure 2

of the double bonds are uncertain. Those given in Fig. 2 have been inferred (Kepler and Tove, 1967) from the structure of the preceding intermediate and from the results of studies in which the 11,15-isomer would appear to have been the principal non-conjugated diene formed (Shorland, Weenink, Johns and McDonald, 1957). That a non-conjugated cis, trans diene is an intermediate in the hydrogenation of linolenic acid is supported by the results of Czerkawski (1967b) who incubated linseed oil with rumen contents in semi-permeable sacs within the rumen of cows. Czerkawski reported a component whose concentration rose and then fell again during hydrogenation and whose gas chromatographic behaviour was consistent with its being a non-conjugated cis, trans diene. The diene intermediate is then converted to stearic acid via a mixture of trans-18:1 isomers, with the double bond predominantly in the C_{13} and C_{14} positions (Ward, et al., 1964).

Linoleic acid (cis-cis-9,12-18:2), on the other hand, is isomerized by B. fibrisolvens to cis,trans-9,11-18:2, which has been positively identified (Kepler, et al., 1966; Kepler and Tove, 1967). The enzyme responsible for this isomerization has been located in a particulate fraction of the disrupted bacteria, probably cell wall, and its requirements characterised (Kepler and Tove, 1967). No co-factors could be identified; the enzyme had a pH optimum of about 7, did not require anaerobic conditions

(in contrast to the hydrogenation step, Polan, et al., 1964), showed a preference for emulsified substrate and acted on both linoleic and linolenic acids. It would appear, therefore, that the first step in the hydrogenation of both linoleic and linolenic acids by B. fibrisolvens is carried out by the one enzyme. Finally, isomerised 18:2 is converted to trans-9- and trans-11-18:1 (Kepler, et al., 1966). Bacteria other than B. fibrisolvens are required for the final conversion to stearic acid (Ward, et al., 1964).

A comparison of the structures of the intermediates in the hydrogenation of linoleic and linolenic acids (Fig. 2) suggests that the two sequences of reactions may be catalysed by the same enzymes to the level of the non-conjugated diene in the case of linolenic acid and trans-18:1 in the case of linoleic acid. However, the hydrogenation of the non-conjugated diene would appear to involve further bond migration, as judged by the range of positional isomers found in the resulting trans-18:1 (Ward, et al., 1964). It is of interest here that, whereas Ward, et al. (1964) found that hydrogenation of linolenic acid gave rise to trans-18:1 isomers with the double bond predominantly in the C_{13} and C_{14} positions, Katz and Keeney (1966) found that the major trans-18:1 in the rumen digesta of a cow was the C_{11} isomer. The cow had been fed alfalfa hay which contains largely

linolenic acid (Jackson and Kummerow, 1949; Van der Veen and Olcott, 1964, 1967). This apparent contradiction has not yet been resolved.

Shorland, et al. (1957) found an accumulation of conjugated diene during incubation of linoleic acid with rumen contents in vitro. This was interpreted as indicating that conjugated dienes are resistant to hydrogenation. However, no such accumulation was found in vivo (Czerkawski and Blaxter, 1965). It is, therefore, probable that observed accumulations of conjugated dienes during in vitro experiments may be due to failure to maintain strict anaerobiosis, which would result in normal isomerization but inhibited hydrogenation (Kepler and Tove, 1967), leading to the accumulation of conjugated dienes with linoleate but not with linolenate as substrate (see Fig. 2). In this connection, Czerkawski and Breckenridge (1969) suggested that small amounts of oxygen may enter the rumen during rumination and showed that low levels of oxygen caused accumulation of hydrogen, suggesting that the phenomenon may also occur in vivo.

Hydrogenation of unsaturated fatty acids by pure cultures of rumen bacteria is stimulated by the addition of other bacteria (Polan, et al., 1964). This may be related to the finding that the addition of cell-free rumen liquor to washed rumen bacteria markedly stimulates hydrogenation (Wright, 1960; Polan, et al., 1964; Wilde

and Dawson, 1966). It was suggested that this stimulation represented the addition of necessary co-factors, e.g. reduced nicotinamide nucleotides, but the effect could not be reproduced by the addition of a number of such co-factors (Wilde and Dawson, 1966).

Other bacteria have been isolated from the rumen which carry out a similar function to B. fibrisolvens with respect to hydrogenation (Wilde and Dawson, 1966; Kemp and White, 1967). Mills, Scott, Russell and Smith (1969) isolated a rumen micrococcus which hydrogenated linoleic and linolenic acids by a similar pathway to that of B. fibrisolvens. However, unlike B. fibrisolvens, the micrococcus carried the hydrogenation of linolenic acid through to trans-11-18:1. The pure cultures of bacteria isolated so far do not appear to hydrogenate 18:1. However, mixed rumen bacteria are able to hydrogenate oleic acid (cis-9-18:1) to stearic acid, probably via a trans intermediate (Shorland, et al., 1957; Polan, et al., 1964; Ward, et al., 1964; Czerkawski and Blaxter, 1965; Wilde and Dawson, 1966).

Protozoa have been reported to carry out hydrogenation (Wright, 1959; Williams, Gutierrez and Davis, 1963) although this may have been due to bacteria ingested by the protozoa. Two authors have recently attempted to define the role of protozoa in rumen hydrogenation by selectively defaunating the rumen (Lough, 1968; Dawson

and Kemp, 1969). During long term defaunation, polyunsaturated fatty acids in blood plasma lipids were increased, indicating depressed hydrogenation (Lough, 1968). However, acute defaunation caused complete cessation of hydrogenation, but the saturating mechanisms reappeared before protozoa were reestablished in the rumen (Dawson and Kemp, 1969). These results are difficult to interpret but indicate that protozoa probably play at least a symbiotic role in hydrogenation.

Little is known concerning hydrogenation of unsaturated fatty acids other than the C_{18} group. Hay and Morrison (1970) recently reported the presence in bovine milk fat of C_{14}, C_{16} and C_{17} trans monoenes with several positional isomers of each chain length. Some of these fatty acids may be derived from dietary sources (Weenink and Shorland, 1964) but this finding may also indicate that unsaturated fatty acids of these chain lengths are hydrogenated in the rumen. Alternatively, they may be derived by partial oxidation of the C_{18} homologues although it is hard to see how C_{17} trans monoenes could arise in this way.

Lipolysis. Garton, Hobson and Lough (1958) were the first to report that triglyceride added to the rumen was hydrolysed. Since then it has been shown that phospholipids (Dawson, 1959) and galactolipids (Garton, 1964) are also split by rumen contents.

Reports of the extent of lipolysis have varied from about 20% to greater than 90% (Wright, 1961; Garton, Lough and Vioque, 1961). Most authors have failed to detect appreciable levels of mono- or diglycerides in rumen, abomasal or intestinal contents. However, Hawke and Robertson (1964) reported the formation of considerable quantities of the partial glycerides during *in vitro* incubation of rumen contents with linseed oil. Moore, Noble, Steele and Czerkawski (1969) have also found mono- and diglycerides in the rumen and abomasum during the first few hours after the infusion of maize oil into the rumen of a sheep, and Evans (1964) reported monoglyceride in rumen and abomasal contents of a bison. It has been suggested that the variability observed in the extent of lipolysis in the rumen may be due to differences in microbial populations (Hawke and Robertson, 1964). The existence of a number of lipases which produce differing lipolytic patterns within the rumen under various circumstances has also been postulated (Hartmann, 1966). On the other hand, it has been suggested that the lipolytic mechanisms of the rumen can be saturated by large amounts of triglyceride (Bath and Hill, 1967; Moore, *et al.*, 1969) and this may account for the accumulation of appreciable quantities of partial glycerides in some experiments. Examination of abomasal digesta from animals fed increasing levels of oil would give valuable evidence on this point.

It is evident, nevertheless, that, when ruminants are fed normal diets unsupplemented with fat, lipolysis within the rumen goes rapidly to completion. Under these circumstances, FFA makes up about 70% of the lipid released into the duodenum (Bath and Hill, 1967; Lennox, et al., 1968; Leat and Harrison, 1969). Glycerol released from ester combination within the rumen is rapidly fermented to yield volatile fatty acids and carbon dioxide (Garton, Lough and Vioque, 1961; Wright, 1969).

The observation that FFA isolated from rumen contents was more saturated than esterified fatty acids from the same source prompted the suggestion that lipolysis is a necessary prerequisite to hydrogenation (Garton, Lough and Vioque, 1961; Hawke and Robinson, 1964). Garton (1964), on the other hand, suggested that fatty acids in ester combination could be hydrogenated. Hawke and Silcock (1969) have recently re-examined this problem by incubating triglyceride containing ^{14}C- labelled linolenic acid with rumen contents and isolating the resulting FFA and triglyceride. It was found that the only radioactive fatty acid in triglyceride was 18:3 whereas the FFA contained radioactive 18:0, 18:1, 18:2 and 18:3. It was, therefore, concluded that lipolysis is necessary for hydrogenation.

Desaturation. Williams, et al., (1963) demonstrated some desaturation of palmitic acid incubated with rumen

bacteria and Wilde and Dawson (1966) isolated some rumen organisms which desaturated long chain fatty acids. However, this process is masked *in vivo* by the rapid hydrogenation of unsaturated fatty acids and its significance is not known.

Metabolism. Ulyatt, Czerkawski and Blaxter (1966) fed sheep under steady state (continuous feeding) conditions and estimated the output of fatty acids at the pylorus. They obtained 77% recovery of long chain fatty acids fed in dried grass and 100% recovery of fatty acids added to the grass diet as linseed oil. In another experiment, Czerkawski (1967b) incubated emulsions of fatty acids with strained rumen fluid in semi-permeable sacs within the rumen for periods of up to 23 hr. and recovered approximately 88% of the added lipid irrespective of incubation time. Studies in which radioactively labelled fatty acids were incubated with rumen contents did not detect any degradation of long chain fatty acids (Garton, Morehouse and Lough, 1961; Polan, *et al.*, 1964; Ward, *et al.*, 1964). It would appear, therefore, that rumen microorganisms do not oxidize long chain fatty acids of dietary origin.

Synthesis. The observation that rumen protozoa and bacteria contain considerable proportions of branched chain and odd carbon number fatty acids (Keeney, Katz and Allison, 1962) and that dairy cows secrete more

branched chain fatty acids in milk than they ingest in the diet (Kuzdzal-Savoie, 1964) suggested that these fatty acids may be synthesized by the rumen microorganisms. It has since been convincingly demonstrated that rumen bacteria synthesize odd carbon, long chain fatty acids from n-odd carbon volatile fatty acids (Kanegasaki and Takahashi, 1968) and branched, long chain fatty acids from branched, volatile fatty acids (Allison, Bryant, Katz and Keeney, 1962; Tweedie, Rumsby and Hawke, 1966). The biosynthesis of branched, long chain fatty acids by Bacillus species has been extensively studied by Kaneda, who has summarised his findings in a recent report (Kaneda, 1967). The crucial step in synthesis of these unusual fatty acids is the formation of acyl-CoA derivatives from volatile fatty acids and amino acids. In this connection, a propionate activating system has recently been demonstrated in rumen bacteria (Peters and Matrone, 1967).

In addition to synthesis from short chain precursors, rumen bacteria appear to carry out some chain lengthening, since labelled palmitate is incorporated to some extent into stearate (Williams, et al., 1963).

Absorption. It has been shown that long chain fatty acids may be absorbed from the rumen (McCarthy, 1962). However, McCarthy's data suggest that this absorption could not contribute significantly to overall fat

absorption. This was confirmed by Wood, Bell, Grainger and Teekell (1963) who introduced radioactively labelled linoleic acid into the rumen of fasted sheep in which the reticulo-omasal orifice had been ligated and recovered 86-96% of the dose from rumen contents after 48 hr. Those long chain fatty acids which do get taken up by rumen epithelium may be oxidized and released into the blood-stream as ketones (Jackson, Taylor, Hatcher and Carter, 1964; Hird, Jackson and Weidemann, 1966).

Digestion in the Abomasum.

The abomasum performs two functions of relevance to the digestion of fat. The first is the addition of large quantities of acidic gastric juice to the near-neutral digesta entering from the rumen (Hill, 1965), so that chyme from the proximal duodenum has a pH of 2-4 (Heath and Morris, 1963; Lennox, et al., 1968; Leat and Harrison, 1969). The second function is the lysis of most of the microorganisms entering the abomasum from the rumen (Smiles and Dobson, 1956). Proteolytic enzymes may be responsible, at least in part, for this lysis (Hird and Hoogenraad, 1968).

Digestion in the Intestines.

Lipid entering the duodenum. Lipid entering the duodenum consists largely of FFA and may contain about 5-30% of phospholipid with small proportions of triglyceride and virtually no partial glycerides, (Bath and Hill,

1967; Lennox, et al., 1968; Leat and Harrison, 1969). The FFA are predominantly palmitate and stearate whereas phospholipid contains relatively more medium chain (C_9-C_{14}) and unsaturated fatty acid (Bath and Hill, 1967). This observation is consistent with the published composition of microbial phospholipid from the rumen, which is the probable source of phospholipid leaving the abomasum (Keeney, et al., 1962; Katz and Keeney, 1966, 1967).

In view of the importance of the physical state of lipid for absorption from the small intestine, it is pertinent to ask in what physical state lipid enters the duodenum. Samples of rumen contents (personal observation) and intestinal digesta (Garton, 1969) from ruminants do not "oil out" on standing as does digesta from monogastric animals (Borgström, 1962) indicating the absence of an oil phase in ruminant digesta. It appears that the bulk of rumen lipid is bound by particulate plant residues (Hawke and Robertson, 1964; Ward, et al., 1964; Czerkawski, 1967b) although FFA might be expected to precipitate as crystalline aggregates at the low pH encountered in the abomasum and duodenum (cf. Hofmann and Small, 1967). Lipid may also be "taken up" by rumen microorganisms (Williams, et al., 1963) or adsorbed to the surface of bacteria (Maxcy and Dill, 1967). Ninety percent of the FFA entering the duodenum is apparently bound to solid residues (Lennox, et al., 1968; Leat and Harrison,

1969). Phospholipid is not bound by the solid phase to the same extent as FFA.

Role of bile and pancreatic juice. The flow of bile and of pancreatic juice has been measured in unanaesthetised sheep and cows. Approximately 400-900 ml./24 hr. of bile (Adams and Heath, 1963; Heath and Hill, 1969) and 300-500 ml./24 hr. of pancreatic juice (Taylor, 1960) enter the duodenum of the sheep. In cows, 5-9 l./24 hr. and 6-16 l./24 hr. of bile were secreted under stall-feeding and grazing conditions, respectively (Tashenov and Nikitin, 1966). Similarly, these latter workers reported flow rates for pancreatic juice in cows of 4.0-4.3 l./24 hr. It is evident that the flow of bile and pancreatic juice in sheep and cattle is comparable on a bodyweight basis. However, the flow/Kg. bodyweight and enzyme content of pancreatic juice in the sheep is lower than in the dog (Taylor, 1962). The flow of pancreatic juice exhibits diurnal variation, being higher at night (Tashenov and Nikitin, 1966), and appears to be controlled by humoral stimuli from the intestine (Taylor, 1962) whereas bile flow is dependent on an intact enterohepatic circulation of bile salts and the presence of food in the rumen (Harrison and Hill, 1962). The secretion of both bile and pancreatic juice is enhanced during stimulation of the udder in lactating sheep and goats and this response appeared to be mediated by the

nervous system (Tashenov, Simbinov and Kusebaeva, 1966). Secretion of biliary constituents in the ruminant is stimulated by secretin and is depressed by interruption of the entero-hepatic circulation of bile salts (Heath and Hill, 1969) as in the non-ruminant (Baxter, 1966; Nahrwold and Grossman, 1967; Nilsson and Schersten, 1969).

Heath and Morris (1963) studied the role of bile and pancreatic juice in unanaesthetised sheep by draining one or other of these secretions to the exterior and monitoring fat transport in the intestinal lymph. Diversion of bile caused the absorption of fat into lymph to cease and diversion of pancreatic juice reduced fat absorption to a low level. It was recently demonstrated that, during bile deprivation, up to 30% of labelled FFA administered into the abomasum or duodenum was absorbed although very little of this appeared in the lymph (Heath and Hill, 1969). It appears, therefore, that diversion of bile causes a change in the route of absorption of fat in the ruminant similar to that observed in monogastric animals (Morgan and Borgström, 1969). Heath and Morris (1963) also demonstrated an active lipase in sheep pancreatic juice with characteristics similar to that of other species. Thus, bile and pancreatic juice are necessary for optimal fat absorption in the ruminant.

A considerable amount of lipid is added to the intestinal contents in bile. Thus, the output of fatty

acids in the lipids of bile from sheep with interrupted entero-hepatic circulation is about 3 g/24 hr. (Heath and Hill, 1969; Leat and Harrison, 1969) whereas that from sheep with intact entero-hepatic circulation has been reported to be 6 g/24 hr. (Heath and Hill, 1969) but may be as high as 15 g/24 hr. (Adams and Heath, 1963). The significance of this contribution of lipid to the intestine may be seen when it is compared with the dietary fatty acid intake of sheep, which may be as low as 12 g/24 hr. when fed processed diets (Czerkawski, 1966; Heath and Hill, 1969).

Lipids in ruminant bile have been studied extensively. The first report appeared in 1963 (Adams and Heath) and dealt with the bile phospholipids from lambs, sheep and steers. Bile from sheep and cattle contains approximately 1 g/100 ml. of phospholipid, together with small amounts of FFA and free and esterified sterol (Adams and Heath, 1963; Balint, Kyriakides, Spitzer and Morrison, 1965; Leat, 1965; Lennox, et al., 1968; Garton, 1969). Biliary phospholipids from sheep have been reported to consist of 40-45% (Adams and Heath, 1963) or less than 10% (Lennox, et al., 1968) lysophosphatidyl choline although there is agreement that phospholipids from ox bile contains little lysophosphatidyl choline (Adams and Heath, 1963; Garton, 1969). Phosphatidyl choline and lysophosphatidyl choline from ruminant bile have a similar fatty

acid composition, both containing more 18:1 than 18:0 in contrast to rumen contents (Table 2) as well as appreciable levels of 18:2 (Adams and Heath, 1963; Balint, et al., 1965; Nakayama and Kawamura, 1967; Lennox, et al., 1968).

The addition of phospholipid in bile accounts for the increased content of phospholipid in digesta obtained distal to the common bile duct compared with that leaving the abomasum (Lennox, et al., 1968; Leat and Harrison, 1967, 1969). This also explains the finding that lipids from small intestinal contents contain higher levels of unsaturated fatty acids than do those from rumen or abomasal contents (McCarthy, 1962; Feliński, Garton, Lough and Phillipson, 1964; Ward, et al., 1964; Bath and Hill, 1967). Thus, bile secretion results in the addition to the intestinal digesta of considerable amounts of lipid which is more unsaturated than lipid entering the duodenum.

Biliary phosphatidyl choline, in the presence of pancreatic juice, is hydrolysed to lysophosphatidyl choline to the extent of 50% or more (Leat and Harrison, 1967; 1969) so that lysophosphatidyl choline is the predominant phospholipid in jejunal contents (Lennox, et al., 1968; Leat and Harrison, 1969). It would appear that both the 2- and 3- esters of phosphatidyl choline may be hydrolysed by pancreatic juice, indicating the presence of phospholipases A and B (Leat and Harrison, 1967).

Bile from adult sheep contains approximately 6 g/100 ml. of bile salts, the major bile acid being cholate, with taurine conjugates predominating (Peric-Golia and Socic, 1968; Sheriha, Waller, Chan and Tillman, 1968). The predominance of taurine conjugates is interesting in view of the greater acid stability of these compounds (Hofmann and Small, 1967) and the lower pH of duodenal contents from ruminants compared with non-ruminants (Lennox, et al., 1968). Intestinal contents from sheep collected 0.5 m. distal to the entrance of the common bile duct were reported to contain 0.5 g/100 ml. of bile salts (Lennox, et al., 1968). Calculated as taurocholate (M.W. = 516 g), 0.5 g/100 ml. of bile salt represents a concentration of approximately 10 mM, which is well above the CMC for mixed bile salts (Hofmann, 1963). The concentration of bile salts increased over the proximal half of the sheep small intestine (Lennox, et al., 1968) in a similar fashion to that observed in monogastrics (Boyd, Eastwood and Kermack, 1963; Dietschy, 1967). From the above it may be concluded that a micellar phase exists in the intestinal lumen of the ruminant as in the monogastric animal.

Reference has already been made to the role played by monoglyceride in micellar solubilization of FFA in the non-ruminant intestine. Intestinal digesta from sheep does not usually contain appreciable levels of

monoglycerides (Bath and Hill, 1967). It has, therefore, been suggested that lysophosphatidyl choline derived by hydrolysis of phosphatidyl choline under the influence of pancreatic phospholipases may replace the function of monoglyceride for micelle formation in the ruminant (Bath and Hill, 1967; Leat and Harrison, 1967, 1969; Lennox, et al., 1968). In this connection, it may be noted that lysophosphatidyl choline is a soluble amphipath and will, therefore, form micelles by itself (Hofmann and Small, 1967).

A major role of pancreatic juice in ruminant fat digestion is obviously the provision of phospholipases to catalyse production of lysophosphatidyl choline. However, it is difficult to assign a role to pancreatic lipase in ruminants. As previously mentioned, it would seem that lipolysis within the rumen may not always be complete. Thus, from time to time, sufficient glyceride material may enter the intestines to require the presence of pancreatic lipase.

Absorption from the Intestines.

Luminal phase. The composition of the micellar solution present in ruminant gut has been determined and it is evident that it differs from that of non-ruminant species. In the human, micellar FFA and monoglyceride are present in the approximate ratio of 2:1 (Hofmann and Borgström, 1964) whereas in the sheep, approximately equal

amounts of FFA and lysophosphatidyl choline are present in micellar solution (Lennox, et al., 1968). However, the concentration of lipid present in micellar solution in the sheep gut is comparable with that in human gut.

It is of interest that, whereas in the non-ruminant lipids are partitioned predominantly between an oil phase and micellar solution, in the ruminant lipids are partitioned between a solid phase and micellar solution. Approximately 40% of the bile salts, 70% of the phospholipids and 90% of the FFA present in the contents of the proximal half of the sheep intestine is associated with the solid phase (Lennox, et al., 1968; Leat and Harrison, 1969). Lipids present in the solid phase may be bound to plant residues (Eastwood and Hamilton, 1968) or may be precipitated at the acid pH of the ruminant intestine (Borgström, 1967). It is evident, therefore, that only a small proportion of FFA is available for absorption at any one time. Despite this fact, fat absorption is almost completed by the time the digesta reach the ileum (Lennox and Garton, 1968). There is a progressive hydrolysis of neutral lipids and phospholipids as they pass down the intestine although the concentrations of the major lipids do not change much over most of the intestinal length (Lennox and Garton, 1968; Leat and Harrison, 1969).

Mucosal phase. There is no reason to believe that the uptake of lipid from the lumen by intestinal epithelial cells differs between the ruminant and the non-ruminant. However, it was suggested by Hartmann, Harris and Lascelles (1966) that, in view of the almost complete absence of monoglycerides from intestinal digesta of the ruminant, the predominant pathway for esterification of absorbed fatty acid may be the glycerol-3-phosphate pathway, in contrast to the monogastric in which the monoglyceride pathway predominates. Two short communications from other laboratories followed, one of which reported that there was an active monoglyceride esterifying mechanism in sheep intestine (Leat and Cunningham, 1968) whereas the other reported no esterification of monoglyceride by homogenates of sheep intestinal mucosa (Bickerstaffe and Annison, 1968). These authors have since amplified their original communications.

Bickerstaffe and Annison (1969a) studied incorporation of radioactively labelled substrates into lipids by homogenates and subcellular fractions isolated from the intestines of sheep, pigs and chickens. Using glycerol-3-phosphate as an acyl acceptor, it was found that ^{14}C-palmitate was incorporated into triglyceride by preparations from all three species. Palmitate esterification

required ATP, CoA and Mg^{++}, the maximum esterifying activity being located in the microsomes. As for other species (Hübscher, et al., 1967) synthesis of triglyceride from glycerol-3-phosphate by microsomes was stimulated by the addition of particle-free supernatant. This was shown to be due to the presence of phosphatidate phosphohydrolase in the particle-free supernatant. The enzymes necessary for esterification of absorbed fatty acid by the glycerol-3-phosphate pathway were thus demonstrated in all three species.

On the other hand, when 1-monoglyceride was used as acyl acceptor, subcellular fractions from sheep intestine did not esterify ^{14}C-palmitate but there was some esterification by the total homogenate (Bickerstaffe and Annison, 1969a). This latter observation was attributed to the presence of monoglyceride lipase activity which was demonstrated in the particle-free supernatant and microsomes of sheep intestine, although the direct esterification of monoglyceride could not be excluded. Microsomes from pig and chicken intestine, however, displayed normal esterifying activity towards monoglycerides.

These results were corroborated by those of Cunningham and Leat (1969) who found that approximately 70% of doubly labelled 1-monoglyceride incubated with segments of sheep intestine in vitro was hydrolysed. 2-monoglyceride, on the other hand, was not hydrolysed to

the same extent. These authors calculated from their results that at least 28% and 43% of the total glycerides synthesised from 1- and 2-monoglyceride, respectively, by the sheep intestine were derived via the monoglyceride pathway. Using 1-monoglyceride the comparable contribution of the monoglyceride pathway in the rat intestine was 45%.

The calculations by Cunningham and Leat, (1969) involved the assumptions that free glycerol is incorporated into glycerides to the same extent as glycerol hydrolysed from monoglycerides and that labelled glycerol incorporated into di- and triglycerides came largely from unhydrolysed monoglyceride. It remains to be determined whether these assumptions are entirely justified. Whether or not the intestinal epithelium of the ruminant possesses the enzymes of the monoglyceride pathway is, therefore, uncertain. It is suggested that the use of glyceryl monoethers (non-hydrolyseable analogues of the monoglycerides) may resolve this point. Nevertheless, it is obvious that the glycerol-3-phosphate pathway must be responsible for the synthesis of almost all of the triglyceride released into lymph by the ruminant intestine in vivo (Bickerstaffe and Annison, 1969a; Cunningham and Leat, 1969).

Bickerstaffe and Annison (1969b) have demonstrated the presence of a glycerokinase in the intestinal mucosa of sheep so that free glycerol derived from the blood or

from the hydrolysis of phosphoglycerides in the intestinal lumen is probably utilised for the esterification of fatty acids by the intestinal epithelium. Lysophosphatidyl choline may be absorbed intact and incorporated into phosphatidyl choline by intestinal epithelium (Nilsson and Borgström, 1967; Subbaiah, Sastry and Ganguly, 1969). Evidence has been presented that lysophosphatidyl choline may also be incorporated into lymph triglyceride without prior hydrolysis of the acylglycerol ester bond (Nilsson, 1968; Paris and Clement, 1969). These observations suggest that, in addition to glycerol and blood glucose, lysophosphatidyl choline derived from the phosphatidyl choline of bile and rumen bacterial lipid by hydrolysis within the intestinal lumen may be a significant acyl acceptor for esterification of absorbed FFA within the intestinal mucosa of the ruminant (Garton, 1969).

Extent of absorption. Reports of the efficiency with which the ruminant gut is able to digest and absorb lipid introduced into the abomasum or duodenum have differed considerably. Phillips and Roberts (1966) reported that administration of sunflower oil into the duodenum of sheep gave apparent digestibilities for fat of only 50%. Following duodenal or abomasal administration of ^{14}C-labelled tripalmitin in olive oil, 12-71% of the labelled fat was recovered in the faeces (Heath and Morris, 1962). On the

other hand, Erwin, Sterner and Marco (1963) reported that when safflower oil was given into the abomasum of sheep, the apparent digestibility of fat was 96%. In a recently reported experiment, Mills, Cook and Scott (1970) made use of a technique for protecting triglyceride from rumin-

al lipolysis and hydrogenation with formaldehyde-treated protein (Scott, et al., 1970). When protected fat was fed to goats, more than 90% entered the abomasum unchanged and labelled fat was not detected in the faeces whereas, with unprotected fat, approximately 8% of the administered radioactivity was recovered in the faeces. Heath and Hill (1969) reported that ^{14}C-labelled palmitate, stearate or oleate injected into the abomasum of sheep were absorbed to the extent of about 90%. Furthermore, labelled glycer-

yl tripalmitate injected into the rumen was absorbed with an efficiency of 90% and this extent of absorption did not change as the intake of fatty acids increased from 12 g/24 hr. to 44 g/24 hr. This increase in dietary in-

take was achieved by supplementing oaten chaff with in-

creasing levels of maize oil. When this was done, the apparent digestibility of dietary fatty acid increased to approach that of the labelled fat on the highest intake (Heath and Hill, 1969). This finding was not consistent with the results of Czerkawski (1966) who found that when the fatty acid intake of sheep was increased from 13 to 74 g/24 hr. by supplementing a concentrate-dry grass meal

ration with linseed oil fatty acids, the digestibility of fatty acids in the diet did not change consistently. However, the digestibility of fatty acids was 85-90% in most cases. Roberts and McKirdy (1964) reported that digestibility of crude fat by steers was increased when rapeseed oil and tallow but not sunflower seed oil was added to a grain ration.

The efficiency of fat absorption in ruminants is likely to be a complex function of the nature of the dietary lipid, the physical state in which the fat enters the intestine and the amount of roughage in the diet. It would seem, nevertheless, that the ruminant intestine has the capacity to absorb fat efficiently when it is presented in an appropriate form.

Incorporation of Dietary Fatty Acids into Lymph Lipids.

Absorption of lipid into lymph. The application to ruminants of surgical techniques for the chronic collection of lymph was first reported by Lascelles and Morris (1961). High levels of fat were noted in the thoracic duct lymph of sheep. It was also evident that intestinal lymph contributed the bulk of the flow to the thoracic duct. It has since been found that very little fat enters the thoracic duct in lymph from the hepatic (Heath and Morris, 1962) and cervical (Feliński, et al., 1964) regions of sheep or the mammary gland of cows (Lascelles, Cowie, Hartmann and Edwards, 1964) and that the bulk of

the lipid in the thoracic duct lymph of sheep comes from intestinal sources (Heath and Morris, 1962; Feliński, et al., 1964). The lipid composition of thoracic duct lymph from sheep (Feliński, et al., 1964) and cows (Hartmann and Lascelles, 1966) resembles that from monogastric species, indicating that fat is transported as chylomicrons in lymph from the ruminant intestine. Variable proportions of absorbed fatty acid are transported in the lymph of sheep, recoveries averaging about 50% (Heath and Morris, 1962). This was attributed to the presence of lymphatico-venous anastomoses preventing complete collection of the lymph. However, subsequent experiments failed to demonstrate anastomotic channels between the lymphatic and blood vascular systems and it was concluded that a significant proportion of absorbed fat must be transported via the portal vein in sheep (Heath, 1964). Indeed, Heath and Hill (1969) reported that, in a sheep in which the bile was prevented from entering the intestine, 32% of ^{14}C-palmitic acid injected into the abomasum was absorbed but only 1.7% of the labelled fatty acid appeared in thoracic duct lymph.

It may be recalled that, in monogastric species, feeding a fat meal results in a characteristic absorption pattern, the transport of fat in lymph rising rapidly to attain a maximum and declining thereafter. Fat absorption

may be completed in as little as 9 hr. A similar absorption pattern is observed in sheep when ^{14}C-triglyceride is administered into the abomasum or duodenum, absorption being almost complete 6 hr. after the fat is given (Heath and Morris, 1962). However, fat absorption is much slower when the fat is given intraruminally, maximum absorption being attained about 22 hr. after administration and radioactivity continuing to appear in lymph for several days. These results were corroborated by the study of Hartmann, et al., (1966) who administered safflower oil to cows in a single dose and measured the output of total esterified fatty acid (TEFA) in thoracic duct lymph. The output of TEFA almost doubled between 6 and 7 hr. and did not begin to decline until 17 hr. after feeding the oil. Fat transport in lymph did not return to normal until 2-3 days later. This extended pattern of absorption has been attributed to the mixing of the administered fat with the large volume of rumen contents and the subsequent release of small quantities of ruminal digesta at regular intervals (Lascelles, Hartmann and Harris, 1966). The reproducibility of the response to oil feeding of lymph lipid from the three cows used by Hartmann, et al., (1966) contrasted with the findings in sheep (Heath and Morris, 1962). This observation, together with the overall level of fat transport in lymph, suggested that most of the absorbed fat was

transported by way of the lymph in cows (Hartmann, et al., 1966).

Saturated vs. unsaturated fatty acids - Balance studies. Clarke and Roberts (1967) found that when the proportion of palmitic plus stearic acids in the fat fed to sheep was varied from 10% to 90%, no change in apparent digestibility of crude fat was observed. Czerkawski (1966) calculated that the apparent digestibility of stearic acid leaving the rumen (derived largely by hydrogenation of the unsaturated C_{18} fatty acids) was about 93%. This calculation involves a number of assumptions but gives at least an indication of the efficiency of utilization. He further reported unpublished experiments in which the diets of sheep were supplemented with stearic acid. At levels of 20-39 g/24 hr. the apparent digestibility of the added fat was 80-90%. However, when the stearic acid intake was increased to 50-60 g/24 hr., the digestibility dropped to about 60%. These results suggest that the addition of larger amounts of stearic acid may exceed the capacity of the rumen digesta to disperse this fatty acid.

Lennox and Garton (1968) measured the rates of flow and fatty acid content of digesta at different levels of the sheep intestine. They concluded that, while there were no gross differences in the overall composition of the FFA in different parts of the intestine, there may

have been slightly more absorption of 18:1 (especially the trans isomer) than of 16:0 and 18:0. These results were supported by those of Leat and Harrison (1969). Heath and hill (1969) have recently determined the extent of absorption of ^{14}C-labelled palmitic, stearic and oleic acids injected into the abomasum of sheep. All three acids were absorbed to the extent of about 90%. Thus it would appear that, under most circumstances, saturated, long chain fatty acids are as well absorbed as their unsaturated homologues in the ruminant.

Fatty acids in lymph lipids. Of the labelled palmitic acid absorbed into the lymph of sheep, greater than 90% is present as neutral glycerides (largely triglyceride) and small amounts are incorporated into phospholipid and FFA (Heath and Morris, 1962). In this respect, ruminants do not appear to differ from non-ruminants. The interpretation of data on the incorporation of dietary fatty acids into lymph FFA must be approached with care, since fatty acids from chylomicron triglyceride are rapidly incorporated into blood plasma FFA (Havel and Goldfien, 1961) which may appear in the capillary filtrate.

Feliński, et al. (1964) observed in sheep that the major fatty acid of triglyceride in lymph from intestinal sources was stearic acid and estimated that the daily transport of stearate in lymph was 5.6-7.4 g. This

probably represents an underestimate since the lymph was collected on the first day after surgery (see Hartmann and Lascelles, 1966). The high level of stearate in absorbed lipid was interpreted as evidence of efficient absorption of this fatty acid in the sheep (Feliński, et al., 1964) and was contrasted to findings in the rat (Carroll and Richards, 1958). The fatty acid composition of triglyceride from the thoracic duct lymph of sheep closely resembled that of FFA in jejunal digesta (Feliński, et al., 1964) but differed from that of blood plasma triglyceride in having less 18:1 and more 18:0 (Garton and Duncan, 1964). This suggests that the high desaturase activity observed in intestinal mucosa from sheep in vitro (Bickerstaffe and Annison, 1969b) is of little quantitative significance in vivo.

Comparison of the fatty acid composition of lymph and dietary lipids was consistent with the hydrogenation of dietary lipid in the rumen of sheep (Heath, Adams and Morris, 1964; Miller, et al., 1967). That trans-18:1 isomers arise during hydrogenation of unsaturated C_{18} fatty acids was elegantly demonstrated in an experiment in which maize oil (largely 18:2, no trans fatty acids) was introduced into the rumen or abomasum of sheep (Heath, et al., 1964). Abomasal administration caused almost complete disappearance of trans-18:1 from the

lymph due to dilution of lipid containing trans isomers leaving the rumen. However, when the oil was introduced into the rumen, the trans-18:1 content of lymph more than doubled, due to partial hydrogenation of the fat load.

Little change in the gross fatty acid composition of lymph lipids was seen after administration of maize oil into the rumen, but when the rumen was by-passed, the fatty acid pattern of lymph lipid changed toward that of the maize oil (Heath, et al., 1964). The content of C_{15} and C_{17} fatty acids in lymph lipids during these latter experiments was higher than that in the diet (lucerne chaff), consistent with the microbial synthesis of these fatty acids in the rumen. Thus, it may be seen that the fatty acid composition of lymph triglyceride reflects events occurring in the rumen as well as the addition of unsaturated fatty acids in bile to the intestinal contents.

Cholesterol ester and phospholipid from intestinal and thoracic duct lymph was characterised by high levels of the C_{18} unsaturated fatty acids (Feliński, et al., 1964). The levels of 18:2 and 18:3 in phospholipid and the levels of 18:1, 18:2 and 18:3 in cholesterol ester exceeded those found in triglyceride from lymph. Heath, et al. (1964) found similar results for phospholipid but observed that the fatty acid composition of cholesterol ester resembled that of triglyceride from lymph. These

workers also reported that, during the absorption of maize oil injected into the abomasum, the proportion of the total fatty acids from lymph transported as phospholipid was less than during normal feeding. When the content of the individual fatty acids in lymph phospholipids was expressed as a proportion of the corresponding fatty acids in lymph total lipid, it became evident that 18:0, 18:2 and 18:3 may have been selectively incorporated into phospholipid (Heath, et al., 1964). This conclusion takes no account of the endogenous contribution, particularly lysophosphatidyl choline of biliary origin, to lymph phospholipid so that it should be regarded with caution.

ASSIMILATION OF LIPID IN YOUNG RUMINANTS

Some aspects of this topic have been dealt with in recent reviews (Huber, 1969; Porter, 1969).

Development of Rumen Function.

It is well-known that the reticulo-rumen of the newborn is smaller than the omaso-abomasum but grows rapidly until it occupies nearly 90% of the total stomach volume in the adult. This growth is dependent on the ingestion of solid food and the production of volatile fatty acids in the rumen. Newborn ruminants given free access to solid matter such as hay or grass, develop a rumen that is

fully functional with respect to fermentative and cellulolytic processes by about 6 weeks after birth. Rumen development may be prevented by maintaining the young on liquid diets but proliferation of rumen tissue will commence as soon as solid feed is included in the diet (Huber, 1969).

It has been found that the fatty acid composition of rumen lipid obtained from calves 15 days after they were put on a solid diet had changed only a little towards that of the adult whereas after 30 days the fatty acid pattern of rumen lipid was similar to that of the adult (Viviani, Borgatti, Monetti and Mordenti, 1967).

When the development of the rumen in calves is retarded by feeding a liquid diet, the fatty acid composition of depot fat, liver, muscle and peripheral nervous tissue reflects that of the diet (Sirén, 1962; Erwin and Sterner, 1963; Garton and Duncan, 1969). However, when rumen development is induced by feeding a solid diet, the fatty acid composition of adipose tissue changes towards that of the adult, acquiring high levels of 18:0, trans-18:1 and fatty acids of microbial origin (Garton and Duncan, 1969).

It would appear, therefore, that the capacity to hydrogenate unsaturated fatty acids and synthesise odd carbon number and branched chain fatty acids develops

synchronously with the other functions of the rumen microorganisms during rumen development.

Fat Absorption in the Milk Fed Ruminant.

Pregastric esterase. Milk is mixed with salivary lipase (or "pregastric esterase") during ingestion by the calf, resulting in lipolysis of milk fat within the abomasum (Otterby, Ramsey and Wise, 1964). Salivary lipase has a marked specificity for short chain fatty acids and the composition of long chain fatty acid in glycerides remaining from its action was found to be quite close to that of the ingested milk fat (Siewert and Otterby, 1968). These latter workers concluded that salivary lipase is probably similar to pancreatic lipase in its mode of action. They found that 0.5-2.0 hr. after ingestion of milk, the glycerides of abomasal contents consisted of approximately 50% triglyceride, 35% diglyceride and 15% monoglyceride, on a molar basis. It would appear, therefore, that salivary lipase plays an important role in the digestion of fats by the young ruminant.

Pancreatic juice. Heath and Morris (1963) showed that in 2-3 week-old lambs, pancreatic juice is essential for fat absorption. Huber, Jacobson, Allen and Hartman (1961) found that the lipase activity/g. dry pancreas from one day-old calves was about one third of that in tissue from

8 day-old calves but did not change from 8 to 44 days of age. Koref and Mũnoz (1943) reported that the lipase concentration in foetal calf pancreas was low compared with suckling calves and cows. These observations suggest that the secretion of pancreatic lipase in the ruminant at birth may be low but increases to adequate levels within the first week of life. In this connection, it has been reported that the flow of pancreatic juice is scant and its enzyme content low in the newborn calf (R.S. Comline, unpublished observations, quoted by Hardy, 1969) whereas the flow rate increased linearly from 3 to 180 days of age with an average flow of 9-10 ml./kg./ 24 hr. (McCormick and Stewart, 1967). Since the concentration of secretory products in glandular tissue is not necessarily related to their rate of secretion, it must be concluded that the amount of pancreatic lipase entering the gut lumen in the very young ruminant remains to be determined.

Bile. Bile secretion in lambs was studied by Adams and Heath (1963) who found comparable levels of phospholipid in lambs and adult sheep. The principal phospholipid in lamb bile was phosphatidyl choline. A flow of 5 ml./hr. and an output of 1-2 g/24 hr. of phospholipid in bile was reported. The predominant bile acids in bile from bovine and ovine foetuses are cholic and chenodeoxycholic acids but chenodeoxycholate declines after birth,

leaving cholate as the predominant bile acid in the adult (Peric-Golia and Socic, 1968; Sheriha, et al., 1968). Taurine conjugates predominate at all ages (Peric-Golia and Socic, 1968).

Interestingly, the pH of intestinal digesta from preruminant calves (Mylrea, 1966a) shows a similar pattern to that reported for adult ruminants (Lennox, et al., 1968). A micellar phase probably exists in the intestinal lumen of young ruminants, although no data is available on this subject. The fatty acid composition of lymph glyceride from lambs closely reflects that of dietary lipid with respect to both total fatty acids and trans isomers (Heath, et al., 1964).

Esterification of absorbed fatty acid. Hansen (1965a,b) studied the incorporation into triglyceride of several fatty acids incubated separately with suspensions of intestinal epithelium. Palmitate and oleate were incorporated to a greater extent than stearate and linoleate by both lamb and rat mucosa. These findings are not in accord with observations on the relative incorporation of fatty acids into lymph triglycerides from rats in vivo (Karmen, et al., 1963). Furthermore, labelled palmitate incubated with intestinal epithelium was found to be distributed approximately randomly amongst the three positions of the glycerol backbone (Hansen, 1965b),

which contrasts with the distinct localisation of palmitate in the 2-ester position of lymph triglyceride from adult sheep (Garton and Duncan, 1965).

Hansen (1965b) found that the percentage of added fatty acid esterified by suspensions of intestinal epithelium from lambs decreased with the age of the lamb. More recently, Cunningham and Leat (1969) observed that glyceride synthesis /g. wet weight in segments of intestine decreased in the order: foetal lambs, 6-13 day old lambs, adult sheep. It would appear, therefore, that the capacity to synthesize triglyceride per unit weight of intestine decreases with age in ruminants. However, neither of these authors related their results to tissue protein or length of intestine or attempted to define the kinetics of the esterification. The interpretation of their results must, therefore, await further characterisation of the esterification process under these conditions.

Cunningham and Leat (1969) also reported that the contribution of the monoglyceride pathway to glyceride synthesis was less in foetal and newborn lambs than in adult sheep. This finding is surprising in view of the virtual absence of monoglyceride from the adult sheep gut and the probable availability of monoglyceride to the intestinal wall in suckling ruminants.

Incorporation of lipid into lymph. It might be expected that fat assimilation in the preruminant animal would be closely similar to that in the adult monogastric. However, Heath and Morris (1962) found that when glycerol-tri-^{14}C-palmitate was given into the abomasum of 2-3 week old lambs fed diluted milk, the pattern of recovery of radioactivity in the intestinal lymph closely resembled that of the adult sheep. Radioactivity in the lymph attained a maximum about 17 hr. after administration and was still present at 54 hr. after feeding. These results were confirmed and extended in the preruminant calf by Shannon and Lascelles (1967). These workers found that when calves were fed whole milk twice daily, the flow of lymph and output of neutral lipid (largely triglyceride) from the thoracic duct changed little between feeds. When the calves were fed the same quantity of milk once daily, a more distinct pattern of absorption emerged, the output of neutral lipid decreasing after feeding and then increasing to attain a maximum at 10 hr. and falling off slowly thereafter. Nevertheless, similar quantities of neutral lipid were transported in lymph during the two 12 hr. periods between feeds. The relatively continuous nature of fat absorption in calves was similar to that observed in cows (Hartmann and Lascelles, 1966). Mylrea (1966b) studied the flow of digesta along the digestive tract of calves which were fed twice daily by inserting

re-entrant fistulae at various levels of the intestine. It was found that milk ingested by the calf clotted rapidly, the whey fraction escaping from the abomasum before the milk clot. Fat and nitrogen were released from the abomasum at a fairly steady rate. These results have been confirmed by Mathieu (1968). The failure to observe marked absorption patterns for lipid in lymph such as are found in the monogastric was, therefore, ascribed to the entrapment of fat in the casein curd formed when milk enters the abomasum and its subsequent slow release under the influence of proteolytic secretions (Shannon and Lascelles, 1967).

Recoveries of radioactive fat in lymph after administration to lambs was higher than for adult sheep (Heath and Morris, 1962). The output of neutral lipid in lymph from the thoracic duct of calves represented a high recovery of dietary fat (Shannon and Lascelles, 1967). It may be concluded, therefore, that the bulk of absorbed fat is transported in the lymph of young ruminants.

CHAPTER 2. MATERIALS AND METHODS

SURGICAL

Anaesthesia.

Pentothal sodium (5% solution, Abbott) was injected intravenously to induce anaesthesia before intubation with a Magill endotrachaeal tube. In suckled calves, a dose of 8-10 mg. pentothal/Kg. liveweight was administered, whereas in newborn, unsuckled calves, a minimal dose of 4-6 mg/Kg. liveweight was employed. A mixture of cyclopropane and oxygen was used to complete the induction of anaesthesia and subsequently to maintain it. The cyclopropane was discontinued approximately 30 min. before the end of the operation and the system flushed several times with oxygen just before the completion of surgery. Recovery of the calves was rapid, with the animals standing steadily within 6-12 hr. of the operation.

Thoracic Duct.

Anatomical considerations. The thoracic duct in the bovine courses through the anterior mediastinum usually as a single duct (Sisson and Grossman, 1953). It opens into the origin of the anterior vena cava or left common jugular vein at a point immediately adjacent to the first rib and brachial vein. The terminal part of the duct is ampullate but the lymphatico-venous opening is generally

small. Variations of the above are sometimes seen, e.g. in the anterior mediastinum there may be 2-4 ducts (with connecting branches) which usually unite just before the lymphatico-venous opening, but may open separately.

Technique. The thoracic duct was cannulated in the neck as described by Shannon and Lascelles (1967). The calves were laid on the operating table with their left side uppermost. The head and neck were extended and the left forelimb held back to allow access to the region of the first rib. A skin incision extending from approximately midway along the neck to the point of the shoulder was made immediately dorsal to the external jugular vein. The external jugular vein was exposed in the anterior section of the wound and the deep incision extended posteriorly to expose it to its point of junction with the internal jugular vein. The communicating branch of the cephalic vein was located and the accompanying inferior cervical artery was ligated close to its origin from the carotid artery. The artery distal to the point of ligation was reflected to allow better access to the common jugular vein. Careful blunt dissection of the dense connective tissue over the origin of the common jugular vein was carried out until the ampullate end of the thoracic duct was located immediately adjacent to the pleura. The

duct was ligated at its venous entrance and dissected free for a distance of 2-3 cm. Two further ligatures were passed around the exposed duct and left loose. After the duct had dilated somewhat a small opening was made in the wall and a polyvinylchloride cannula (1.5 mm. ID, 2.5 mm. OD, Dural Plastics and Engineering Pty. Ltd., Dural, N.S.W.) was passed into the duct for a distance of 5 cm. The cannula was firmly secured with the ligatures, care being taken to ensure that the end of the cannula was approximately midway between two lymphatic valves. In calves possessing more than one duct, the accessory ducts were ligated, together with the cervical and brachial ducts.

A catheter, of the same dimensions as that introduced into the thoracic duct, was passed into the anterior vena cava by way of an incision in the communicating branch of the cephalic vein. Blood was flushed out of the catheter with sterile 0.9% (w/v) sodium chloride and its end blocked with a plug. Both the thoracic duct and venous cannulas were sutured securely to the fascia at several points before being led out through the anterior end of the incision. They were each sutured to the skin in a semi-circle and the lymphatico-venous shunt was completed by pushing the ends of the two cannulas into a short length of polyvinylchloride cuff tubing of

suitable size. Four ligatures placed around the cuff and secured firmly prevented the cannulas pulling apart.

Post-operative management. The calves were kept in small pens on straw bedding during the experimental period. Each calf was given 1 x 10^6 i.u. procaine penicillin and 0.5g. streptomycin intramuscularly daily for 5 days. Whole milk (1.14 l.) was warmed to body temperature by the addition of hot water and given by stomach tube soon after completion of the operation. The calves recovered quickly and 6-8 hr. later were able to stand and drink from a bucket. There was no evidence of infection of the operative region in the calves.

Intestinal and Hepatic Lymph Ducts and Common Bile Duct.

Anatomical considerations. The intestinal lymph duct is usually formed from the union of the two large lymphatic ducts coming from the intestines and the stomach. This common duct runs dorsally through the pancreas and after passing backwards for a short distance, unites with the lumbar trunk to form the cisterna chyli which may be found adjacent to the vena cava.

The hepatic lymph ducts are formed from efferent lymphatic ducts arising from the hepatic lymph nodes located in the region of the portal fissure. In the calf, a variable number of fine ducts were seen running

posteriorly and then bending dorsally over the surface of the pancreas to run parallel to the intestinal trunk. The largest of these ducts could usually be seen entering the intestinal duct or cisterna chyli. The smaller ducts either united with the main hepatic duct just prior to emptying into the intestinal duct, or had separate openings. A considerable degree of anatomical variation was observed in the number of ducts and their relationship with the intestinal duct.

In the calf, the cystic duct draining the gall bladder and the hepatic bile duct unite to form a very short common bile duct which enters the duodenum separately from the pancreatic duct.

Technique. Intestinal and hepatic lymph ducts were cannulated in newborn, unsuckled calves following the procedures described by Shannon and Lascelles (1968) for older calves. The animals were laid on the operating table with their right side uppermost and the hind limbs extended posteriorly to permit access to the abdominal region between the thigh and the last rib. The skin was incised from the transverse process of the lumbar vertebrae ventrally for a distance of about 25 cm. along a line immediately behind the last rib. The incision was continued through the musculature and the peritoneal cavity opened.

The caudate lobe of the liver is very large in the

calf and is attached to the ventral surface of the right kidney by a well-developed ligament. This ligament was cut and the caudate lobe retracted to expose the dorsal surface of the pancreas as far forward as the region of the portal fissure. The dorsal surface of the pancreas was separated from the kidney and the intestinal lymph duct located emerging from the substance of the pancreas immediately adjacent to the adrenal gland. All hepatic ducts were ligated and the longest cleared of peritoneum prior to cannulation. The intestinal duct was cannulated with clear vinyl tubing (1.5 mm. ID, 2.7 mm. OD) and the hepatic duct with similar tubing of smaller dimensions (0.75 mm. ID, 1.45 mm. OD). In each case the cannula was securely fixed to the dorsal commisure of the wound by a number of sutures. It was sometimes found that lymph would not flow unless some tension was placed on the cannula at this stage, probably due to the end of the cannula locating against the lymphatic wall at a bend in the duct.

The intestinal cannula was carried along the mid-dorsal line and sutured to the skin. A cannula of the same dimensions was introduced into the jugular vein and fixed to the skin. The two cannulas were joined by a piece of cuff tubing, as for the thoracic duct prepara-

tion, thus completing the lymphatico-venous shunt. A calf prepared in this way is pictured in Fig. 3. The hepatic lymph, on the other hand, was not recirculated but collected continuously into a plastic bottle sutured to the skin of the flank.

The common bile duct was ligated as it entered the duodenal wall and the duct cannulated with clear vinyl tubing (1 mm. ID, 2 mm. OD). Bile was collected into a plastic bottle sutured to the flank.

Collection and Storage of Samples.

The lymphatico-venous shunt was disconnected, sterile 0.9% sodium chloride was flushed through the venous cannula and its end blocked with a plug. Lymph was collected into a measuring cylinder for a measured period of time (2-4 min.). Occasional blood samples were withdrawn by syringe from the venous cannula. Heparin (Pularin, Evans) was added to the samples in powder form as an anti-coagulant. After once again flushing the venous cannula with sterile saline, the shunt was re-established. The calves quickly became accustomed to the sampling routine and could be sampled in either the standing or recumbent position without noticeable disturbance. A balanced electrolyte solution (McSherry and Grinyer, 1954) was regularly introduced intravenously to replace the fluid and electrolyte lost

FIGURE 3.

A young calf in which an intestinal lymphatic duct-venous shunt was established shortly after birth.

The cannula may be seen leaving the abdominal cavity to pass along the back and enter the jugular vein in the neck.

Figure 3

as a result of lymph collection.

Lymph and blood were centrifuged to remove cells and stored at -15°C until analysed.

ANALYTICAL

All determinations were carried out in duplicate. Spectrophotometric methods were tested and found to obey Beer's Law within the optical density ranges employed in the determinations.

Solvents.

Solvents used in procedures preparatory to gas-liquid chromatography (GLC) were tested for contaminants as follows: 10 ml. of the solvent in a clean, dry beaker was evaporated to dryness at room temperature under a stream of nitrogen. The residue was taken up in 1 ml. of the same solvent and 5μl aliquots were submitted to GLC. It was found that the following solvents were contaminated at a level which would interfere with GLC of fatty acid esters: AR grade chloroform and methanol (Univar, By-Products and Chemicals Pty. Ltd., Sydney, N.S.W.), "chromatographic" hexane (E. Merck AG. Darmstadt, Germany), anhydrous ethyl ether (Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.) and anaesthetic grade ether (Drug Houses of Australia Ltd., Sydney, N.S.W.). These solvents were further purified before use in

preparing samples for GLC. On the other hand, n-heptane (Univar, By-Products and Chemicals) and carbon disulphide (analytical grade, E. Merck) showed no such contamination and were used without further purification.

Chloroform. Technical grade chloroform was purified by a procedure adapted from Gross and Saylor (1931). The chloroform was allowed to stand in contact with 10% (w/v) aqueous sodium hydroxide for 1 hr. and was then washed successively with water, concentrated sulphuric acid and water. The product was dried over anhydrous calcium chloride with a little anhydrous sodium carbonate added and then over anhydrous calcium sulphate ("Drierite"). The Drierite was filtered off and the chloroform fractionally distilled through a 75 cm. Hempel column packed with 0.64 cm. Raschig rings (Vogel, 1961). The distillate was stored in a dark bottle in the presence of 0.5% (v/v) methanol at 4°C.

Methanol. AR grade methanol (Univar, By-Products and Chemicals) was redistilled before use. Absolute methanol was prepared from the AR grade product by the iodine-activated magnesium methylate procedure as described by Vogel (1961).

Butanol. AR grade n-butanol (Analar, British Drug Houses, Poole, England) was dried over Drierite and fractionally distilled as described for chloroform.

Diethyl ether. Technical grade diethyl ether was purified as described by Weissberger, Proskauer, Riddick and Toops (1965). The ether was shaken intermittently for 1 hr. with 10% (w/v) sodium bisulphite solution. This treatment was repeated until the ether was free of peroxides (test for peroxides according to Vogel, 1961). The product was washed successively with saturated aqueous sodium chloride containing 0.5% (w/v) sodium hydroxide, saturated aqueous sodium chloride containing a little sulphuric acid and finally with saturated aqueous sodium chloride. The ether was then dried over sodium metal and fractionally distilled in an atmosphere of nitrogen. The distillate was stored in a dark bottle in the presence of nitrogen and sodium at 4°C.

Protein Determinations.

Plasma proteins. Determinations of the total protein and albumen concentrations of plasma were carried out by the method of Gornall, Bardawill and David (1949). Globulin concentrations were obtained by difference. To 0.5 ml. of plasma was added 9.5 ml. of 22.6% sodium sulphate solution. The resultant mixture was shaken vigorously. For the determination of total protein, 2 ml. of the mixture was transferred to another test tube to which was added 8 ml. of Biuret reagent (1.5 g. cupric sulphate, 6.0 g. potassium sodium tartrate, 300 ml. 10%

sodium hydroxide, made up to a final volume of 1 l.) and the colour allowed to develop for 30 min. For the determination of albumen, the precipitated globulins were removed from the remaining original mixture by a number of filtrations through Whatman No. 42 filter paper to produce a clear filtrate. To a 2 ml. aliquot of the clear filtrate was added 8 ml. of Biuret reagent and the colour allowed to develop for 30 min. The optical density of the resultant colour was measured at 540 mμ.

Calculation:-

Optical density of unknown x 35.0 = g./100 ml. protein.

A standard solution of bovine plasma albumen (Cohn Fraction V) was used to obtain the regression coefficient of 35.0.

Lymph proteins. Total protein, albumen and globulin concentrations in hepatic lymph were determined as described above for plasma. Due to the high lipid content of thoracic duct and intestinal lymph a modification of the procedure described by Gornall, et al. (1949) was adopted for the determination of total protein and albumen (Shannon and Lascelles, 1968). After the addition of the Biuret reagent, the reaction mixture was incubated for 2-4 hr. at 37°C. During this time any lipid in the mixture flocculated and could be largely taken out by

centrifugation and removal of the lipid layer at the surface. Any remaining lipid was then removed by filtration of the solution through a sintered glass disc (Gooch crucible, porosity 2 or 3). The optical density of the resultant solution was measured at 540 mμ and the protein concentration calculated as above. Globulin concentration was determined by difference.

Total Esterified Fatty Acid.

TEFA was determined by the method of Stern and Shapiro (1953). Samples of the plasma and lymph (0.2 ml.) were extracted with 10 ml. boiling ethanol-ether (3:1 v/v). The precipitated protein was removed by filtration through Whatman No. 43 filter paper. A 3 ml. aliquot of the filtrate was taken in the case of determinations on plasma and hepatic lymph. A 1 ml. aliquot of filtrate was diluted to 3 ml. with ethanol-ether (3:1 v/v) in the case of thoracic duct and intestinal lymph determinations. The aliquots of filtrate were reacted with 0.5 ml. 2M hydroxylamine hydrochloride and 0.5 ml. 3.5 N sodium hydroxide. After standing at room temperature for 20 min., the solution was acidified with 0.6 ml. hydrochloric acid solution (1:2 v/v, concentrated hydrochloric acid - water). The addition of 0.5 ml. 0.37 M ferric chloride (in 0.1N hydrochloric acid) completed colour development. The optical density of the

resultant colour was measured at 525 mμ. A standard, containing 0.5μ equiv./ml. glycerol trioleate (purified by TLC) was included in each series of estimations.

Calculation: the average molecular weight of fatty acids in lymph and plasma triglyceride from calves is close to 260 g. (see Chapter 6). This value was used in the calculation of TEFA concentrations.

Phospholipid.

Phospholipid was determined on aliquots of the filtrate from the ethanol-ether extraction described under Total Esterified Fatty Acids. The solvent was removed under vacuum and the dry fat was digested.

One of two methods was employed for the estimation of lipid phosphorous. In the procedure of Zilversmit and Davis (1950), digestion of the lipid extract was accomplished by adding 1 ml. 60% perchloric acid and boiling until the solution was clear. After cooling, 5-6 ml. distilled water was added followed by 1 ml. 4% (w/v) ammonium molybdate solution. Colour development was achieved by the addition of 1 ml. of 2/5 dilution of reducing solution (0.5 g. 1,2,4-amino naphthol sulphonic acid, 30 g. sodium metabisulphite and 6.0 g. sodium sulphite made up to 250 ml. with water) and the final volume adjusted to 10 ml. by the addition of distilled water. The colour was allowed to develop for 20 min. and the optical density read at 810 mμ Standards

containing 0.02 mg. phosphorous (2 ml. 0.04391 g./l. potassium dihydrogen orthophosphate) were included in each series of estimations.

Calculation: a factor of 25 was used to convert lipid phosphorous to phospholipid.

The second procedure used was a micro-adaption from the method of Allen (1940). Digestion of the dried ethanol-ether extract was achieved by adding 0.45 ml. 70% perchloric acid and boiling until a clear solution was obtained. If the solution did not clear, it was cooled, 1-2 drops of 30% hydrogen peroxide ("100 volumes") was added and the digestion was continued for a further 5 min. This procedure was repeated if necessary. After cooling, 4.0 ml. distilled water was added followed by 0.4 ml. amidol reagent (2.0 g. 2,4-diaminophenol hydrochloride and 40.0 g. sodium bisulphite made to 200 ml. with distilled water and filtered) and 0.2 ml. 8.3% (w/v) ammonium molybdate. After 10-15 min. the optical density was read at 660 mμ in a spectrophotometer or using a red filter in a colorimeter. Reagent blanks and standards containing 0.4 ml. 70% perchloric acid were included in each series of estimations.

Calculation: as before.

Free Fatty Acid.

FFA was determined by a modification of the method of Dole (1956). The FFA was extracted by shaking 1 ml. of plasma or lymph with 5 ml. extraction mixture (40:10:1, v/v/v, iso-propyl alcohol-heptane-1 N sulphuric acid). After standing for 10 min. 3 ml. heptane and 2 ml. water were added and the phases were allowed to separate. 2 ml. of the heptane phase containing the FFA was transferred to a test tube together with 1 ml. indicator solution (0.01%, w/v, thymol blue in 90% ethanol). The addition of 3 ml. iso-propyl alcohol produced a single-phase system for titration (Schnatz, 1964). A stream of nitrogen was bubbled through the solution, prior to and during the course of the titration to eliminate carbon dioxide and to mix the solution. Titration was carried out with 0.01 N sodium hydroxide introduced through a fine polythene tube from an "Agla" micrometer syringe. Titration was continued until the first appearance of a yellow-green colour. At the beginning and end of each series of estimations, titrations were carried out on blanks (2 ml. heptane, 1 ml. indicator solution and 3 ml. iso-propyl alcohol) and on a standard solution (0.5 μ equiv. palmitic acid).

Calculation: the average molecular weight of the FFA was taken as 280, and the results were expressed as

mg./100 ml.

Cholesterol.

Total cholesterol in plasma and lymph was determined by the method of Rappaport and Eichhorn (1960). However, this method was found to overestimate the concentration of total cholesterol.

A modification of this method was used routinely to determine free cholesterol eluted from the thin layer chromatograms. After evaporation of the eluant the residue of free cholesterol was dissolved in 1.4 ml. acetic acid (glacial). The Leiberman-Burchardt colour was developed by the addition of 3 ml. acetic anhydride and 0.4 ml. concentrated sulphuric acid. The solution was shaken vigorously and the colour allowed to develop for 10 min. The optical density was read at 540 mμ. A standard containing 0.4 mg. free cholesterol was included in each series of estimations. Close agreement was found between these spectrophotometric estimations and those obtained by the gravimetric procedure.

Milk Fat.

Milk fat was determined by the Babcock method as described by Davis and MacDonald (1953).

Iodine Value (Hanus Method).

The following procedure was scaled down from the Association of Official Agricultural Chemists (1965) procedure by a factor of 10.

Reagents: Approximately 15% (w/v) potassium iodide solution.

0.1 N Sodium thiosulphate

Starch Indicator. Mix approximately 1 g. soluble starch with enough cold water to make a thin paste, add 100 ml. boiling water and boil for about 1 min. while stirring.

Hanus iodine solution. Measure 825 ml. glacial acetic acid and dissolve in it with the aid of heat 13.6 g. iodine. Cool and titrate 25 ml. of this solution with 0.1 N sodium thiosulphate. Measure another 200 ml. portion of acetic acid and add 3 ml. bromine. To 5 ml. of this solution add 10 ml. 15% potassium iodide solution and titrate with 0.1 N sodium thiosulphate. Calculate the quantity of bromine solution required to double the halogen content of the remaining 800 ml. iodine solution as follows:

$A = {}^{B}/_{C}$, where A = ml. bromine solution required, B = 800 x thiosulphate equivalent of 1 ml. iodine solution. Make the mixed solution to 1 l. with acetic acid.

About 50 mg. fat dissolved in 1 ml. chloroform was placed in a 150 ml. glass-stoppered flask. Hanus iodine solution (2.5 ml.) was added and the mixture allowed to stand in the dark for 30 min. with occasional shaking. This time must be adhered to closely to obtain accurate

results. Excess of iodine remaining after reaction must be at least 60% of the quantity added. Determinations in which this was not the case were repeated with adjustment of the amount of fat used so that at least 60% excess iodine remained. Stoppers were kept in the flasks at all times to minimise losses of iodine.

Potassium iodide solution (1 ml.) was added, the flasks were shaken thoroughly and 10 ml. freshly boiled and cooled water was added, washing down any free iodine on the stopper. The free iodine remaining from the reaction was titrated with 0.1 N sodium thiosulphate, adding it gradually, with constant shaking, until the yellow solution turned almost colourless. The flask was stoppered and shaken vigorously so that any iodine remaining in the chloroform was taken up by the potassium iodide solution. A few drops of starch indicator was added and the titration continued until the blue colour just disappeared. Two reagent blanks were included in each set of determinations.

Calculation: The volume (ml.) of 0.1 N sodium thiosulphate required by the blank (B) minus the volume (ml.) required by the sample (S) gave the thiosulphate equivalent of iodine absorbed by the fat. The percentage by weight of iodine absorbed by the fat (iodine value) was calculated as follows:

$$\text{Iodine Value} = \frac{(B - S) \times 1.269}{\text{weight of sample (g.)}}$$

Extraction of Lipid.

Lipid was extracted according to the method of Folch, Lees and Sloane-Stanley (1957) which uses 2:1 (v/v) chloroform-methanol. Tissue samples were homogenised with 17 volumes (assuming 1 g. tissue occupies 1 ml.) of chloroform-methanol in a top-drive blender and the mixture was filtered (Whatman No. 43 filter paper previously extracted with boiling chloroform-methanol). A further 3 volumes of solvent was used to rinse the blender and filter paper. These steps were carried out at 4°C.

Liquid samples (e.g. lymph and blood plasma) were extracted by addition of the sample to 20 volumes of cold chloroform-methanol held in an ice-jacketed flask and stirred magnetically for 45 min. The extraction mixture was filtered as above and the flask and filter paper rinsed with 6 volumes of solvent. Alternatively, the samples were placed in glass-stoppered flasks containing glass beads (4-5 mm. diameter) and 17 volumes of chloroform-methanol, and agitated violently in a mechanical shaker for 15 min. The extraction mixture was filtered and the flask and filter paper rinsed with 3 volumes of solvent.

To the filtered extraction mixture was added 0.2 of its volume of 0.9% (w/v) sodium chloride solution and the mixture was shaken thoroughly. The phases were

separated either by allowing the mixture to stand in a stoppered measuring cylinder overnight or by centrifuging, in the cold. The chloroform phase containing lipid was obtained either by suction through a fine plastic tube or by removing as much of the upper phase as possible and rinsing the interface three times with pure-solvents-upper-phase (chloroform-methanol-water, 3:48:47, v/v/v). The chloroform was removed on a rotary evaporator and the lipid transferred to tared vessels with several washings of chloroform. Solid contaminants were removed at this stage by passing through a Pasteur pipette packed with fat-free glass wool. The chloroform was removed under vacuum and the lipid weighed. Lipid samples were stored in solvent under nitrogen sealed in freeze drying ampoules (Edwards High Vacuum Ltd., Crawley, Sussex, England) at -15°C.

Thin Layer Chromatography.

TLC was conducted according to Mangold (1965) on 20 x 20 cm. glass plates coated with 0.25 mm. layers of Silica Gel H (E. Merck AG.). Rhodamine 6G (20 mg.) was added to every 30 g. Silica Gel before preparation of the thin layers. Lipid was dissolved in chloroform and applied along a 12 cm. band near the base of the plate, using the apparatus of Roughan and Tunnicliffe (1967). The plates were developed with petroleum ether (b.p. 60-80°C)-diethyl ether-glacial acetic acid (90:10:1,

v/v/v). This procedure separated into distinct bands free cholesterol, FFA, triglyceride and cholesterol ester in ascending order of R_f values. Phospholipids remained at the origin. The chromatograms were removed from the solvent tank when the solvent front was approximately 12 cm. from the origin. The lipid bands were located under ultra-violet light, scraped from the plate and formed into small columns. Phospholipids were eluted with methanol and the other lipid fractions with chloroform into tared vessels.

The esterified fatty acid content of the triglyceride fraction was checked by the method of Stern and Shapiro (1953). The cholesterol ester content was determined by the method of Abell, Levy, Brodie and Kendall (1952). Close agreement was obtained between the colorimetric and gravimetric determinations.

Silicic Acid Columns.

Phospholipid was separated from the other lipids by passing a chloroform solution of the total fat through a small column of silicic acid. The following procedure was adapted from Horning (1964). Purified silicic acid was prepared by acid treatment of Florisil (Carroll, 1963). The silicic acid so obtained was activated at 110°C for 1 hr. and 1 g. was packed into a small glass column using 4:1 (v/v) chloroform-methanol. The column was washed with 10 ml. chloroform-methanol and then with

chloroform until the column became transparent. Up to 50 mg. fat was placed on the column in a small volume of chloroform and lipids other than phospholipid were eluted with 20 ml. chloroform. The phospholipids were then eluted with 30 ml. methanol. For amounts of lipid greater than 50 mg. the quantities of silicic acid and solvents were increased proportionately. Recovery of lipid from these columns as measured gravimetrically was in excess of 100%, indicating the presence of contaminants. However, recovery of lipid phosphorous in the methanol eluate was 95% or better and the extraneous material did not interfere with GLC.

Preparation of Fatty Acid Esters.

Dialkyl-carbonate induced transesterification. The procedure of Glass, Jenness and Troolin (1965) for the preparation of fatty acid esters was adapted and scaled down for 10 mg. quantities of lipid. Dry reagents were freshly prepared at regular intervals and stored at $4^{o}C$ under anhydrous calcium chloride.

Methyl esters of triglyceride and phospholipid fatty acids were prepared by adding 0.5 ml. 3:2 (v/v) absolute methanol-dimethyl carbonate to 10 mg. or less of lipid in a 5 ml. volumetric flask. The mixture was warmed until the lipid dissolved and 0.02 ml. 2N sodium methoxide (prepared by dissolving sodium metal in absolute methanol)

was added. It was found that, with polyester stationary phases in the gas-chromatograph, on-column injection of the alkaline reaction mixture resulted in artifactual peaks on the chromatogram, probably arising from stationary phase decomposition catalyzed by the sodium methoxide. Therefore, after allowing 90 sec., the reaction mixture was neutralized with 0.02 ml. 2N HCl-methanol (prepared with hydrogen chloride gas generated from concentrated hydrochloric and sulphuric acids as described by Vogel, 1961). Furthermore, it was observed that methanol produced a prolonged elevation of the baseline during GLC. To completely exclude methanol from the esters, saturated sodium chloride solution was added to the neutralized reaction mixture and the esters were extracted with heptane or diethyl ether (Metcalfe, Schmitz and Pelka, 1966). Butyl esters of glyceride fatty acids were prepared in a similar manner, using dry butanol and dibutyl carbonate. It was apparent that the interesterification of triglyceride was complete, because triglyceride was not detected on TLC of the heptane extract from the methyl- or butyl-interesterification of whole lymph fat, butter oil or cod liver oil. In agreement with Whyte, et al. (1963), a variable recovery of methyl esters from the interesterification of phospholipid was observed. It was assumed on the basis of the report by Whyte, et al. that interester-

ification of phospholipid was complete and, accordingly, more vigorous extraction with heptane was employed to ensure maximum recovery. Also, 90% saturated sodium chloride solution was used to prevent the precipitation of sodium chloride.

The method was further tested by putting a mixture of fatty acid methyl esters of known composition (Mixture KC, Applied Science Laboratories) through the methyl inter-esterification procedure. Some small loss of C_8 methyl ester was observed but the relative proportions of the longer chain components remained unchanged.

Esterification of FFA. FFA are not esterified under the alkaline conditions of the above procedure. Glass and Troolin (1966) effected methyl esterification of FFA by acidifying the reaction mixture after alkaline trans-esterification of glycerides. The following procedure was used to esterify FFA under conditions similar to those described by Glass and Troolin.

To 10 mg. or less of FFA in a 5 ml. volumetric flask was added 0.5 ml. 3:2 (v/v) absolute methanol-dimethyl carbonate followed by 0.07 ml. 2N HCl-methanol. After 15 min. the reaction mixture was neutralised with 0.07 ml. 2N sodium methoxide and extracted as previously. Using this method, 95% esterification of free palmitic acid was obtained.

Esterification of cholesterol ester fatty acids.

Submission of purified cholesterol ester or total lipid from lymph and blood plasma to dimethyl carbonate induced transesterification gave no apparent decrease in the cholesterol ester fraction on TLC. Furthermore, close to quantitative (Leiberman-Burchardt reaction) recovery of cholesterol ester was obtained after TLC of the transesterified lipid from cow plasma.

Cholesterol esters were, therefore, saponified essentially according to Abell, et al. (1952) before esterification. Not more than 5 mg. cholesterol ester was placed in a 10 ml. glass-stoppered tube together with 2.5 ml. alcoholic potassium hydroxide (6 ml. 33g./100 ml. aqueous potassium hydroxide and 94 ml. absolute ethanol). The tube was stoppered and incubated with shaking at 37°C for 55 min. After cooling, 5 ml. heptane, 1.5 ml. saturated sodium chloride solution and 1.0 ml. distilled water was added and the tube shaken. The heptane layer was discarded and the lower phase washed with a further 2.5 ml. heptane. The aqueous phase was then acidified to a pH of less than 2 with concentrated sulphuric acid and the FFA were extracted with 3 x 1 ml. aliquots of heptane. The FFA were esterified as above.

Using this method, 93% of the fatty acids of cholesterol ester from cow plasma were recovered as methyl

esters (measured by TEFA estimations of the fractions isolated by TLC).

Sealed tube methanolysis. In the later studies reported here, sealed tube methanolysis was employed as an alternative method to prepare methyl esters from some lipid samples. The lipid was placed in small freeze-drying tubes (capacity about 2 ml.) together with 0.5-1.0 ml. absolute methanol, 1-2 drops concentrated sulphuric acid and 0.2 ml. heptane. The tubes were gassed out with nitrogen, sealed in a flame and heated at 80°C until the lipid dissolved. The heptane layer was sampled directly for GLC.

Bromination. For the purposes of fatty acid identification, aliquots of methyl ester solutions were brominated as described by Burchfield and Storrs (1962). A 2% (v/v) solution of bromine in ether was added dropwise to the methyl ester solution until the yellow colour persisted. The mixture was injected directly into the gas chromatograph.

Gas-Liquid Chromatography.

GLC was carried out with a Pye model 104-24 twin column gas chromatograph (W. G. Pye and Co. Ltd., Cambridge, England) equipped with flame ionization detectors, constant gas-flow controllers and facilities for linear temperature programming.

Stainless steel columns (1.52 m. by 0.64 cm. OD) were employed and were deactivated by several hours' treatment with a 5% (v/v) solution of dimethyldichlorosilane (Applied Science Laboratories Inc., State College, Pa., U.S.A.) in toluene prior to packing. Column packing was prepared by adding the solid support to a solution of stationary phase in an appropriate solvent and removing the solvent on a rotary evaporator. Care was taken to ensure minimum abrasion of the column packing. The columns were packed by connecting an aspirator pump to the outlet and introducing small amounts of packing into the inlet end. Between each addition the column was tapped gently for 1 min. while lying horizontally on the bench followed by 1 min. while in a vertical position. As far as was possible, equal amounts of column packing were introduced into the two columns to facilitate balancing of the bleed rates during operation.

Routine separations were done on columns packed with 10-15% diethylene glycol succinate polyester (DEGS) on 80-100 mesh Gas Chrom P or 60-80 mesh Gas Chrom Q (Applied Science). GLC was also carried out using organosilicone polyester columns (10% EGSP-Z on Gas Chrom Q, Applied Science) prepared in this laboratory or 10% ethylene glycol adipate polyester (EGA) and 10%

Apiezon L grease (APL) on Celite 545 packed in 1.52 m. by 0.64 cm. (OD) glass columns obtained pre-packed from Pye. Each set of columns was conditioned at high temperature before use.

Samples were introduced by direct on-column injection of 0.1-5.0 μl aliquots of a solution of fatty acid esters in heptane, ether or carbon disulphide. The fatty acid composition was calculated by internal normalization of peak areas derived either with the aid of a ball and disc mechanical integrator (Disc Instruments Inc., Santa Ana, Calif., U.S.A.) or by computing peak height x retention time (Bartlet and Iverson, 1966). Under isothermal conditions, semilog plots of retention time (relative to methyl palmitate) versus carbon number were not completely linear in the shorter chain region (C_8-C_{14}). Hence the application of the peak height x retention time method of estimating peak area was not strictly valid for these fatty acids, but since they represented only a small proportion of the total in most cases, the results were accepted.

To assess the characteristics of the GLC system, mixtures of fatty acid methyl esters of known composition were used. NIH type mixtures KA and KB (Horning, et al., 1964) obtained from Applied Science Laboratories were used to determine the overload limits of the

apparatus. The quantities of unknown methyl esters analysed in the studies reported in the following chapters were well within the overload limits of the GLC system. In addition NIH type mixtures KC and KD and a complex methyl ester mixture prepared in this laboratory covering the range C_8-C_{22} and including some unsaturated components were used to assess the accuracy and repeatability of GLC. Comparison of the repeated analyses of the NIH mixtures with the stated compositions gave relative errors of not more than 9.4% for KD and of not more than 7.0% for components in KC of carbon number 14 and greater. The repeated analysis of the complex mixture prepared in this laboratory gave coefficients of variation for minor components (10% of the total mixture) of not more than 7.4% and for major components (10% of the total mixture) of not more than 2.1%.

Gerson, Shorland and McIntosh (1966) reported the loss of methyl esters of polyunsaturated fatty acids during GLC. To ascertain losses under the present conditions, a mixture containing the methyl esters of stearic (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.), linolenic and arichidonic (Applied Science Laboratories) acids were prepared by weighing the pure (99%) esters, and aliquots of the mixture were injected into the DEGS column used for the studies of Chapter 3. By comparison with the methyl stearate peak, it was shown

that 19% of methyl linolenate and 50% of methyl arachidonate were lost. These losses were shown to be repeatable over an extended period and appropriate correction factors have been applied to the results reported in Chapters 3 and 4. Unsaturated fatty acids containing more than four double bonds were probably completely lost.

The fatty acid methyl esters from samples analysed in the present studies were tentatively identified in the following manner:

1) The retention times (relative to methyl palmitate) of the unknowns were compared with a semilog plot of relative retention time versus carbon number prepared using mixtures of known standard esters.

2) The behaviour of the methyl esters on several columns was compared (cf. Fig. 4 and 5). It may be seen that the branched chain and unsaturated components moved in different positions on the various columns.

3) The composition before and after removal of the unsaturated fatty acids by bromination was compared (Fig. 5).

4) The fatty acid patterns obtained were compared with published results for ruminant fats (Magidman, Herb, Barford and Riemenschneider, 1962; Hansen, 1966a).

During analysis of biological samples under isothermal conditions, methyl esters of C_{10}-C_{24} fatty acids could be detected and estimated and C8 esters could be

detected but not estimated. Temperature programming allowed the detection and estimation of fatty acid butyl esters from C_4 upwards. The analyses were not extended beyond C_{24}.

Isolation of Lipoproteins.

Centrifugation was carried out in a Beckman model L preparative ultracentrifuge using the SW 41 swinging bucket rotor.

Chylomicrons were separated by overlaying samples of lymph (10 ml. per tube) with 2 ml. 0.9% (w/v) saline and centrifuging at 17,000 rpm for 30 min. (cf. Nestel, et al., 1962). The contents of the tube below the saline layer were removed by puncturing the bottom of the tube.

The lipoprotein fractions of lymph and blood serum were separated by a method modified from that of Wurm and Straus (1968). An 8 ml. aliquot was layered over 2 ml. 54% (w/v) sucrose (d = 1.20) and the tube carefully filled with 2 ml. 0.9% (w/v) saline (d = 1.005) to form a top layer. Centrifugation was carried out at 2.4×10^6 g. hr. (usually 12 hr. at 41,000 rpm). A light scattering lipoprotein floated to the top, usually occurring as a dense band in the upper half of the saline layer (see Fig. 11). The region between the saline and sucrose layers was slightly pigmented and a strongly pigmented band was present in the region of the sucrose

interface. Accordingly, three fractions were collected by puncturing the bottom of the tube. Fraction 1 (ca. 2 ml.) contained the sucrose layer and the pigmented band, fraction 2 (ca. 8 ml.) the infranatant, and fraction 3 (ca. 2 ml.) the cloudy lipoprotein in the saline layer.

CHAPTER 3.

FATTY ACID COMPOSITION OF LIPID IN THE THORACIC DUCT LYMPH OF GRAZING COWS.

INTRODUCTION

Although the fatty acids of pasture lipids are highly unsaturated, the lipid entering the ruminant small intestine contains mainly saturated fatty acids. This is due to extensive hydrogenation in the rumen (cf. Bath and Hill, 1967). In accordance with these findings, the fatty acids of the triglyceride in thoracic duct and intestinal lymph of sheep have been reported to be considerably more saturated than those in the diet (Feliński, et al., 1964; Heath, et al., 1964).

Lipid absorption in the cow has recently been studied in this laboratory using a technique which allowed the periodic collection of lymph from the thoracic duct (Hartmann and Lascelles, 1966). The results indicated that for the lactating cow grazing pasture, at least 400 g. esterified fatty acid was transported daily in the lymph. The present experiment extends the previous findings by analysing the fatty acid composition of lipid in the thoracic duct lymph of cows grazing pasture.

METHODS

Experimental Protocol.

The samples of plasma and lymph for fatty acid analyses were taken from three cows used in previous experiments (Hartmann and Lascelles, 1966). Cow 1 (a three-year-old Ayrshire cross weighing 420 Kg.) was dry. Cow 2 (a five-year-old Jersey weighing 390 Kg.) was in the second month of lactation, and Cow 3 (a four-year-old Guernsey weighing 418 Kg.) was in the sixth month of lactation. Cows 2 and 3 were producing an average of 11.4 and 9.1 l. milk/24 hr., respectively. Lymph and blood were collected from thoracic duct and jugular vein cannulas. The cows grazed a good quality pasture of paspalum, ryegrass, and white clover, and each cow was given an additional 2 Kg. of a concentrate mixture (high energy) together with 1.5 Kg. lucerne chaff each day. Cows 2 and 3 were milked twice daily by machine.

Analytical.

Lipid was extracted from lymph and plasma samples with chloroform-methanol (2:1, v/v) and separated into the major lipid classes by TLC. Lipid fractions for analysis by GLC were obtained by elution from thin-layer plates. Lipid samples were stored free of solvent under nitrogen at -15°C until analyzed. Methyl esters of fatty acids were prepared by dialkyl carbonate-induced trans-esterification.

RESULTS

Comparison of samples of fatty acid methyl esters using three different columns during GLC enabled most of the fatty acids to be identified (Fig. 4 and 5). Columns containing EGA as stationary phase gave the most complete resolution. Using DEGS columns, several composite peaks were observed which contained C_{12}-C_{17} monounsaturated together with branched chain, saturated fatty acids (upper part of Fig. 5). Bromination of a lymph triglyceride sample from Cow 3 (lower part of Fig. 5) indicated that the branched chain acids formed the largest part of these peaks except for the peak designated 17:1, 18:Bri, in which 17:1 was the largest component (cf. EGA column, Fig. 4).

Some samples of lymph triglyceride from Cows 1 and 3 were also analysed on open-tubular (capillary) columns by Dr. R. G. Ackman of the Fisheries Research Board, Nova Scotia, Canada, in connection with another study (Ackman and Hooper, 1969). One of the chromatograms is illustrated in Fig. 6. The other samples showed similar patterns.

The presence of the following homologous groups of fatty acids in the triglyceride of thoracic duct lymph from cows is consistent with the results of the analysis done in this study.

FIGURE 4.

Comparison of the analysis of methyl esters from lymph triglyceride (Cow 3) using EGA or APL stationary phases during gas chromatography.

GLC conditions: EGA - 15% EGA on 80-100 mesh Chromosorb W packed in 1.524 m. x 0.54 cm. (OD) stainless steel columns and operated at 170°C.

APL - 10% APL on Celite 545 packed in 1.524 m. x 0.54 cm. (OD) glass columns and temperature programmed from 140 to 250°C at 3 C°/min.

Figure 4

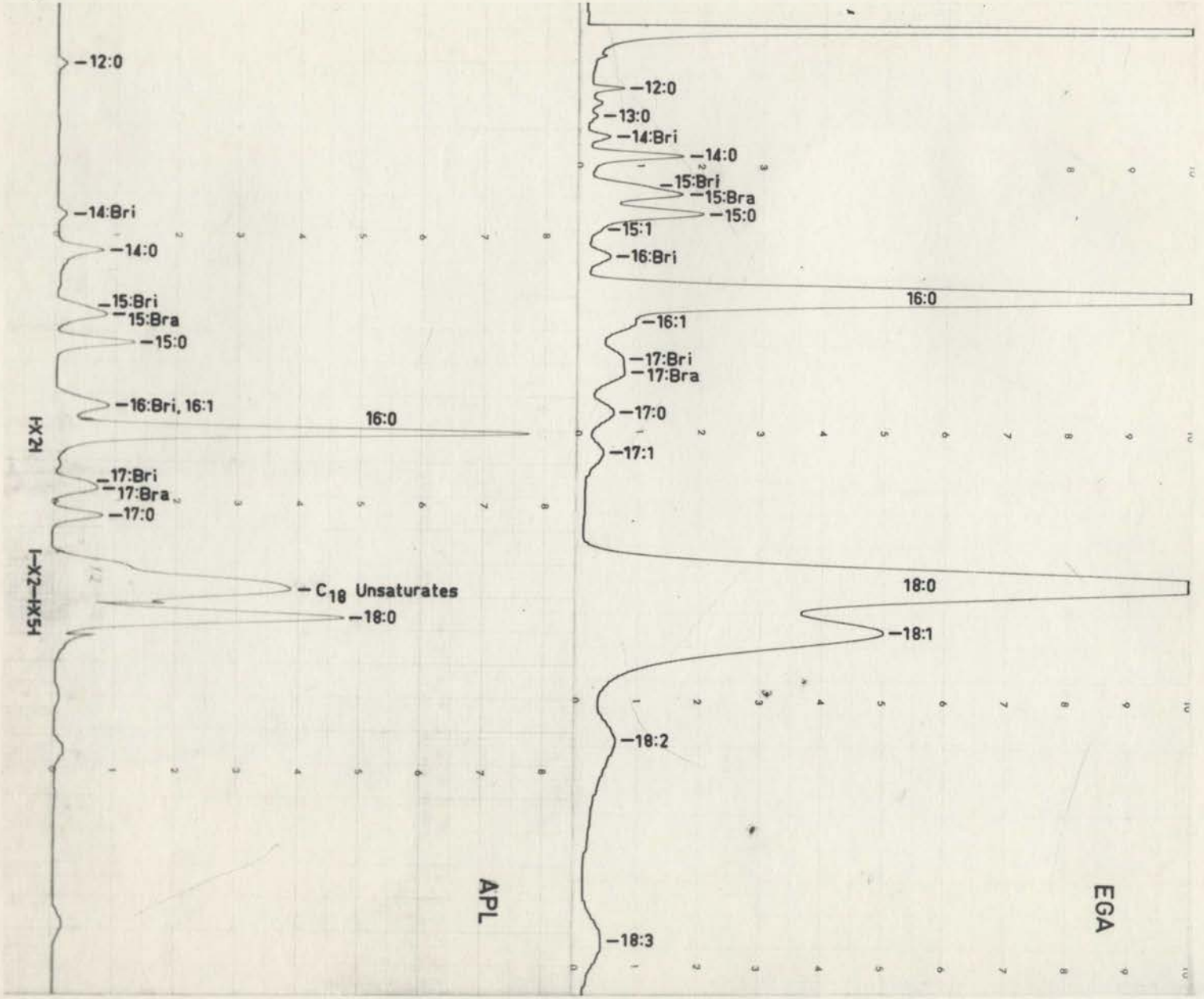

12:0
14:Bri
14:0
15:Bri
15:Bra
15:0
16:Bri, 16:1
16:0
17:Bri
17:Bra
17:0
C18 Unsaturates
18:0
HX2
I-X2-HXH
APL
12:0
13:0
14:Bri
14:0
15:Bri
15:Bra
15:0
15:1
16:Bri
16:0
16:1
17:Bri
17:Bra
17:0
17:1
18:0
18:1
18:2
18:3
EGA

FIGURE 5.

Gas chromatographic analysis of methyl esters from a sample of lymph triglyceride (Cow 3) before and after bromination.

GLC conditions: 12% DEGS on 80-100 mesh Gas Chrom P packed in 1.524 m. x 0.54 cm. (OD) stainless steel columns and operated at 180°C.

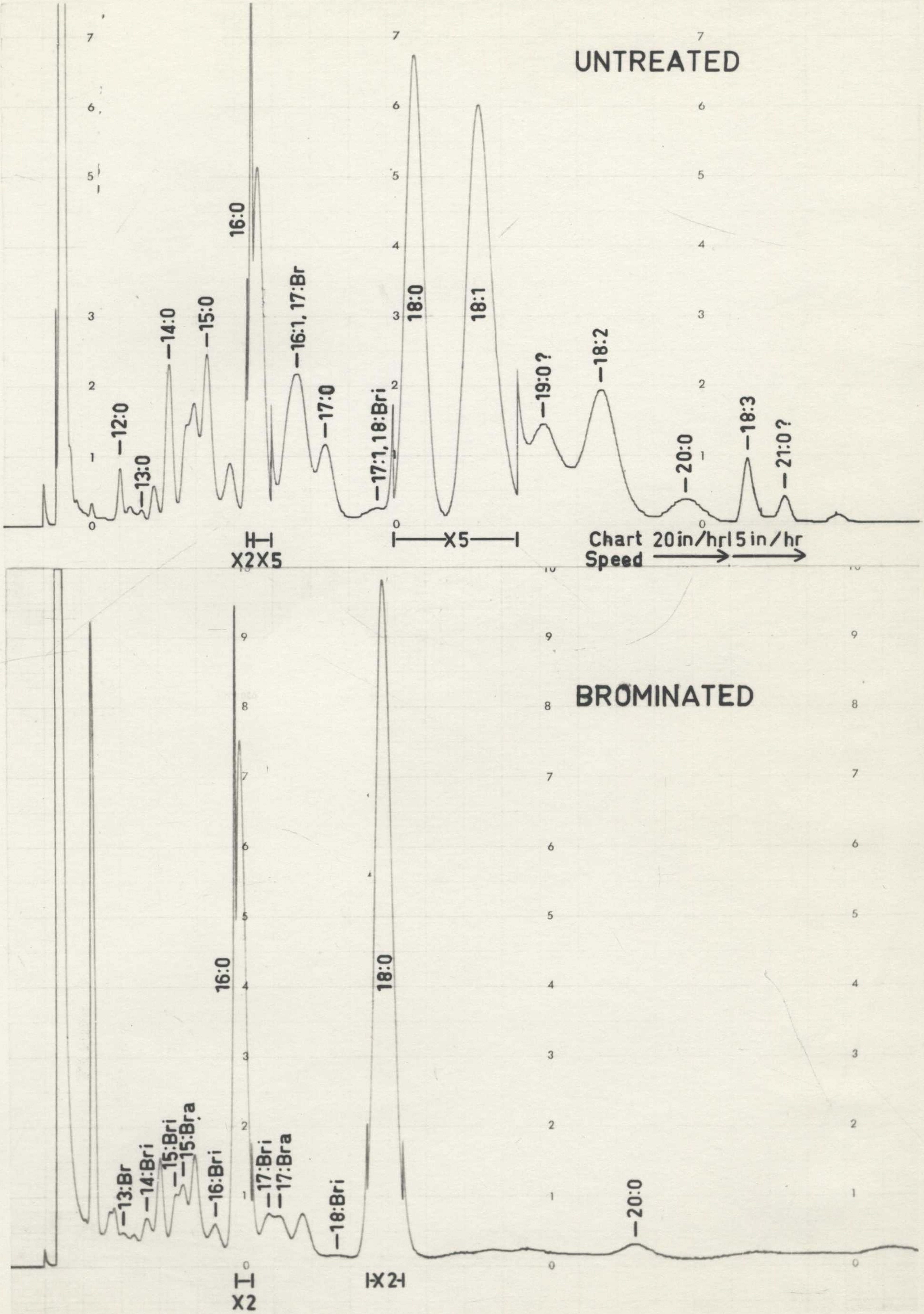

UNTREATED
12:0
13:0
14:0
15:0
16:0
16:1, 17:Br
17:0
17:1, 18:Bri
18:0
18:1
19:0 ?
18:2
20:0
18:3
21:0 ?
X2 X5
X5
Chart Speed 20 in/hr 5 in/hr
BROMINATED
13:Br
14:Bri
15:Bri
15:Bra
16:Bri
16:0
17:Bri
17:Bra
18:Bri
18:0
20:0
X2
X2

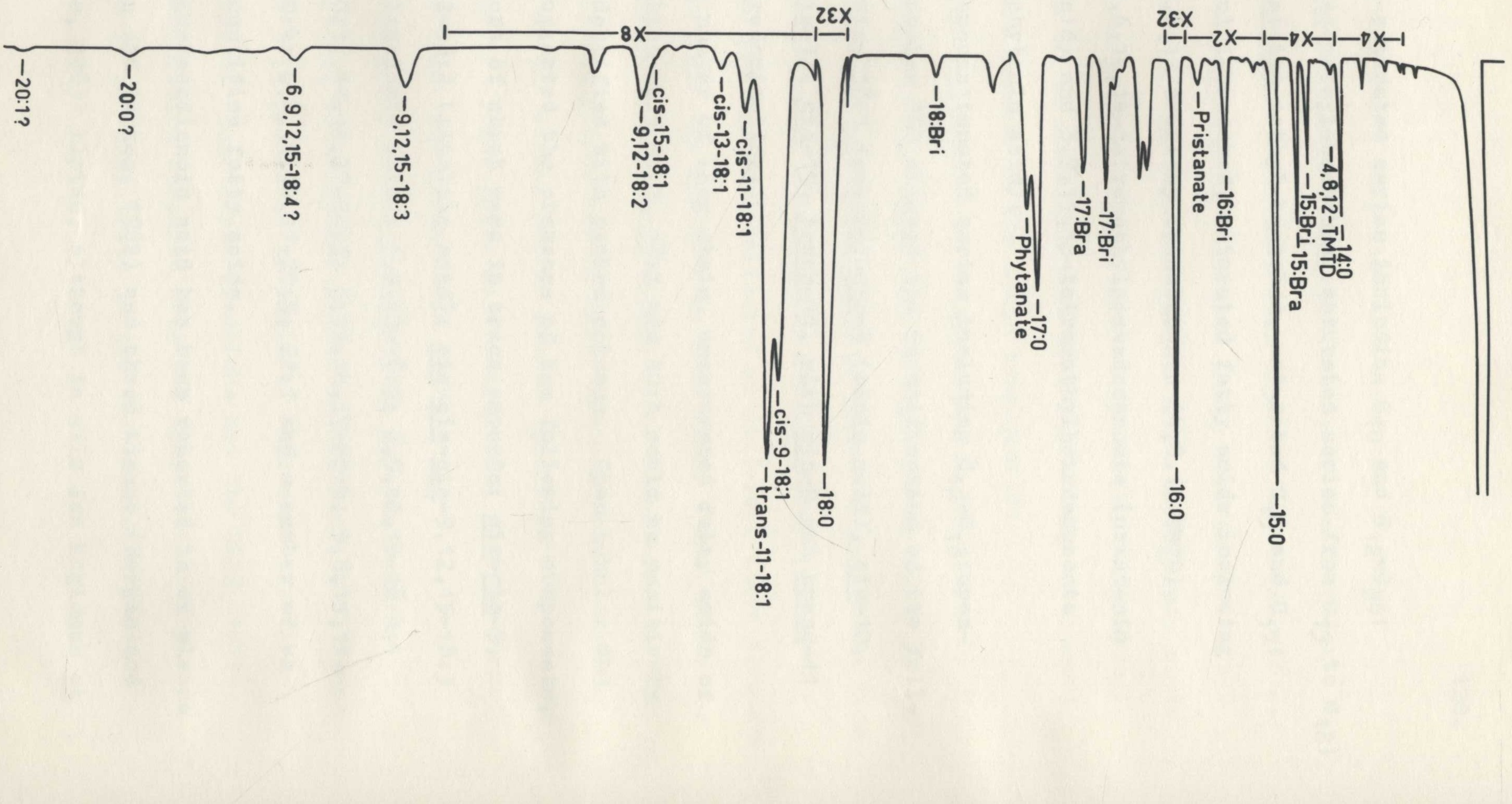
Figure 5

FIGURE 6.

Analysis of methyl esters from lymph triglyceride (Cow 3) on open tubular columns.

GLC conditions: 150 ft. x 0.01" (ID) column coated with butanediol succinate polyester and operated at 170° and 50 psig heluim (Perkin-Elmer 900 gas chromatograph).

Figure 6


X4
X4
X2
X32
X32
14:0 TMTD
15:Bri
15:Bra
16:Bri
Pristanate
17:Bri
17:Bra
Phytanate
18:Bri
X32
X8
cis-11-18:1
cis-13-18:1
cis-15-18:1
9,12-18:2
9,12,15-18:3
6,9,12,15-18:4 ?
20:0 ?
20:1 ?
15:0
16:0
17:0
18:0
cis-9-18:1
trans-11-18:1

1) n-saturated series including C_{10} and C_{12}-C_{24};

2) iso-methyl-branched, saturated series from C_{13} to C_{18};

3) anteiso-methyl-branched, saturated C_{15} and C_{17};

4) multi-branched, saturated fatty acids including 4,8,12 trimethyltridecanoate (4,8,12-TMTD), 2,6,10,14-tetramethylpentadecanoate (pristanic acid) and 3,7,11,15-tetramethylhexadecanoate (phytanic acid);

5) monounsaturated series including C_{12}-C_{18}:open-tubular GLC allowed the identification of the following 18:1 isomers:-cis-9 (oleic acid), cis-12, cis-13, cis-15, trans-11, with cis-9 and trans-11 predominating;

6) a number of long chain, unsaturated fatty acids of which only 18:2, 18:3 and 20:4 could be positively identified with packed columns. Open-tubular GLC suggested the presence of the following components, most of which were in trace amounts: cis-cis-9, 12-18:2 (linoleic acid); cis-cis-cis-9,12,15-18:3 (linolenic acid); 6,9,12-18:3; 6,9,12,15-18:4; 20:1; 11,14,17-20:3; 8,11,14,17-20:4; 5,8,11,14-20:4; 5,8,11,14,17-20:5; 22:1 and a number of unidentified fatty acids.

Hexadecadienoic acid has been reported in ox plasma (Duncan and Garton, 1962) and sheep tissue (Horgan and Masters, 1963) lipids, although in milk fat Magidman et

al. (1962) could only find trace amounts of diene using ultra-violet spectrophotometry of the fraction from GLC which would have been expected to contain 16:2. Under the conditions described here, 16:2 should have emerged with the peak designated 17:1, 18:Bri and, since this peak comprised only about 0.2% of the total methyl esters, it is concluded that 16:2 could only be present in trace amounts in the lymph triglyceride.

The composition of triglyceride in the thoracic duct lymph of the three cows is reported in Table 3, together with the composition of plasma triglyceride from one of the cows. The results for the three cows were derived from samples collected at different times throughout the day. It may be seen from Table 3 that there was no consistent variation within cows. Also noted is the significant proportion of fatty acids having a branched chain or odd carbon number (Heath, et al., 1964) and the similarity of the fatty acid composition of triglyceride in the lymph and plasma from Cow 3.

The fatty acid composition of phospholipid and cholesterol ester in the plasma and thoracic duct lymph of Cow 3 is presented in Table 4. The most striking features of these lipid fractions are the high degree of unsaturation compared with the lymph triglyceride (Table 3) and similarity to the corresponding lipid

TABLE 3. FATTY ACID COMPOSITION OF TRIGLYCERIDE FROM PLASMA AND THORACIC DUCT LYMPH OF COWS GRAZING ON PASTURE

Results (weight percentage of total fatty acid methyl esters) are expressed as means, and ranges are shown in brackets.

[a] Determined as described by Woodford and van Gent.

[b] Cow 1 was dry; Cows 2 and 3 were lactating. Two, four, and two samples were collected from Cows 1, 2, and 3, respectively, at different times throughout the day.

[c] Plasma from one sample of jugular vein blood.

[d] This fraction consisted of small proportions of 10:0, 13:0, 12:1 + 13:Br *iso* in lymph and plasma and two unidentified components of carbon-number 21.5 and 21.8 in plasma.

TABLE 3

Fatty acid	Carbon-number[a]	Lymph						Plasma[c] Cow 3
		Cow 1[b]		Cow 2		Cow 3		
12:0	12:0	0.1	(0.1-0.2)	0.3	(0.3-0.4)	0.2	.....	0.4
13:1, 14:Br iso	13.5	0.2	.....	0.3	(0.2-0.3)	0.3	(0.3-0.4)	0.2
14:0	14.0	1.1	.....	1.4	(1.2-1.6)	1.2	(1.1-1.3)	2.5
14:1, 15:Br iso, 15:Br anteiso	14.7	0.7	.....	1.7	(1.5-1.9)	1.5	.....	1.8
15:0	15.0	1.9	.....	2.2	(2.0-2.4)	2.0	(2.0-2.1)	2.1
15:1, 16:Br iso	15.5	0.3	.....	0.9	(0.8-0.9)	0.8	(0.8-0.9)	...
16:0	16.0	21.1	(19.9-22.2)	22.2	(21.0-23.5)	22.3	(21.2-23.4)	24.4
16:1, 17:Br iso, 17:Br anteiso	16.6	2.1	(1.9-2.3)	3.0	(2.9-3.1)	2.2	(2.0-2.3)	2.8
17:0	17.0	1.2	(1.0-1.3)	1.3	(1.1-1.3)	1.6	(1.5-1.6)	1.9
17:1, 18:Br iso	17.6	0.2	.....	0.2	(0.2-0.3)	0.2	(0.2-0.3)	0.2
18:0	18.0	45.8	(45.6-45.9)	37.7	(36.2-38.8)	42.9	(41.1-44.6)	42.6
18:1	18.6	17.2	(16.2-18.2)	19.9	(19.3-20.7)	14.8	(14.5-15.2)	12.0
19:0	19.1	1.0	.....	1.2	(1.1-1.2)	1.2	(1.2-1.3)	1.6
18:2	19.5	2.1	(2.0-2.2)	2.2	(2.0-2.3)	2.5	(2.2-2.7)	2.1
20:0	20.0	0.7	(0.6-0.7)	0.5	(0.5-0.6)	0.7	(0.7-0.8)	0.7
18:3	20.6	2.8	(2.6-3.0)	3.5	(3.4-3.6)	3.0	(2.8-3.1)	1.4
21:0	21.0	0.3	(0.2-0.3)	0.3	(0.2-0.5)	0.3	(0.2-0.3)	0.3
22:0	22.0	0.4	(0.4-0.5)	0.4	.....	0.6	.....	0.6
20:4	22.6	...	.....	0.2	.....	0.3	.....	...
23:0	23.0	0.3	.....	0.5	(0.4-0.5)	0.5	.....	0.6
24:0	24.0	0.4	(Trace-0.4)	0.3	(0-0.5	0.5	(0.4-0.5)	0.6
Remainder[d]	...	0.1	.....	0.2	.....	0.2	.....	1.2

TABLE 4. FATTY ACID COMPOSITION OF THE PHOSPHOLIPID AND CHOLESTEROL ESTER IN PLASMA AND THORACIC DUCT LYMPH FROM A LACTATING COW GRAZING PASTURE (COW 3)

Results represent weight percentage of total fatty acid methyl esters.

[a] Determined as described by Woodford and van Gent.

[b] Plasma from one sample of jugular vein blood.

[c] This fraction consisted of several long chain components, probably polyunsaturated, together with small proportions of 8:0-11:0, 13:0, 21:0, 12:1 + 13:Br *iso*, and 13:1 + 14:Br *iso*. Not all of these components were detected in every sample.

TABLE 4

Fatty acid	Carbon-number[a]	Phospholipid		Cholesterol ester	
		Plasma[b]	Lymph	Plasma	Lymph
12:0	12.0	0.1	Trace	0.1	0.5
14:0	14.0	0.2	0.2	0.9	2.0
14:1, 15:Br iso 15:Br anteiso	14.7	0.3	0.4	1.1	1.4
15:0	15.0	0.4	0.7	0.7	1.1
15:1, 16:Br iso	15.5	0.2	0.3	0.6	1.0
16:0	16.0	15.2	16.7	4.1	8.3
16:1, 17:Br iso 17:Br anteiso	16.6	2.0	1.6	2.4	4.4
17:0	17.0	1.9	1.4	0.3	1.1
17:1, 18:Br iso	17.6	0.3	0.3	0.6	3.4
18:0	18.0	31.3	28.3	3.6	14.8
18:1	18.6	14.0	12.5	4.7	8.6
19:0	19.1	0.5	0.4	Trace	Trace
18:2	19.5	24.9	23.9	53.5	24.2
20:0	20.0	...	0.2	1.0	1.0
18:3	20.5	4.6	7.9	22.2	15.6
22:0	22.0	1.5	1.5	0.3	Trace
20:4	22.5	2.6	2.8	1.8	6.5
Remainder[c]	...	0.1	1.2	2.2	6.6

FIGURE 7.

Methyl esters of FFA from duplicate extractions (A and B) of lipid from a single lymph sample.

The difference in the proportions of the C_{18} unsaturates relative to 18:0 may be accounted for by varying contributions of fatty acid from the hydrolysis of phospholipid during preparation.

GLC conditions: 12% DEGS on 80-100 mesh Gas Chrom P packed in 1.524 m. x 0.54 cm. (OD) stainless steel columns and operated at 180°C.

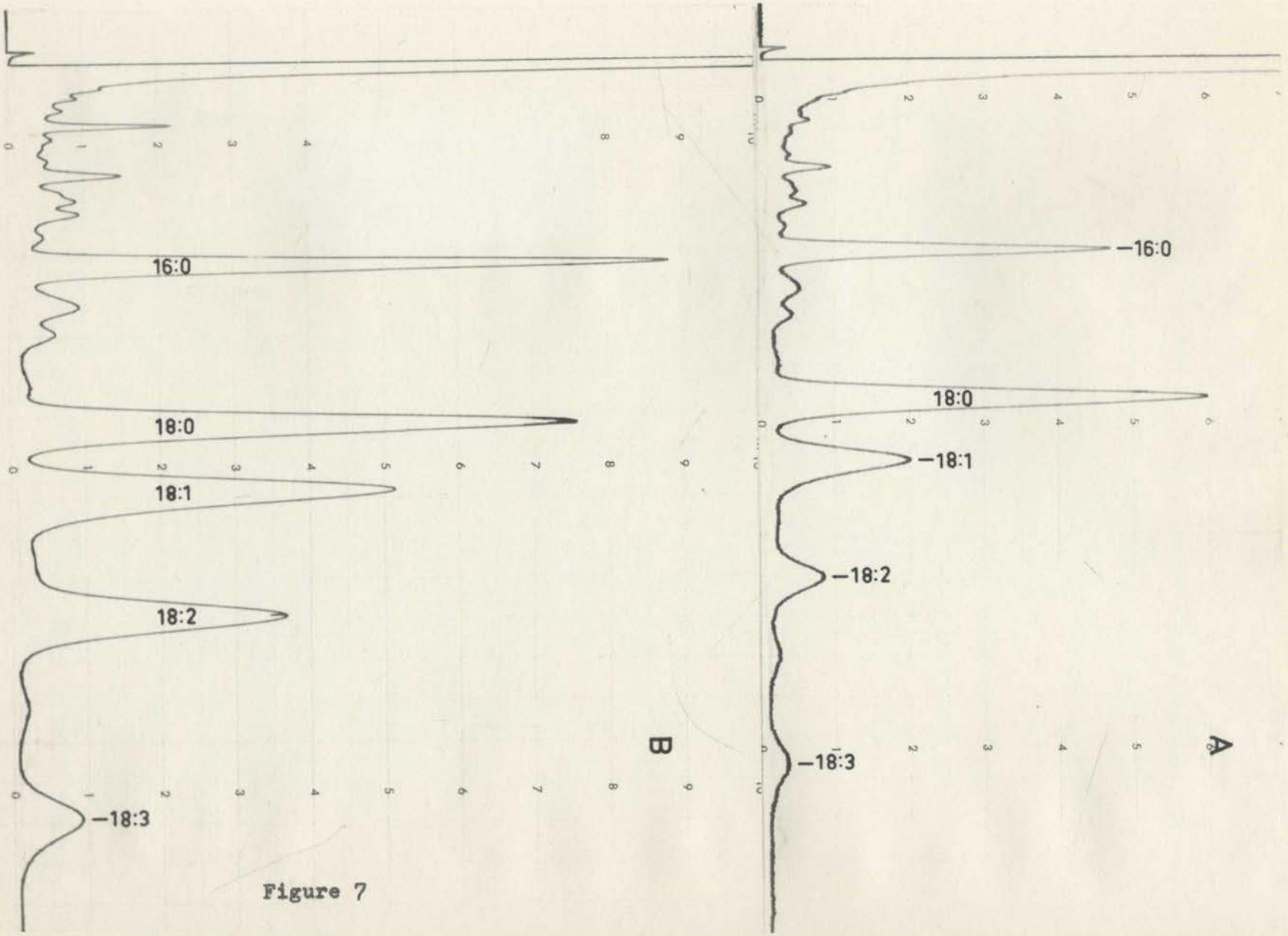


Figure 7

fractions of plasma.

The FFA fraction eluted from thin layer plates was contaminated to a variable extent with phospholipid fatty acids (see Fig. 7). Therefore, the composition of FFA is not reported. It was evident, however, that the lymph FFA contained a much smaller proportion of 18:2 and 18:3 than the phospholipid (Duncan and Garton, 1962).

DISCUSSION

The identification of fatty acid methyl esters from the results of GLC alone must be treated with caution (Horning, et al., 1964). Thus, peaks usually identified as unsaturated or branched chain monocarboxylic esters could be contaminated with acetals from the esterification of aldehydes, or diesters of dicarboxylic acids (Karmen, 1967). In this connection, bovine rumen lipids have been reported to contain significant concentrations of aldehydogenic material (Katz and Keeney, 1964). The occurrence of methoxystearic acid artifacts derived from hydroxystearic acid during the rigorous esterification of sheep perinephric fat has also been reported (Hansen, 1966b). In addition, cyclic fatty acids may be present in plant lipid (Hilditch and Williams, 1964). It would appear that any of these fatty acids may be present in

one or more of the methyl ester peaks observed but, if present, they would significantly contaminate only the minor components. It was felt that the use of three different stationary phases during GLC minimised the risk of confusion in identifying fatty acids in the present study. Although the three cows were of different age, breed, and stage of lactation, there was little difference between them in the fatty acid composition of the triglyceride in lymph (Table 3).

Another study of fatty acid patterns in lymph lipid from cows has recently been reported (Leat and Hall, 1968). These workers found less 18:0 and more 18:1 in triglyceride from thoracic duct and intestinal lymph than reported here (Table 3) but the lymph samples used were collected under anaesthesia and the interpretation of the results is, therefore, complicated by the effects of operative trauma and anaesthesia (see Chapter 8 for a discussion of this point).

The fatty acid composition of triglyceride in thoracic duct lymph from cows (Table 3) is similar to that reported for the intestinal lymph of sheep (Felíński, et al., 1964; Heath, et al., 1964). The saturated nature of lymph triglyceride is consistent with the extensive hydrogenation of the highly unsaturated pasture lipid which has been shown to be accomplished by rumen microorganisms

(Garton, 1964). Erwin, et al. (1963) reported that abomasal administration of safflower oil in the sheep resulted in a higher digestibility of crude fat than did ruminal administration. They postulated that hydrogenation of a proportion of the unsaturated C_{18} fatty acids to stearic acid reduced the digestibility of fat leaving the rumen. This hypothesis originated from the observation that the digestibility of stearic acid is very low when fed as the sole source of fat in the diet of rats (Carroll, 1958; Carroll and Richards, 1958). However, the similarity of the proportion of stearic acid in the lymph triglyceride of pasture-fed cows (Table 3) to that in its major precursor, the lipid of rumen digesta (Table 2), suggests that this fatty acid is as efficiently absorbed as the others. Recent work on the effect of dietary content and site of administration of unsaturated fatty acids on the digestibility of fat in sheep (Little and Mitchell, 1965; Phillips and Roberts, 1966; Clarke and Roberts, 1967) has supported this suggestion.

The predominance of cis-9-18:1 and trans-11-18:1 amongst the 18:1 isomers of lymph triglyceride (Fig. 6) is consistent with the results of Katz and Keeney (1966) for lipids from bovine rumen digesta. Trans-11-18:1 is a known product of the hydrogenation of linoleic acid by a rumen bacterium (B. fibrisolvens; Kepler, et al., 1966).

Phytanic acid has been reported in total rumen contents, rumen bacteria, blood serum, adipose tissue and milk fat from cows (Lough, 1964; Avigan, 1966; Hansen, 1966a; Patton and Benson, 1966). Pristanic acid has also been isolated from most of these sources (Hansen and Morrison, 1964; Avigan, 1966) with the notable exception of rumen bacteria (Hansen, 1966a). Adipose tissue from sheep contains 4,8,12-TMTD (Hansen, 1968) although, as far as is known, this fatty acid has not previously been reported in bovine lipids. It has been shown that the major pathway for the degradation of phytanic acid is an initial α-oxidation yielding pristanic acid followed by successive β-oxidations to yield lower branched chain homologues, prominent amongst which is 4,8,12-TMTD (Mize, et al., 1969). The presence of both pristanate and 4,8,12-TMTD in thoracic duct lymph (Fig. 6) strongly suggests that the intestinal mucosa of the cow oxidizes phytanic acid absorbed from the gut lumen.

The fatty acid composition reported for triglyceride (Table 3), cholesterol ester, and phospholipid (Table 4) in plasma is comparable to that found by other workers (Evans, Patton and McCarthy, 1961; Duncan and Garton, 1962; Brown and Stull, 1966). It may be seen by comparing the data in Tables 3 and 4, that the fatty acid composition of lipid fractions in lymph resembled the fatty acid composition of corresponding fractions in plasma.

This was especially evident for phospholipid present in higher concentration in the lymph than cholesterol ester (Hartmann and Lascelles, 1966). One interpretation of the resemblance of lymph and plasma phospholipid would be that most of the phospholipid in lymph originated from the plasma. However, the findings of Hartmann and Lascelles (1966) indicated that considerable quantities of lymph phospholipid are derived from sources other than the capillary filtrate. Thus, the similarity in the fatty acid composition of phospholipid in lymph and plasma probably arose as a result of selective incorporation of certain of the absorbed fatty acids into the phospholipid of lymph.

Cholesterol ester in the lymph can be quantitatively accounted for by filtration from plasma (Hartmann and Lascelles, 1966). On this basis, the fatty acid composition of the cholesterol ester in lymph would be expected to resemble closely that in plasma. Nevertheless, while the fatty acid patterns of lymph and plasma cholesterol ester were qualitatively similar, they differed quantitatively (Table 4). It may be concluded that not all the cholesterol ester in the thoracic duct lymph of this cow was derived from the capillary filtrate. It is suggested that the differences in composition were due to dilution of plasma cholesterol ester with cholesterol ester derived from the esterification of absorbed fatty acids within the intestinal epithelium (Karmen, et al., 1963).

CHAPTER 4.

EFFECT OF FEEDING SAFFLOWER OIL ON THE COMPOSITION OF ABSORBED FATTY ACID IN GRAZING COWS

INTRODUCTION

Attention was drawn in Chapter 3 to the fatty acid composition of the triglyceride in thoracic duct lymph from pasture fed cows, which reflected the very extensive hydrogenation of unsaturated fatty acids occurring in the rumen.

The output of lipid in thoracic duct lymph has been shown to rise substantially several hours after feeding a single dose of safflower oil to lactating cows, and to return to normal slowly over the following 2-3 days (Hartmann, et al., 1966).

It is the objective of this investigation to report changes in the degree of unsaturation in the fatty acids of the lymph triglyceride in response to oil feeding.

METHODS

Experimental Protocol.

Samples of lymph for fatty acid analysis were obtained from a previous experiment in which two lactating cows (a Jersey and a Guernsey) were used (Hartmann, et al., 1966). The Jersey cow (a five-year-old, weighing 390 Kg.) was in the second month of lactation and pro-

ducing 11.4 l. milk/24 hr. The Guernsey cow (a four-year-old, weighing 418 Kg.) was in the sixth month of lactation and producing 9.1 l. milk/24 hr. The cows grazed on good quality pasture of paspalum, ryegrass and white clover and each was given an additional 2 Kg. of a concentrate mixture (high energy) together with 1.5 Kg. lucerne chaff each day. Safflower oil (commercial grade) was administered orally to each cow in a single dose and samples of thoracic duct lymph were collected from a catheter at intervals thereafter. The Jersey cow received 380 g. and the Guernsey cow 480 g. oil. Both cows were milked twice daily by machine.

Analytical.

Lipid was extracted from lymph samples with chloroform-methanol (2:1 v/v) and separated into the major lipid classes by TLC. Lipid fractions for analysis by GLC were obtained by elution from thin-layer plates. Lipid samples were stored free of solvent under nitrogen at -15°C until analyzed. Methyl esters of fatty acids were prepared by dialkyl carbonate-induced transesterification. Reference samples of conjugated diene for GLC were obtained by alkali isomerization of safflower oil (Brice, et al., 1952).

RESULTS

The changes in the fatty acid composition of tri-

glyceride in lymph from the Guernsey cow after oil feeding are shown in Table 5. It may be seen that a single, oral dose of safflower oil has a prolonged and marked effect on the composition of the triglyceride in thoracic duct lymph. It is interesting to note that the absorbed fatty acid contained very little 18:2 in comparison to the safflower oil which contained 79% linoleic acid. Oil feeding produced an increase in the proportion of the fatty acids designated 18:1, 18:2, 18:3, 19:0 and 21:0 and a decrease in the proportion of all other fatty acids. Analysis of a number of triglyceride samples from the Jersey cow gave similar results. The sum of the proportions of the methyl esters of the C_{18} fatty acids from lymph triglyceride increased in response to oil feeding from 63 to 75% for the Guernsey cow and from 65 to 79% for the Jersey cow. This is consistent with the influx into the rumen of C_{18} fatty acids which comprise the bulk of safflower oil.

The output of each fatty acid in the triglyceride of thoracic duct lymph was computed and expressed as a percentage of the corresponding output immediately before oil feeding. In both cows there was a striking increase in the relative output of the fatty acids designated 18:1, 18:2, 18:3, 19:0 and 21:0 following oil feeding. The changes observed in one of the cows are shown in Fig. 8.

TABLE 5. EFFECT OF OIL FEEDING ON THE FATTY ACID COMPOSITION OF TRIGLYCERIDE IN THORACIC DUCT LYMPH FROM THE GUERNSEY COW

[a] Only those components which comprised 1% or more of the total are reported, with the exception of 21:0.

[b] In addition, the safflower oil contained small proportions of 12:0, 14:Br, 20:0 and 22:0, together amounting to 0.7% of the total methyl esters.

[c] 480 g. of safflower oil was administered orally at zero hr.

[d] Results are expressed as weight percentage of total methyl esters.

[e] Br iso, Br anteiso refer to the iso- and anteiso-isomers, respectively of methyl-branched, saturated fatty acids.

TABLE 5

Fatty acid[a]	Saf-flower oil[b]	Time after oil feeding[c] (hr.)										
		-10	0	2	4	7	10	14	19	35	54	105
14:0	0.2[d]	1.3	1.1	1.1	1.2	0.8	0.7	0.8	0.9	1.2	1.5	1.1
14:1, 15:Br iso,[e] 15:Br anteiso	...	1.5	1.5	1.4	1.6	0.9	0.7	0.7	0.9	1.4	1.9	1.5
15:0	...	2.1	2.0	1.9	2.0	1.3	1.1	1.1	1.3	1.7	2.1	1.8
16:0	6.3	23.4	21.2	20.1	20.5	16.1	14.7	15.0	16.4	17.0	19.3	21.1
16:1, 17:Br iso, 17:Br anteiso	0.1	2.3	2.0	2.1	2.1	1.5	1.4	1.5	1.8	2.0	2.7	1.8
17:0	0.1	1.6	1.5	1.6	1.5	1.1	0.8	0.8	0.9	1.1	1.5	1.4
18:0	2.4	41.1	44.6	46.2	39.2	38.0	32.4	34.1	37.3	34.9	34.6	46.9
18:1	11.6	15.2	14.5	14.6	16.2	22.9	33.1	35.8	30.6	30.1	23.8	13.8
18:2	77.9	2.7	2.2	2.1	5.9	8.4	6.9	2.8	2.4	2.9	3.4	2.5
18:3	0.8	3.1	2.8	2.6	3.9	3.6	3.0	2.4	2.3	3.0	4.0	2.8
19:0	...	1.2	1.3	1.4	1.3	1.4	1.6	1.9	1.8	1.6	1.6	1.3
21:0	...	0.2	0.3	0.3	0.3	0.5	0.8	0.9	0.7	0.5	0.3	0.3

FIGURE 8.

Output of several fatty acids in the triglyceride of thoracic duct lymph from the Guernsey cow before and after oil feeding.

Outputs are expressed as a percentage of the output immediately before oil feeding. Arrows indicate the time at which safflower oil (480 g.) was fed.

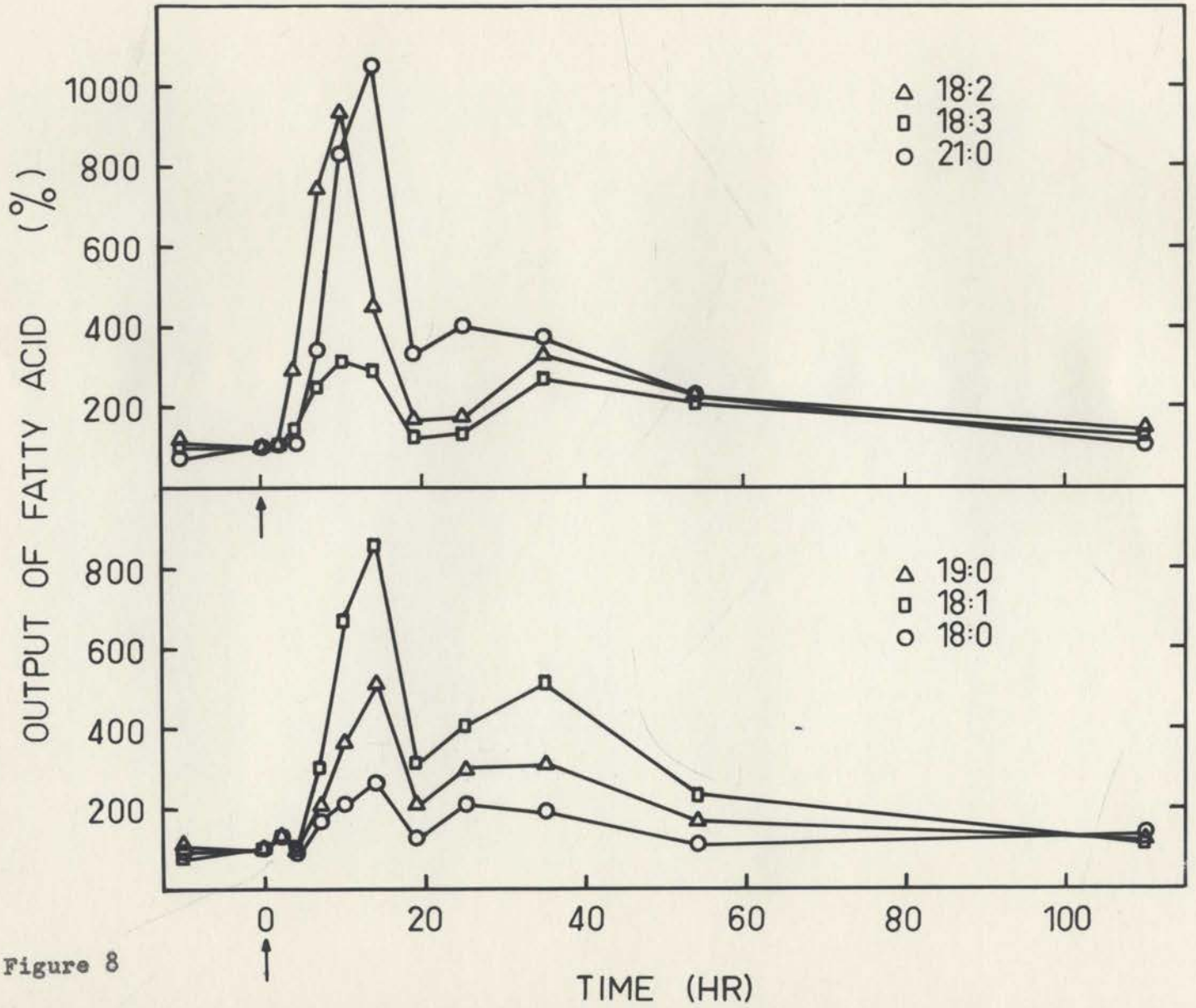

OUTPUT OF FATTY ACID (%)
1000
800
600
400
200
18:2
18:3
21:0
800
600
400
200
19:0
18:1
18:0
0
20
40
60
80
100
TIME (HR)

Figure 8

There was a similar increase in the relative output of the same fatty acids for the Jersey cow. The relative output of the remaining components showed no consistent variation from prefeeding levels when the data from both cows were compared. There was a consistent increase in the relative output of 18:0 after oil feeding (Fig. 8) but this was small and equivocal when compared with the range of variation observed for the other components. The increase in relative output of the fatty acids designated 18:1, 19:0 and 21:0 was large, the output of each reaching a peak about 14 hr. after oil feeding. The peak output of 18:2 and 18:3 occurred somewhat earlier at approximately 10 hr. after oil feeding. When considering the significance of the changes in output of 19:0 and 21:0 it should be borne in mind that these fatty acids formed only a small percentage of the total fatty acids in the lymph triglyceride (Table 5). However, the computed change in relative output for each fatty acid was large and these results were confirmed for the Jersey cow in which 21:0 formed a greater proportion of the total methyl esters, rising from 0.5% before to 1.3% after oil feeding.

Of the other lipid fractions in lymph, only phospholipid was present in sufficient quantities to allow an examination of the effect of oil feeding on fatty acid composition. The composition of phospholipid samples

obtained from the Guernsey cow is reported in Table 6. The proportion of 18:0 and 18:3 decreased and that of 18:2 increased in response to oil feeding, whereas that of 18:1 did not change.

DISCUSSION

Since the output of most of the fatty acids of lymph triglyceride did not change after oil feeding, it is reasonable to assume that those fatty acids whose output did increase after oil feeding were derived from the oil fed. It is known that 18:1 is an intermediate in the hydrogenation by rumen microorganisms of linoleic acid (Fig. 2) which is the predominant fatty acid of safflower oil (Table 5). It is suggested that the time-course of absorption of 18:1 illustrated in Fig. 8 is in accord with the production of 18:1 by hydrogenation of the linoleic acid fed in safflower oil. The fact that there was a large increase in the output of 18:1 in response to feeding safflower oil indicated that hydrogenation of oil in the rumen had been incomplete. The extent of hydrogenation may be related to the amount of unsaturated fatty acid being presented to the rumen microorganisms. Thus, the *in vitro* experiments of Shorland, *et al.* (1957), Polan, *et al.* (1964), and Ward, *et al.* (1964) demonstrated that small amounts of linoleic acid incubated with rumen contents were almost completely converted to stearic acid

TABLE 6. EFFECT OF OIL FEEDING ON THE FATTY ACID COMPOSITION OF PHOSPHOLIPID IN THORACIC DUCT LYMPH FROM THE GUERNSEY COW

[a] Only those components which comprised 1% or more of the total are reported.

[b] 480 g. of safflower oil was administered orally at 0 hr.

[c] Results are expressed as weight percentage of total methyl esters.

[d] Br iso, Br anteiso refer to the iso- and anteiso-isomers, respectively, of methyl-branched, saturated fatty acids.

TABLE 6

Fatty acid[a]	Time after oil feeding[b] (hr.)		
	-10	7	14
14:0	0.2[c]	1.5	0.5
15:0	0.7	1.0	0.8
16:0	16.7	17.6	17.5
16:1, 17:Br *iso*,[d] 17:Br *anteiso*	1.6	1.4	0.8
17:0	1.4	1.3	1.5
18:0	28.2	23.3	23.1
18:1	12.5	12.3	12.7
18:2	23.9	24.3	31.9
18:3	7.9	5.7	4.5
20:4	2.8	3.8	2.5
22:0	1.5	1.1	0.9

whereas hydrogenation was much less complete with larger amounts. That safflower oil was administered in a single, large dose in my experiments probably accounts for the large increase in unsaturation of lymph fatty acids in response to oil feeding.

The output of 18:2 reached a peak which was relatively greater and occurred 4 hr. earlier than that of 18:1 but was, in absolute terms, much smaller. It is suggested that the early rise in the output of 18:2 may have been due to some escape of undigested oil from the rumen, associated with the feeding of a large amount of the oil in one dose.

The marked increase, in response to oil feeding, of the relative output of fatty acids designated 18:3, 19:0 and 21:0 (Fig. 8) was unexpected because safflower oil contains neither 19:0 nor 21:0 and only small proportions of 18:3 (Table 5). Comparison of the increase in the output of these fatty acids with the increase in the output of 18:1 suggests that these fatty acids are involved as intermediates or products in the hydrogenation of linoleic acid in the bovine rumen. However, none of these fatty acids have hitherto been implicated in pathways of hydrogenation of linoleic acid in the rumen. It was, therefore, considered that the preliminary identification of these peaks as 18:3, 19:0 and 21:0 (see Fig. 5) may not have been correct.

A component behaving similarly on GLC to that designated here as 19:0 was reported by Kuzdzal-Savoie, et al. (1966) in a study of the effect of feeding linseed oil to cows on the fatty acid composition of butter. This peak only appeared in response to oil feeding. These authors noted that the further a double bond is from the carboxyl carbon, the longer is the retention time on polar GLC stationary phases. They provisionally identified this new component as trans-16-octadecenoic acid. It is known that significant quantities of octadecenoic acids having the double bond near the methyl end of the carbon chain occur in the rumen (Katz and Keeney, 1966) and that they are also involved as intermediates in the hydrogenation of linoleic acid by rumen contents (Ward, et al., 1964). Thus, it is suggested that the component designated 19:0 in Table 1 consists largely of octadecenoic acid having the double bond near the methyl end of the carbon chain. The response of this component to oil feeding is consistent with its being an intermediate in the hydrogenation of linoleic acid by bovine rumen contents.

Since conjugated dienes are known to be involved in the pathway of hydrogenation of linoleic acid by rumen microorganisms (Czerkawski and Blaxter, 1965; Kepler, et al., 1966; Shorland, et al., 1957) and are present in bovine milk fat (Herb, Magidman, Luddy and

Riemenschneider, 1962; Kuzdzal-Savoie, et al., 1966) and plasma triglyceride from sheep (Garton and Duncan, 1964) it was considered that the peaks designated here as 18:3 and 21:0 may have contained significant quantities of conjugated 18:2. In order to confirm this point, safflower oil was alkali-isomerized and the products were analyzed by GLC under the same conditions as the lymph lipids. The cis, trans and trans-trans conjugated isomers of linoleic acid had carbon numbers (Woodford and van Gent, 1960) of 20.7 and 21.3, respectively, compared with carbon numbers of 20.6 and 21.0 (see Chapter 3) for 18:3 and 21:0 respectively. It is therefore suggested that the gas chromatographic behaviour and response to oil feeding of 18:3 and 21:0 are consistent with these peaks containing significant proportions of cis, trans and trans-trans conjugated 18:2, respectively.

The fatty acids in the lymph contribute to the circulating pool of fatty acid from which the mammary gland derives long chain fatty acid for milk fat synthesis (Glascock, Duncombe and Reinius, 1956; Lascelles, Hardwick, Linzell and Mepham, 1964). Thus changes in the degree of unsaturation of the fatty acid in lymph following the feeding of safflower oil would be expected to be reflected in the composition of milk fat. This is borne out by the many reports of marked changes in the

composition of milk fat when cows are fed large quantities of unsaturated vegetable oils. Of particular pertinence to this experiment are two studies in which safflower oil was fed daily to lactating cows in similar amounts to those used here (Parry, Sampugna and Jensen, 1964; Smith, Dairiki, Dunkley and Renning, 1966). It was reported that there were marked increases in the proportions of the C_{18} unsaturated fatty acids in milk fat, consistent with the changes in the fatty acid composition of lymph reported in this publication.

The changes in the fatty acid composition of the lymph phospholipid in response to oil feeding (Table 6) were not as marked as those seen in the lymph triglyceride (Table 5). This suggests that a situation is present in the cow similar to that reported for the rat in which the resemblance between the fatty acid composition of chylomicron phospholipid and the test meal is much less than that between chylomicron triglyceride and the test meal (Goodman, 1965).

CHAPTER 5.

THE EFFECT OF THE PERIODIC FEEDING OF LINSEED OIL ON THE PRODUCTION AND IODINE VALUE OF MILK FAT

INTRODUCTION

The poor spreadability of refrigerated butter is largely due to the highly saturated nature of its component fatty acids. Indeed, spreadability of butter is closely correlated with its iodine value (Hill and Palmer, 1938; Dixon, 1964).

Recent studies in this laboratory on the absorption of fat by grazing cows have shown, however, that the feeding of a single, large dose of vegetable oil produces an increase in the amount and degree of unsaturation of absorbed fat and this increase is prolonged over several days (Hartmann, et al., 1966; Chapter 4). These observations, coupled with the fact that the mammary gland can utilize chylomicron triglyceride either directly or indirectly for the synthesis of milk fat (Lascelles, Hardwick, Linzell and Mepham, 1964) suggests that a significant softening of butter may be achieved by feeding a large, single dose of oil to dairy cows.

That oil feeding increases the degree of unsaturation of milk fat is well known, but previous experiments have usually involved daily administration of 200 g. or

more of oil (see, for example, Brown, Dustman and Weakley, 1941; Parry, et al., 1964). At current prices this would not be economic. However, consistent with my observations on thoracic duct lymph, Aylwood, Blackwood and Smith (1937) reported that the feeding of a single dose (300 g.) of linseed oil to a cow gave rise to an increase in the iodine value of the milk fat which reached a maximum of 7 units above pre-feeding levels and was prolonged for more than a week.

This prompted investigation of the possibility of obtaining a commercially significant rise in the iodine value of milk fat by dosing lactating cows with a highly unsaturated vegetable oil at less frequent intervals than have previously been used. Accordingly, a trial was instigated to assess the effect on the iodine value of milk fat of periodic administration of approximately 450 g. linseed oil to dairy cows.

METHODS

Animals.

Three cows from the Dairy Research Unit herd were used. Details of their age, breed and stage of lactation at the commencement of the experiment, together with the results of the monthly herd recording before the experiment are given in Table 7.

TABLE 7. DETAILS OF THE ANIMALS AT THE BEGINNING OF THE EXPERIMENT.

Cow	Breed	Age	No. of calvings	Date of commence-ment	Stage of lactation, week	Milk production, lb./day	Fat %
1	Ayrshire	2 years 9 months	2	27. x. 67	11	29.2	2.9
2	Friesian	8 years 1 month	6	2. i. 68	15	40.4	3.9
3	Guernsey*	5 years 11 months	4	5. i. 68	10	42.6	4.3

* Cross bred

The cows grazed a good quality pasture of rye grass and clover. Cow 1 received no supplements whereas Cows 2 and 3 received 10 and 12.5 lb. of dairy pellets (15% protein), respectively, each day during the first 2 sampling periods but no supplements during the last period. The cows were milked with the herd twice daily by machine.

Experimental Protocol.

The experiment followed a similar design to that used by Shannon and Lascelles (1969b), consisting of 3 collection periods. Oil was administered during the middle period and the other 2 periods served as controls. Time was allowed between periods for the animals to adjust to the change in diet.

Samples of milk were taken at each milking for 6 days (period 1). The cows were then dosed with 450 g. linseed oil (Meggit Pty. Ltd., Parramatta, N.S.W., Australia) at 6-day intervals. Beginning immediately after the third dose of oil, samples were collected for a further 6 days (period 2). The cows were thus on the oil feeding regime for 12 days before the start of period 2. No more oil was given and 12 days were allowed after the completion of period 2 before commencing a final 6-day collection (period 3).

The milk from each milking was weighed. It was then thoroughly mixed and a ca. 50 ml. sample taken and stored

at 4°C in the presence of a few drops of formalin. Twice weekly the samples were warmed and stirred thoroughly, and the morning and evening milks from each day were mixed in proportion to yield. A sample of each mixture was then taken for lipid extraction.

Analytical.

Total fat in the milk was determined by the Babcock method. Lipid was extracted with 2:1 (v/v) chloroform-methanol and iodine values were determined in duplicate on 20-60 mg. lipid by the Hanus method.

RESULTS

Mean values for milk and fat production, fat percentage and iodine value of milk fat for each cow and each period are presented in Table 8. The iodine value rose by an average of 5 units during oil feeding and returned to pre-feeding levels after the withdrawal of oil. By contrast, the other parameters either declined throughout the experiment (milk and fat production) or rose during the last period (fat percentage).

An analysis of variance of the results was carried out and is summarized in Table 9. The experimental design allowed the comparison of the 3 periods as though they were separate treatments. Since there was one oil feeding and 2 control periods, partition of the 'treatments' source of variation using polynomial coefficients

TABLE 8. MILK AND FAT PRODUCTION, FAT PERCENTAGE AND IODINE VALUE OF MILK FAT FROM COWS RECEIVING 450 g. OF LINSEED OIL AT 6 DAY INTERVALS

The experiment consisted of 3 collection periods, each of 6 days' duration, with 12 days between periods. Three doses of oil (6 days apart) were given between periods 1 and 2, the last dose being given immediately before the start of period 2. Values represent the means of daily determinations.

TABLE 8

	Period	Cow 1	Cow 2	Cow 3	Mean
Milk production, lb./day	1	26.3	41.8	42.0	36.7
	2	24.0	39.3	40.8	34.7
	3	21.5	29.9	32.1	27.8
Fat production, lb./day	1	0.87	1.47	1.55	1.30
	2	0.77	1.28	1.49	1.18
	3	0.77	1.14	1.23	1.05
Fat, %	1	3.3	3.6	3.7	3.5
	2	3.2	3.2	3.6	3.4
	3	3.6	3.8	3.8	3.8
Iodine value	1	36.8	36.9	33.3	35.6
	2	41.7	42.9	38.4	41.0
	3	37.4	36.9	35.0	36.4

TABLE 9. SUMMARY OF THE ANALYSIS OF VARIANCE OF MILK AND FAT PRODUCTION, FAT PERCENTAGE AND IODINE VALUE OF MILK FAT FROM 3 COWS FED 450 g. OF LINSEED OIL AT 6 DAY INTERVALS.

The experiment consisted of 3 collection periods ('treatments'), each of 6 days' duration, with 12 days between periods. Three doses of oil (6 days apart) were given between periods 1 and 2, the last dose being given immediately before the start of period 2. Periods 1 and 3 served as controls.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

To calculate the variance ratio for the 'treatments' term and components thereof, the mean squares for the 'treatments x cows' interaction and its corresponding components have been used as the denominator, while the mean square for 'treatments x cows x times' has been used as the denominator in the calculation of the variance ratios for the remaining sources of variation.

TABLE 9

Source of variation	Degrees of freedom	Mean squares			
		Milk production	Fat production	Fat %	Iodine value
Cows	2	1050.3***	1.937***	0.55	62.3***
Treatments	2	332.8*	0.281*	0.78*	150.9**
Linear	1	562.5	0.560	0.64*	5.4
Quadratic	1	103.1	0.001	0.93	296.3*
Treatments x cows	4	33.1*	0.039	0.06*	2.8
Linear x cows	2	39.9*	0.053	0.015*	2.0
Quadratic x cows	2	26.3	0.025	0.10	3.5
Times	5	19.1	0.021	0.102	2.3
Treatments x times	10	12.6	0.046	0.364	14.4**
Times x cows	10	7.9	0.047	0.282	5.8
Treatments x cows x times	20	11.4	0.058	0.357	3.3

allowed differentiation of responses to oil feeding (quadratic effects) from lactational trends (linear effects).

There were significant treatments effects for all parameters. However, the quadratic component of the treatments terms for milk and fat production and fat percentage was not significant. While the linear component was not significant for milk or fat production, it accounted for 85 and 99.9% respectively, of the variation attributable to treatments effects. It may thus be seen that the significant changes in the yield and content of fat and production of milk were due to differences between the 2 control periods rather than to any effect of oil feeding. Further information on this point was obtained when milk yield curves for the present and previous lactations were compared. All 3 cows were in declining lactation during the experiment. No anomalies attributable to oil feeding were observed, although the yields during periods 1 and 2 were slightly above the comparable values of the previous lactation. It is concluded that the differences between the control periods may be largely accounted for by normal decline in lactation.

On the other hand, the significant ($P < 0.01$) treatments term for iodine value of milk fat was almost entirely accounted for by a significant ($P < 0.05$) quadratic component (Table 9), indicating that the

increase of 5 units during period 2 (Table 8) was due to the feeding of linseed oil. It is interesting that the treatments term accounted for 42% of the total variation for iodine value whereas it only contributed 8-19% of the total variation for milk and fat production and fat percentage.

There was also a significant ($P < 0.01$) 'treatments x times' interaction for iodine value of milk fat (Table 9). The changes in daily iodine values are illustrated in Fig. 9, from which it is evident that the significant treatments x times term was due to a different pattern of change during the oil feeding period compared with the control periods. Although there was an apparent change in iodine value during period 1, separate analyses of variance of the results for the 3 periods (using polynomial coefficients) showed that there were no significant changes in iodine value during periods 1 and 3 whereas there was a significant ($P < 0.001$) linear decrease in iodine value during period 3.

DISCUSSION

The average increase of 5 units in the iodine value of milk fat evoked by oil feeding (Table 8) is comparable to the range of seasonal variation in iodine value observed in butters from Australia and New Zealand (Mitchell, 1959; Dixon, 1964). The present observations (Fig. 9)

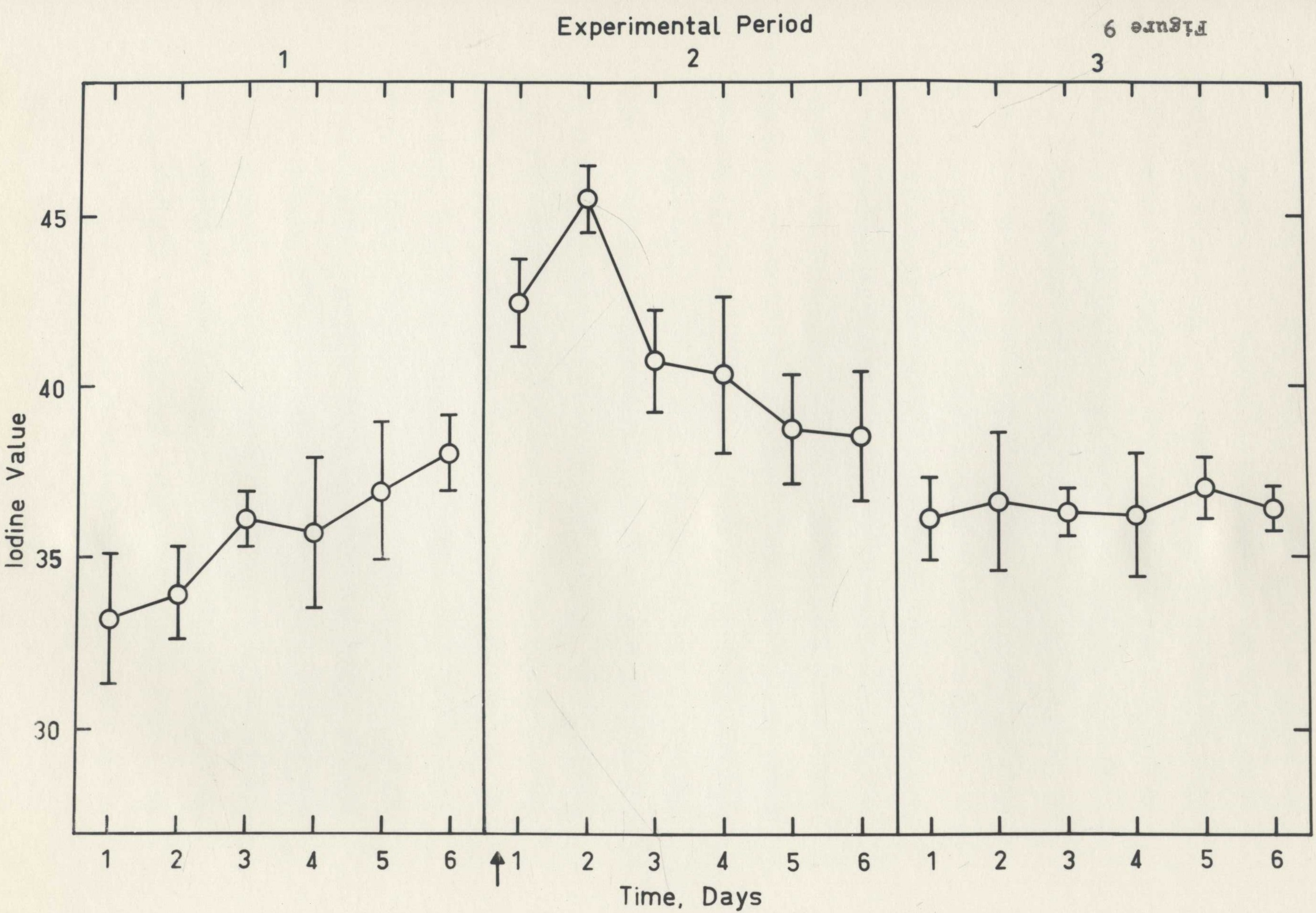
FIGURE 9.

Iodine value of milk fat from cows fed 450 g. linseed oil at 6 day intervals.

The experiment consisted of 3 collection periods, each of 6 days' duration, with 12 days between periods. Three doses of oil (6 days apart) were given between periods 1 and 2. The last dose of oil is indicated by the arrow.

Values plotted represent means ± S.E. for the results from 3 cows.

Figure 9


Experimental Period
1
2
3
Iodine Value
45
40
35
30
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
Time, Days

are in agreement with previous studies on the transfer of single doses of fat into milk (Aylwood, et al., 1937; Glascock, McWeeny and Smith, 1958; Glascock, et al., 1966) which reported a rapid incorporation of the labelled fatty acid given orally, followed by a slow decrease in the specific activity of the milk fat over a period of several days. The principal factors responsible for these effects would appear to be (i) that large doses of unsaturated oils exceed the capacity of the rumen microorganisms to hydrogenate lipid (Moore, Hoffman and Berry, 1945; Chapter 4) and (ii) that ingested lipid is released only slowly from the rumen (cf. Lascelles, et al., 1966).

Extrapolation of these results to practical situations must be approached with care for a number of reasons. First, it has been found that growing steers fed liberal allowances of oil daily over prolonged periods show no increase in the unsaturation of the body fat (Thomas, Culbertson and Beard, 1934; Willey, et al., 1952). This suggests that the rumen microflora may adapt to long-term feeding of unsaturated oils, resulting in increased efficiency of hydrogenation. No data appear to be available on the long-term effects of oil feeding on the iodine value of milk fat. However, oil has been fed daily at high levels for periods up to 6 weeks with no evidence of decline in iodine value (Allen, 1935; Brown, et al.,

1941; Parry, et al., 1964). Furthermore, seasonal variation in iodine value is probably due to dietary factors (McDowall and McGillivray, 1963) which does not support the idea of adaption to highly unsaturated diets. Nevertheless, this possibility cannot be excluded.

Secondly, the effect of fat supplementation on the gross composition and production of milk by dairy cows is not well understood, despite the voluminous literature on the subject (see, for example, Van Soest, 1963). Indeed, Storry, Rook and Hall (1967) concluded from their review of the literature that the effect of feeding tallow and vegetable oils was variable depending on the experimental conditions employed. However, there is evidence to support the view that, at relatively low levels of lipid intake, increases in dietary fat exert a positive effect on the secretion of milk and milk fat, whereas at high levels of fat supplementation the fat content of milk is depressed and the yield of milk and fat may be decreased (Orth and Kaufmann, 1957; Larsen, Klausen and Frederiksen, 1966; Storry, Hall and Johnson, 1968; Dr. A. K. Lascelles, private communication). In the present experiment, the feeding of linseed oil had no effect on the yield and content of fat or production of milk (Tables 8 and 9) in agreement with the observations of Hartmann, et al. (1966) who fed

similar amounts of safflower oil. It would appear, therefore, that the infrequent feeding of large doses of oil avoids the possible detrimental effects of continuous supplementation with fat at high levels.

Finally, the effect of stage of lactation on response to oil feeding is not clear, but since the contribution of blood lipid to milk fat synthesis appear to vary with stage of lactation (Decaen and Adda, 1966; Stull, Brown, Valdez and Tucker, 1966) this may be an important factor. In this connexion it should be pointed out that the 3 cows in this experiment were all in a similar phase of lactation (Table 7).

CHAPTER 6.

THE FATTY ACID COMPOSITION OF LYMPH LIPIDS AND THE FEEDING OF MILK REPLACER TO PRE-RUMINANT CALVES.

INTRODUCTION

The young, milk-fed calf, unlike the adult bovine, derives most of its energy from dietary fat. Previous studies from this laboratory have shown that most of this lipid is absorbed by way of the lymph (Shannon and Lascelles, 1967). The opportunity was taken to use samples from these studies to compare the fatty acid composition of lymph lipid with that of the dietary fat.

In addition, the previous studies in young calves were extended to examine the efficiency of absorption of a mixture of animal and vegetable fats included in a commercial milk replacer with that of fat in fresh, whole milk. Particular attention was paid to the relative efficiency of absorption of the different fatty acids in the diet.

METHODS

Animals.

Samples were obtained from calves used in previous studies (Shannon and Lascelles, 1967, 1968) in which recirculating lymphatico-venous shunts were established in either the thoracic or intestinal lymph ducts. The

calves were Friesians of either sex and surgery was carried out in the first week of life. Fresh whole milk (4.54 l.) was fed either in one or two equal feeds daily. In addition, for studies using milk replacer, thoracic duct-venous shunts were established in four bull calves of mixed breeds (Friesian, Guernsey and Jersey cross-breeds). These calves weighed an average of 32.0 Kg. at the time of operation and their care and management was as previously described (Shannon and Lascelles, 1967).

The milk replacer ("Denkavit", Permewan Wright Ltd., Abbotsford, Victoria) was prepared from skimmed milk powder, butter milk powder and an homogenised fat mixture which was stated to contain coconut oil, lecithin, mutton tallow, beef dripping and lard, together with vitamin and mineral supplements.

Analytical.

Determinations were made of TEFA, phospholipid, FFA, total protein and albumen in lymph and blood plasma. Fat in milk was determined by the Babcock method. Lipids were extracted with chloroform-methanol (2:1, v/v) and separated by TLC. Total lipid samples were also separated into neutral and phospho-lipid on small columns of silicic acid and methyl esters of fatty acids were prepared by dimethyl carbonate-induced transesterification preparatory to GLC. Methyl esters prepared in this way from the neutral lipid of lymph and blood plasma represent

triglyceride fatty acids since lymph and plasma contain little partial glyceride and dimethyl carbonate-induced transesterification does not esterify cholesterol ester or FFA. Butyl esters were prepared using dibutyl carbonate. A typical separation of butyl esters prepared in this way is shown in Fig. 10.

EXPERIMENTAL PROTOCOL AND RESULTS

Diurnal Variations in Fat Absorption.

In an earlier study (Shannon and Lascelles, 1967) calves with thoracic duct fistulae were used in a study of once daily feeding. It was found that there was a definite pattern of absorption on the once daily regime, lipid concentration and output in lymph falling after feeding to reach a minimum level at 2-6 hr., rising to attain a maximum value at 10 hr. and falling off thereafter to pre-feeding levels. Accordingly, 4 samples were taken from each of the 3 calves for fatty acid analysis. Samples were obtained immediately before feeding, during the periods of minimum and maximum neutral lipid outputs and at the end of the 24 hr. collection period. Triglyceride was separated from lymph lipid by TLC and submitted to GLC. One sample of lymph phospholipid (recovered from TLC) from each calf was also examined. In addition, lipid fractions were obtained from the blood plasma of a calf used in another study (Shannon and

FIGURE 10.

Gas chromatogram obtained from butyl esters of milk fat fed to calves.

GLC conditions: 10% EGSP-Z on 60-80 mesh Gas Chrom Q packed in 1.524 m. x 0.54 cm. (OD) stainless steel columns and temperature programmed from 70 to 200°C at 3C° /min.

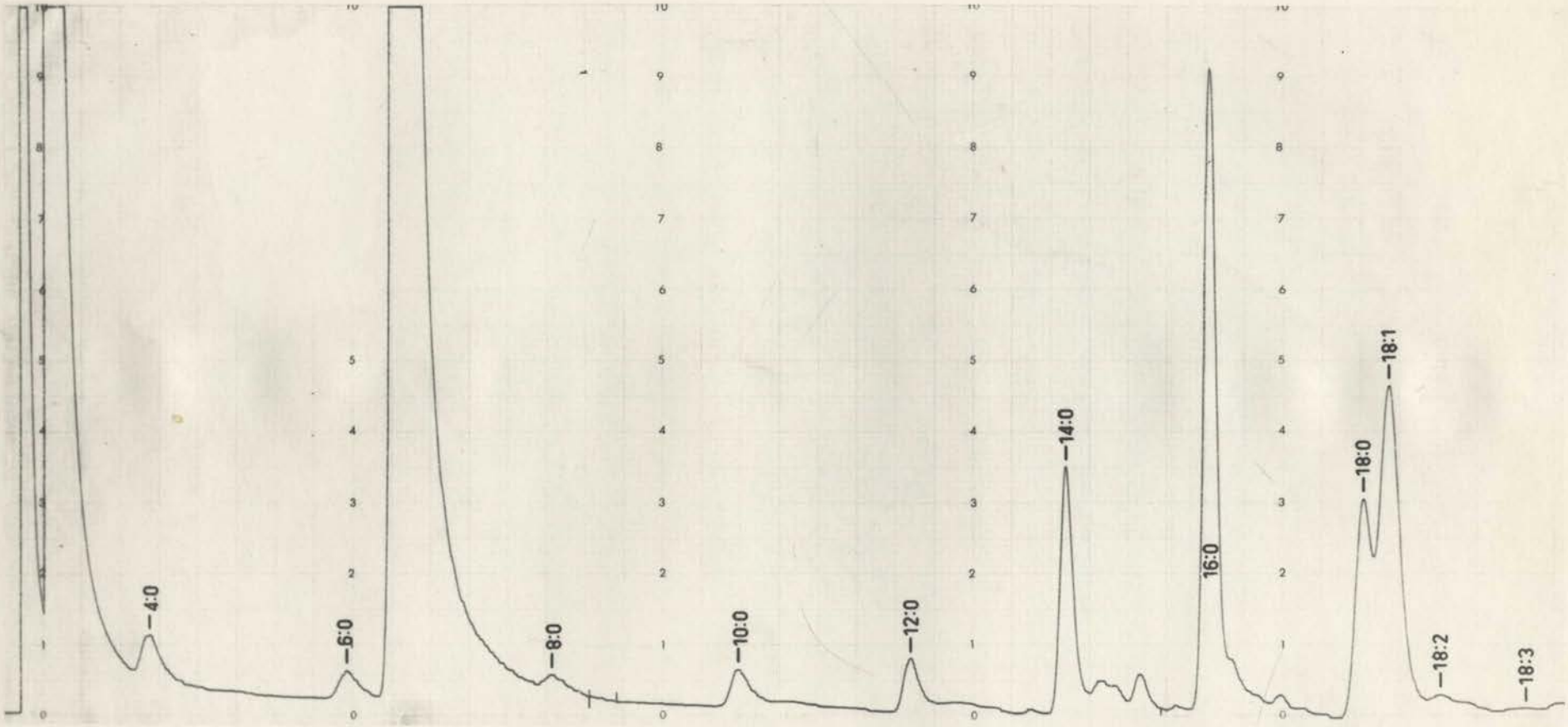


Figure 10

Lascelles, 1968) and analysed.

Analysis of EGA, APL and DEGS columns allowed the following homologous series of fatty acids to be tentatively identified (cf. Chapters 3 and 4) in lymph triglyceride: n-saturated C_8-C_{18} (including odd carbon numbers); iso-methyl-branched, saturated C_{13}-C_{18}; anteiso-methyl-branched, saturated C_{15} and C_{17}; monounsaturated C_{10}, C_{12}-C_{18}; and a number of long chain unsaturated components including 18:2, 18:3 and 20:4 and probably including 18:1 isomers with the double bond near the methyl terminus and 18:2 conjugated isomers. A similar range of fatty acids was tentatively identified in triglyceride from blood plasma, cholesterol ester from blood plasma and lymph and in lymph phospholipid. However, phospholipid from blood was totally deficient in fatty acids of chain length less than C_{12}, contained only small proportions of C_{15} and C_{17} odd carbon number and C_{15}-C_{18} branched chain fatty acids and only one minor, long chain component (18:1 with the double bond near the methyl terminus).

Data for the major components of lymph and plasma lipids are presented in Table 10. The results for lymph triglyceride were submitted to an analysis of variance. There were significant between-calf differences for all fatty acids except 18:0. However, there were no significant between-times differences, indicating that the

fatty acid composition of lymph triglyceride did not change throughout the day on the once daily feeding regime. Accordingly, means and standard errors only are given for lymph triglyceride in Table 10.

Diet and Lymph Lipid.

A comparison of the fatty acid composition of lymph triglyceride from 3 calves with that of the milk fed was made and the results are presented in Table 11. It may be seen that the composition of the lymph triglyceride closely reflected that of the diet, significant differences being small in magnitude.

Milk Replacer Feeding.

The experiment followed the same design as that used previously (Shannon and Lascelles, 1969b), consisting of an experimental period during which milk replacer was fed (230 g. dry powder plus warm water to 2.27 l. fed twice daily), preceded and followed by control periods during which fresh, whole milk (2.27 l.) was fed twice daily. Thus 3-4 days adjustment was allowed at each change of diet before commencing collection of samples. Samples were collected hourly for 12 hr. The average amount of lipid ingested over a 12 hr. period by the calves was 83.4 g., 51.0 g. and 109.2 g. for whole milk, Denkavit and whole milk feeding, respectively.

TABLE 10. FATTY ACID COMPOSITION OF LIPID FRACTIONS FROM THORACIC DUCT LYMPH AND BLOOD PLASMA OF WEEK-OLD CALVES FED ONCE DAILY (MEAN ± S.E.)

TABLE 10

Fatty Acid	Lymph			Blood Plasma		
	Tri-glyceride	Phospho-lipid	Cholesterol ester	Tri-glyceride	Phospho-lipid	Cholesterol ester
No. of Samples	12	4	1	1	1	1
8:0	0-0.5	0	0.1	0	0	0
10:0	1.5 ± 0.2	trace	1.2	1.1	0	0.1
12:0	2.7 ± 0.3	0.2 ± 0.1	2.4	2.3	trace	0.4
14:0	9.5 ± 0.6	2.0 ± 0.5	10.2	10.2	0.5	3.4
16:0	27.0 ± 1.3	18.2 ± 1.5	29.9	33.5	17.8	10.0
18:0	15.1 ± 0.7	25.2 ± 0.4	13.5	12.2	26.7	1.5
18:1	32.1 ± 1.7	34.4 ± 1.6	28.9	26.2	27.4	10.3
18:2	1.8 ± 0.1	10.4 ± 1.7	1.9	2.5	15.9	46.6
18:3	0.7 ± 0.3	1.7 ± 0.3	2.6	2.4	2.0	11.2
20:4	0 - 0.3	1.6 ± 0.1	0.4	0.7	4.7	6.3
Remainder	9.7 ± 0.4	6.5 ± 0.5	9.3	9.0	5.0	10.6

TABLE 11. COMPARISON OF THE FATTY ACID COMPOSITION OF LYMPH TRIGLYCERIDE AND MILK FAT FED TO WEEK OLD CALVES (MEAN ± S.E. FOR 3 CALVES).

* $P < .05$; ** $P < .01$

Significance of differences using Students *t* test

TABLE 11

Fatty Acid	Lymph Triglyceride		Milk Fat
No. of Samples	3		3
10:0	2.0 ± 0.3		2.1 ± 0.3
12:0	3.3 ± 0.5		2.6 ± 0.3
14:0	11.1 ± 1.2		9.4 ± 1.3
16:0	28.8 ± 0.3		26.8 ± 0.9
18:0	10.6 ± 0.3	**	13.2 ± 0.4
18:1	31.9 ± 2.3		31.6 ± 3.3
18:2	1.9 ± 0.2		3.0 ± 0.4
18:3	2.4 ± 0.1	*	1.8 ± 0.2
Remainder	8.2 ± 0.2	*	9.7 ± 0.4

Comparisons of the average lymph flow, concentration of total protein and output of neutral lipid (derived by subtracting values for phospholipid fatty acid from those for TEFA), phospholipid and FFA in thoracic duct lymph when the calves were alternately fed whole milk, Denkavit and whole milk, are presented in Table 12. It may be seen that, whereas total protein concentration changed little during milk replacer feeding, lymph flow and lipid output dropped markedly. Although not shown in Table 12, the protein output and neutral lipid concentration also dropped, as would be expected and the albumen:globulin ratio changed little. These changes were reversed upon returning to the whole milk diet. Analysis of variance (see Shannon and Lascelles, 1969b, Chapter 5) revealed significant quadratic components of the "between-treatments" terms for lymph flow, neutral lipid concentration and the outputs of neutral lipid, phospholipid and FFA, indicating that milk replacer feeding was responsible for the depression of these variates. There were no significant treatment effects for total protein concentration or albumen: globulin ratio in lymph. Since the plasma total protein concentration also remained constant in these calves, it follows that the lymph:plasma ratio for total protein concentration was unaltered by milk replacer feeding.

TABLE 12. LYMPH FLOW, TOTAL PROTEIN CONCENTRATION AND OUTPUT OF NEUTRAL LIPID, PHOSPHOLIPID AND FFA IN THORACIC DUCT LYMPH FROM 4 CALVES FED MILK REPLACER.

The values presented are means ± standard errors derived from a total of 52 samples collected in each of three 12 hr. periods when the calves were being fed whole milk, milk replacer and whole milk, respectively.

TABLE 12

	Whole Milk Feeding	Milk Replacer Feeding	Whole Milk Feeding
Lymph flow (ml./hr.)	387.3 ± 12.1	274.6 ± 6.8	373.8 ± 7.4
Total protein concentration (g./100 ml.)	3.83 ± 0.06	3.75 ± 0.05	3.64 ± 0.07
Neutral lipid output (g./hr.)	4.855 ± 0.276	1.999 ± 0.113	6.395 ± 0.391
Phospholipid output (g./hr.)	0.738 ± 0.030	0.431 ± 0.017	0.886 ± 0.035
FFA output (g./hr.)	0.077 ± 0.008	0.038 ± 0.002	0.089 ± 0.006

Milk replacer feeding, compared with whole milk feeding, did not significantly alter the relative proportions of the lipid fractions in plasma. On the other hand, the concentrations of total lipid, phospholipid and FFA in the lymph were notably lower when the calves were being fed milk replacer. These changes were reversed when the calves were returned to whole milk feeding. Total cholesterol values in the lymph, however, were unaltered by the changing dietary regimes. The percentage contributions made by phospholipid and total cholesterol to the total lipid concentration in lymph during whole milk feeding (10.5 and 2.5%, respectively) were increased following milk replacer feeding (16.1 and 4.6%, respectively).

Lipids from lymph and plasma were separated on silicic acid columns and submitted to GLC. The compositions of lymph triglyceride and phospholipid obtained during feeding of whole milk or milk replacer are compared in Table 13. It may be seen that the feeding of milk replacer produced significant changes in the composition of lymph triglyceride toward that of the diet. The composition of lymph phospholipid changed little by comparison. The changes induced in lymph triglyceride by milk replacer feeding were reflected in blood plasma triglyceride (Table 14) although most of these changes were not significant.

TABLE 13. EFFECT OF FEEDING MILK REPLACER ON THE FATTY ACID COMPOSITION OF TRIGLYCERIDE AND PHOSPHOLIPID FROM THE LYMPH OF WEEK-OLD CALVES (MEANS AND S.E. FOR 4 CALVES).

* $P < .05$; ** $P < .01$; *** $P < .001$

Significance of differences using Student's *t* test.

TABLE 13

Fatty Acid	Lymph Triglyceride		Milk Replacer	Lymph Phospholipid (one calf only)	
	Before	After		Before	After
10:0	1.7 ± 0.2 *	0.7 ± 0.2	2.4	...	...
12:0	3.6 ± 0.2 **	9.6 ± 1.4	9.7	trace	trace
14:0	11.8 ± 0.5 ***	6.6 ± 0.5	5.3	2.3	1.0
16:0	33.1 ± 2.6	24.5 ± 1.8	17.5	17.7	14.4
18:0	10.6 ± 0.9	14.5 ± 2.2	18.1	24.7	28.4
18:1	26.4 ± 2.8 *	34.3 ± 0.9	34.1	26.1	25.7
18:2	2.5 ± 0.8	2.7 ± 0.7	3.0	16.6	19.9
18:3	0.6 ± 0.5	0.9 ± 0.6	1.0	3.4	3.4
Remainder	9.7 ± 2.2	6.3 ± 1.1	9.0	9.2	7.3

TABLE 14. EFFECT OF FEEDING MILK REPLACER ON THE FATTY ACID COMPOSITION OF BLOOD PLASMA TRIGLYCERIDE FROM WEEK-OLD CALVES (MEANS ± S.E. FOR 3 CALVES).

* $P < .05$; ** $P < .01$

Significance of differences using Student's *t* test.

TABLE 14

Fatty Acid	Plasma Triglyceride		
	Before		After
10:0	1.6 ± 0.8		0.7 ± 0.6
12:0	2.2 ± 0.4		3.0 ± 0.7
14:0	9.2 ± 1.0	*	4.7 ± 0.4
16:0	33.5 ± 2.6		28.1 ± 1.0
18:0	7.2 ± 1.7		12.8 ± 6.9
18:1	21.4 ± 1.3		25.4 ± 1.8
18:2	10.6 ± 1.5		15.1 ± 4.5
18:3	2.1 ± 1.1		2.6 ± 1.4
Remainder	12.1 ± 0.4	**	7.6 ± 0.8

DISCUSSION

Fatty Acid Composition of Lymph Lipid.

The range of fatty acids found in the lipids of thoracic duct lymph from calves fed whole milk was similar to that in lymph from cows (Chapter 3) and to that of dietary milk fat (Tables 11 and 13). This finding agrees with those of Heath, et al. (1964) for milk-fed lambs and Toullec (1968) for 3-6 week old calves. In these latter studies, the lymph contained smaller proportions of C_{10} and C_{12} fatty acids than the diets whereas in the present study there were no significant differences between the two during whole milk feeding (Table 11). It has been assumed, following the work of Bloom, et al. (1951), that fatty acids of chain length C_{10} and smaller are absorbed predominantly via the portal vein. The present results confirm this finding for C_4-C_8 fatty acids but suggest that during whole milk feeding the lymphatics may be the more important pathway of absorption for C_{10} fatty acid in young calves.

Triglyceride from blood plasma showed a similar composition to that of lymph triglyceride on whole milk feeding (Table 10) and changed to some extent towards that of the diet when calves were fed milk replacer (Tables 13 and 14). These observations are probably related to the finding that chylomicrons and very low density lipoproteins

($d < 1.005$) can be readily detected in blood serum of milk-fed calves (Wadsworth, unpublished observations).

It is interesting that lymph cholesterol ester showed striking similarity in fatty acid composition to lymph triglyceride (Table 10). Similar results were obtained with milk-fed lambs and adult sheep (Heath, et al., 1964). However, in the adult bovine, lymph cholesterol ester bears a much closer resemblance to cholesterol ester from blood plasma than to lymph triglyceride (Chapter 3). These results suggest that absorbed lipid contributes more fatty acid to lymph cholesterol ester than does cholesterol ester from blood plasma in the calf and sheep and provides no evidence of selectivity in the incorporation of exogenous fatty acids into lymph cholesterol ester similar to that found in rats (Karmen, et al., 1963). Lymph phospholipid, on the other hand, contained higher levels of 18:0 and 18:2 than lymph triglyceride (Table 10) consistent with the observed selective incorporation of these fatty acids into the phospholipid of rat lymph (Whyte, et al., 1963).

It has previously been assumed in this laboratory that the average molecular weight of fatty acids in calf lipids was 280 g. (Shannon and Lascelles, 1967) but the present data indicate a value close to 260 g. for fatty acid in lymph triglyceride (Tables 10 and 13) whereas that for phospholipid fatty acid was closer to 280 g. (Table 10).

Milk Replacer Feeding.

It can be calculated from the data in Table 3 that the neutral lipid transported in the thoracic duct lymph represented, on average, approximately 83% of the long chain ($>C_8$) fatty acid supplied in the whole milk diet. On the other hand, the estimated recovery in lymph of fatty acid supplied by the milk replacer diet was approximately 50%. It would appear that the relative efficiency of absorption was reduced when the milk replacer was fed. A recent study by Radostits and Bell (1968) found low apparent digestibility of milk replacer lipid with negative digestibility coefficients for palmitic and stearic acids, suggesting selective malabsorption of long chain, saturated fatty acids and, indeed, there was significantly less stearic acid in lymph triglyceride than in dietary milk fat in the present study although the proportions of most of the other fatty acids did not differ between lymph triglyceride and the diet (Table 11). Furthermore, the percentage of stearic acid in lymph triglyceride was not significantly changed by feeding milk replacer, despite the higher level of stearate in milk replacer lipid (Table 13). Boucrot and Clément (1965b) found in rats that stearic and oleic acids fed in equal amounts were incorporated into lymph neutral lipid in the ratio 3:4. However, stearic acid is absorbed and incorporated

into the total lipids of rat lymph to the same extent as other fatty acids (Coots, 1964a) which suggests that the discrimination against stearate in the formation of lymph triglyceride may be due to competition with phospholipid synthesis (Whyte, et al., 1963) for the available substrate. Thus, it may be concluded that there was little, if any, selective malabsorption of individual fatty acids during milk replacer feeding in the present experiments.

On the other hand, lymph flow was considerably reduced during milk replacer feeding compared with whole milk feeding (Table 12), probably reflecting changes in lymph formation in the intestinal region (cf. Shannon and Lascelles, 1969b). If a comparison is made of the results obtained in the present experiment with those obtained by Shannon and Lascelles (1969b) it may be seen that lymph flow following the feeding of skimmed milk and milk replacer was approximately 69% and 72%, respectively, of the flow following whole milk feeding. Thus, feeding of milk replacer caused a reduction of lymph formation comparable with that produced by skimmed milk feeding, despite the fact that lipid intake from the milk replacer was some 15 times greater than that from skimmed milk. It is suggested, therefore, that there was a "toxic factor" (or factors) in the artificial diet which was depressing lymph formation.

It is proposed that the "toxic factor(s)" responsible for the reduction in lymph formation and fat absorption observed in this study may have been derived from oxidation of the milk replacer lipid during manufacture or storage. In this connection, it has been reported that deterioration of milk replacers during storage leads to increased peroxide values indicating oxidation of unsaturated fatty acids (Adams, Gander, Gullickson and Sautter, 1959) and that ingestion of oxidised fats results in reduced recovery of dietary lipid in lymph (Bhalerao, et al., 1963). Indeed, gas chromatography of Denkavit lipid prior to silicic acid chromatography revealed the presence of several unidentified peaks which probably represented the products of oxidative breakdown. Furthermore, it has been shown that short chain organic acids (pentanoic, 4-pentenoic acids) similar to those produced during oxidative decomposition (Wexler, 1964) reduce the levels of intracellular CoA (Brendel, Corredor and Bressler, 1969) and would be expected, therefore, to inhibit fat absorption (Senior, 1964). Also, it appears that the products of fatty acid oxidation have vasodepressor activity (Geyer, Watkin, Matthews and Stare, 1949) which could cause depression of lymph formation.

It has been found that the output of endogenous lipid in lymph is independent of the amount or

composition of dietary lipid (Boucrot and Clément, 1968). Since the output of neutral lipid in lymph was reduced during milk replacer feeding (Table 12) it would be expected that endogenous lipid contributed a greater proportion of the total lipid to lymph during feeding of milk replacer than of whole milk. This is consistent with the increased proportions of phospholipid and total cholesterol in lymph lipid and with the lesser resemblance between lymph triglyceride and dietary lipid during milk replacer feeding (Table 13) than during feeding of whole milk (Table 11). In this connection, an experiment in which a young calf was prepared with a re-entrant bile fistula (Wadsworth and Lascelles, unpublished observation) indicated that 0.5-0.7 g./hr. of bile phospholipid entered the gut irrespective of the level of fat in the diet. This would represent about 10-15% of the daily transport of neutral lipid in thoracic duct lymph (Shannon and Lascelles, 1967), and is probably the major source of endogenous lipid in thoracic duct lymph from calves.

CHAPTER 7.

THE TRANSPORT OF LIPOPROTEIN IN THE INTESTINAL AND HEPATIC LYMPH OF NEWBORN CALVES

INTRODUCTION

In a recent paper from this laboratory Shannon and Lascelles (1969a) drew attention to the high concentration and output of lipid in thoracic duct lymph of newborn, unsuckled calves. Under the conditions of anaesthesia employed by these workers the calves quickly recovered from the effects of the operation and were able to stand and suck from a teat within 2 hr. of its completion. During this period lymph flow rapidly increased to relatively stable values of about 350 ml./hr. (11 ml./Kg./hr.) and similarly the output of lipid in lymph rose to values around 0.9 g./hr. This represents a substantial turnover of lipid for a previously unfed animal.

The high levels of lipid, particularly triglyceride, in lymph relative to blood plasma indicated that only a small fraction of the lipid could have originated from the capillary filtrate. The great majority, therefore, must have been derived from the tissues drained by the thoracic duct.

The main aims of the present experiments have been to determine the origin and character of the endogenously

secreted lipid. This has been achieved by collection and analysis of intestinal and hepatic lymph from normal and bile fistulated, newborn calves.

METHODS

Animals and Their Management.

Successful operations were performed on a total of 10 calves. Intestinal lymph was collected from 6 calves, in 2 of which the common bile duct was also cannulated. Hepatic lymph was obtained from 3 calves and thoracic duct lymph from the remaining calf. The details of the calves and the type of operation performed are shown in Table 15. It may be seen that most of the operations were carried out on animals during the first 3 hours after birth.

Considerable difficulty was experienced in obtaining hepatic lymph uncontaminated with intestinal lymph. In contrast to operations in the older, fed animal, in which the presence of chylomicrons in intestinal lymph made it easy to distinguish between intestinal and hepatic ducts, it was often difficult to make this distinction in operations on the newborn, unfed animal. Thus, small anastomotic ducts between the intestinal and hepatic systems were difficult to see and ligate. However, the compositional differences between hepatic and intestinal lymph in newborn animals was so marked that it was easy to tell

TABLE 15. DETAILS OF THE CALVES, TYPE OF OPERATION PERFORMED AND FEEDING REGIME.

TABLE 15

Calf Number	Breed	Sex	Weight (Kg.)	Surgery (hr. after birth)	Type of Operation	First Fed (hr. after surgery)
1	Friesian	F	32.6	2.0	Intestinal Duct	2.0
2	Friesian Cross	M	33.7	5.0	" "	2.25
3	" "	M	31.7	5.0	" "	2.5
4	Friesian	F	31.7	17.0	" "	2.25
5	"	M	39.2	1.5	Intestinal Duct and Bile Duct	11.0
6	"	M	39.2	6.0	" " " "	15.5
7	Ayrshire	M	38.2	2.0	Hepatic Duct	1.5
8	Friesian	M	30.0	0.75	" "	5.0
9	Ayrshire	M	27.0	7.5	Hepatic and Intestinal Duct	...
10	Friesian	M	33.0	3.5	Thoracic Duct	22.5

after the operation whether pure hepatic lymph was being collected and indeed only lymph which remained free of chylomicrons after feeding was regarded as pure hepatic lymph.

The calves were not fed until they had recovered sufficiently from the operation to stand unaided. Each calf was fed 1.14 l. colostrum at first feeding and similar amounts 6 and 12 hrs. later. Subsequently the calves were fed 2.27 l. whole milk at 12 hr. intervals.

Analytical.

Serum was prepared from blood and lymph samples by allowing them to clot on the bench for 1 hr. and separating the clot by centrifugation. Estimations of total protein, TEFA, phospholipid and FFA were routinely carried out on blood and lymph serum samples. Phospholipid in bile was also determined. Lipid was extracted with chloroform-methanol (2:1, v/v) and fractionated by TLC. Methyl esters of fatty acids were prepared by dimethyl carbonate-induced transesterification or sealed-tube methanolysis. Selected samples of lymph and blood serum were submitted to ultra-centrifugation and the lipoprotein fractions analysed.

RESULTS

The calves rapidly recovered from the operation and were generally able to stand within 3 hr. of its completion.

During anaesthesia the flow of intestinal lymph varied between 40 and 80 ml./hr. but rapidly increased during the first 2 hr. after the operation to levels as high as 200 ml./hr. The flow of hepatic lymph during anaesthesia varied between 5 and 15 ml./hr. for the 3 calves and increased after the operation to values which never exceeded 60 ml./hr. After centrifugation at 400 g for 10 min. to remove cells, intestinal lymph was observed to be distinctly opalescent whereas the cell free hepatic lymph was completely clear.

Composition of Blood and Lymph Serum in Unsuckled Calves.

The concentrations of protein, TEFA, phospholipid and FFA in blood and lymph serum collected during anaesthesia and after recovery from the anaesthetic (immediately prior to first feeding) are given in Table 16. Apart from some decrease in the concentration of TEFA and phospholipid in intestinal lymph, it may be seen that there was little difference in the concentration of the constituents in samples collected during and after recovery from the anaesthetic. Protein concentration in hepatic lymph was somewhat higher than in intestinal lymph, the

TABLE 16. THE CONCENTRATION OF TOTAL PROTEIN, TEFA, PHOSPHOLIPID AND FFA IN SAMPLES OF LYMPH AND BLOOD SERUM FROM NEWBORN, UNSUCKLED CALVES OBTAINED DURING ANAESTHESIA AND AFTER THE CALVES HAD RECOVERED FROM THE OPERATION.

The mean values ± S.E. (g./100ml.) are presented and the number of samples is given in brackets.

TABLE 16

	Anaesthesia				Recovery			
	Total Protein	TEFA	Phospho-lipid	FFA	Total Protein	TEFA	Phospho-lipid	FFA
Blood	4.85 (3) ±.51	0.063 (3) ±.009	0.038 (3) ±.006	0.022 (1) - -	4.41 (3) ±.25	0.058 (3) ±.005	0.029 (3) ±.003	0.014 (1) -
Intestinal Lymph	3.15 (4) ±.19	0.539 (4) ±.132	0.164 (4) ±.035	0.027 (2) ±.006	2.71 (3) ±.03	0.357 (3) ±.120	0.098 (3) ±.022	0.016 (2) ±.006
Blood	4.93 (3) ±.60	0.072 (3) ±.007	0.049 (3) ±.009	0.031 (1) -	4.20 (1) -	0.077 (1) -	0.044 (1) -	...
Hepatic Lymph	3.31 (3) ±.12	0.074 (3) ±.011	0.028 (3) ±.007	0.020 (1) -	3.17 (2) ±.28	0.080 (2) ±.019	0.030 (2) ±.007	0.016 (1) -

FIGURE 11.

Diagram of the separation of lipoproteins in lymph and blood serum from newborn calves.

Serum, from which chylomicrons had been removed, was layered over 54% sucrose and overlaid with 0.9% saline. The tubes were centrifuged at 2.4×10^6 g. hr. The portions of the lipoprotein profile included in the fractions (F1, F2, F3) collected by puncturing the bottom of the tube are indicated.

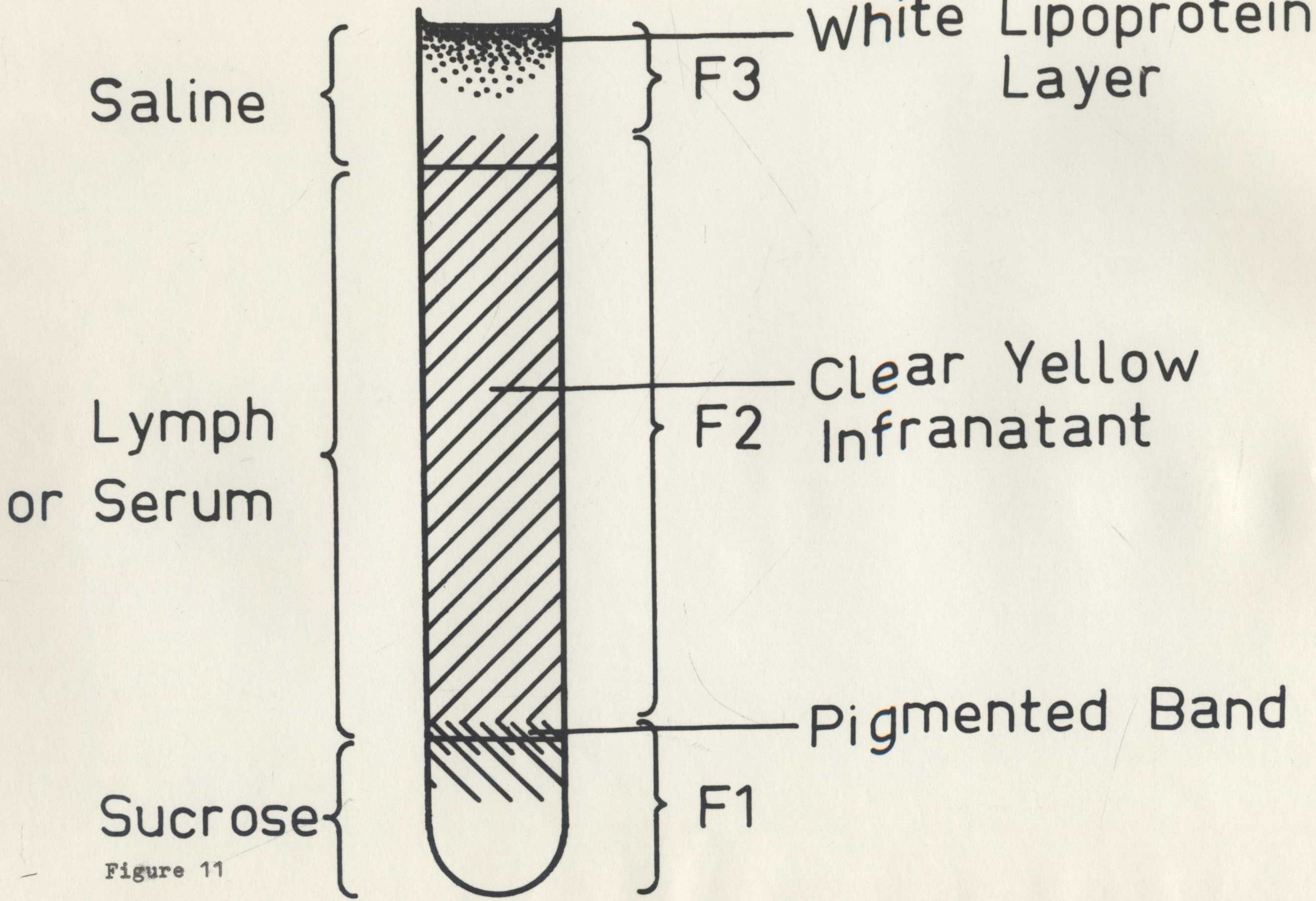

Saline
Lymph
or Serum
Sucrose
F3
F2
F1
White Lipoprotein
Layer
Clear Yellow
Infranatant
Pigmented Band

Figure 11

mean lymph:blood serum ratio being 0.75 and 0.63 respectively. On the other hand, there were striking differences in the lipid concentration in lymph from the two regions. The TEFA and phospholipid concentrations in intestinal lymph collected immediately prior to first feeding were about 6 and 3.5 times their respective values in blood serum whereas in hepatic lymph these constituents were present in similar or lower concentrations than those in blood.

Ultracentrifugal Analysis of Lymph and Blood Serum.

From the results of the preliminary centrifugation (30,000 g for 30 min.) it was apparent that chylomicrons were not present in significant quantities in lymph or blood serum of newborn calves. The second centrifugation (200,000 g for 12 hr.) revealed marked visual differences in the lipoprotein profile of intestinal lymph on the one hand and hepatic lymph and blood serum on the other. Lipoprotein was seen as a white band in the upper half of the saline layer of intestinal lymph samples but could not be seen in hepatic lymph or blood serum. Multiple banding was not observed below the saline layer in any of the samples (Fig. 11) which contrasts with the results obtained in human serum using a similar procedure (Wurm and Straus, 1968)

Approximately 40% of the total lipid in intestinal lymph was present in fraction 3, 50% in fraction 2 and

10-15% in fraction 1. The lymph:blood serum ratio for total lipid in fractions 3, 2 and 1 were, respectively, 24.2, 3.2 and 1.2. Most of the lipid in the hepatic lymph and blood serum occurred in fraction 2 (lymph: blood serum ratio 0.7) with only small amounts in fraction 1 and virtually none in fraction 3. The lipid in fractions 2 and 3 of intestinal lymph was further analysed for triglyceride, cholesterol ester and free cholesterol by TLC. Phospholipid determinations were carried out separately. The results presented in Table 17 show that the content of triglyceride was higher in fraction 3 than in fraction 2 with a higher phospholipid content in the latter. Cholesterol ester and free cholesterol accounted for less than 10% of the total lipid in the lipoprotein fractions and have not been reported. The protein content of fraction 1 measured in 1 sample by a micro-Keldjahl procedure was found to be 18%.

The Flow and Output of Lipid In Lymph.

The mean output of TEFA in intestinal lymph immediately prior to first feeding was approximately 0.6 g./hr. which is about 50% of the output reported for thoracic duct lymph by Shannon and Lascelles (1969a). It is of interest that, although the operation in calf 4 was carried out 17 hr. after birth, the output of lipid in intestinal lymph immediately prior to first feeding was similar to that in other calves which were cannulated

TABLE 17. THE PROPORTIONS OF TRIGLYCERIDE AND PHOSPHOLIPID IN TOTAL LIPID FROM LIPOPROTEIN FRACTIONS OF INTESTINAL LYMPH FROM NEWBORN CALVES.

Values presented are means ± S.E. expressed as weight percentage and the number of samples is given in brackets.

TABLE 18. THE EFFECT OF BILE DEPRIVATION ON THE CONCENTRATION AND OUTPUT OF TEFA IN INTESTINAL LYMPH FROM CALVES 5 AND 6.

Values presented are means ± S.E. for samples obtained soon after surgery (recovered) and after 11-16 hr. bile deprivation.

TABLE 19. THE EFFECT OF BILE DEPRIVATION ON THE CONCENTRATION OF LIPID IN FRACTIONS 1, 2 AND 3 OF INTESTINAL LYMPH FROM CALF 5.

Samples were collected shortly after surgery (recovered) and after 11 hr. bile deprivation. Results are expressed as g./100 ml. lymph.

TABLE 17

	Triglyceride	Phospholipid
Fraction 3	66.5 ± 2.6 (6)	21.0 ± 0.07 (4)
Fraction 2	47.3 ± 0.4 (4)	27.6 ± 0.6 (3)

TABLE 18

	Concentration (g./100 ml.)	Output (g./hr.)
Recovered	0.364 ± 0.147	0.395 ± 0.004
11-16 hr. Bile Deprived	0.113 ± 0.002	0.211 ± 0.010

TABLE 19

	Recovered	11 hr. Bile Deprived
Fraction 3	0.160	0.036
Fraction 2	0.254	0.117
Fraction 1	0.024	0.028

FIGURE 12.

Flow of lymph (upper curves) and output of TEFA (lower curves) from the intestinal (Calf 2, open circles) and hepatic (Calf 7, solid circles) lymphatic ducts of newborn calves.

Samples were collected under anaesthesia (A), after recovery from the operation (R) and at intervals after first feeding.

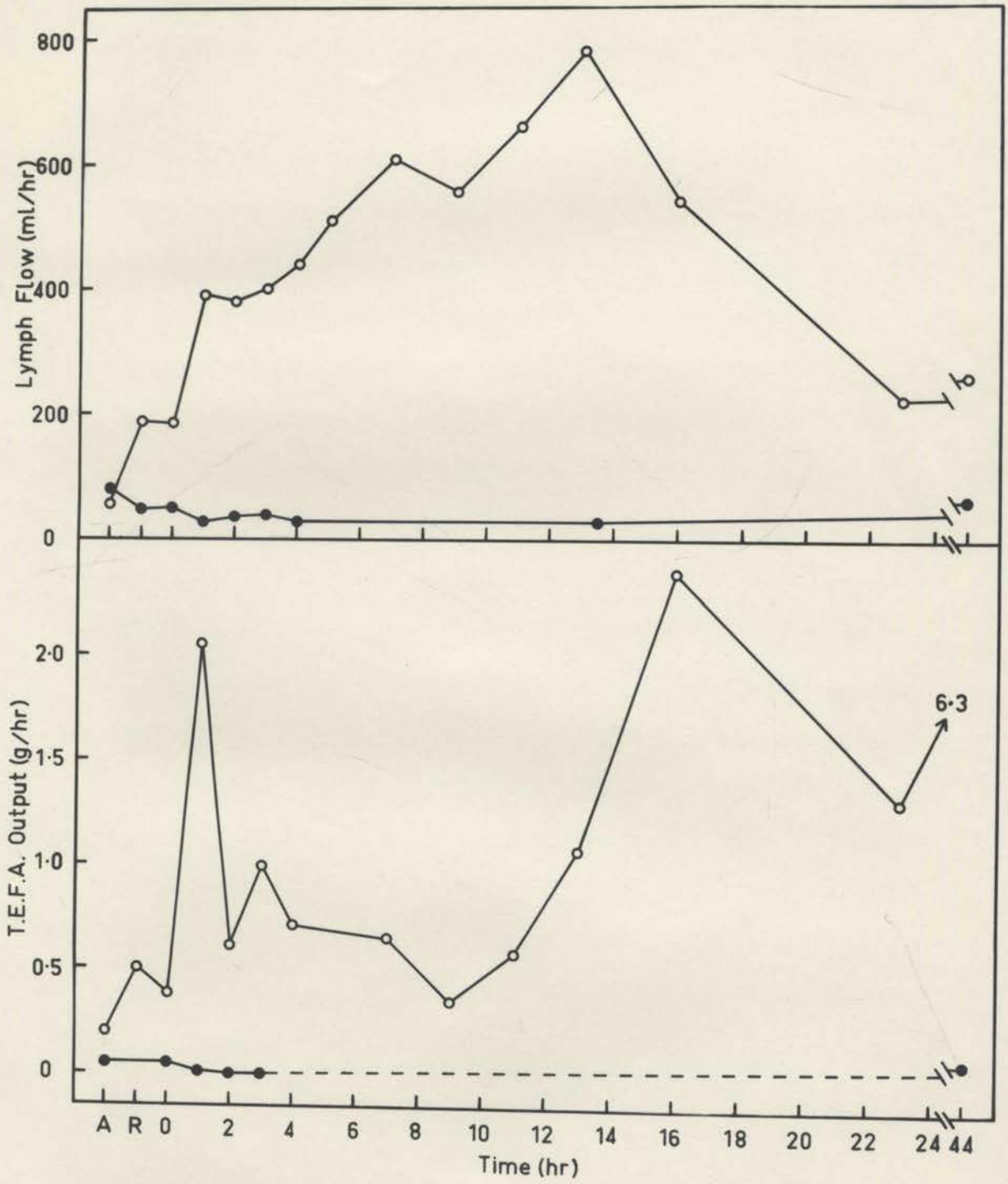

800
600
400
200
0
Lymph Flow (ml/hr)
2·0
1·5
1·0
0·5
0
T.E.F.A. Output (g/hr)
6·3
A R 0 2 4 6 8 10 12 14 16 18 20 22 24 44
Time (hr)

Figure 12

within 5 hr. of birth. This suggests that starvation over a period of 20 hr. did not significantly affect the output of lipid in lymph. Confirmation of this was apparent from the results of calf 10 in which the output of TEFA in thoracic duct lymph after 23 hr. starvation (0.44 g./hr.) was scarcely different from that shortly after surgery (0.50 g./hr.).

The flow and output of TEFA in intestinal and hepatic lymph for calves 2 and 7 before and after feeding are illustrated in Fig. 12. The trends observed for other calves were the same as those depicted in the figure. There was a striking increase in flow of intestinal lymph beginning within 1-3 hr. after first feeding and reaching values of about 400 ml./hr. at 8 hr. Subsequently flow decreased to relatively constant levels of around 250 ml./hr. Both the concentration and output of TEFA in intestinal lymph increased to reach maximum values (output 5-6 g./hr.) 24-48 hr. after first feeding. These results are essentially the same as those reported for thoracic duct lymph by Shannon and Lascelles (1969a). In contrast there was little change in the flow and output of TEFA in hepatic lymph after feeding.

Bile Fistulation.

It is known that bile contains high levels of phospholipid and it was considered that a significant proportion of the lipid in intestinal lymph could have been

derived by absorption of biliary phospholipid (cf. Chapter 6). Accordingly, in calves 5 and 6 the bile as well as the intestinal lymphatic duct was cannulated and bile allowed to drain for 11-16 hr. before first feeding. Changes in the concentration and output of TEFA in lymph before and during bile deprivation are illustrated in Table 18. The concentration and output of TEFA fell steadily during the course of bile deprivation and after 11-16 hr. the output was 55% of comparable values in calves without bile fistulae. The lymph collected from both calves during bile deprivation lost its opalescent appearance and subsequent ultracentrifugal analysis showed that the lipoprotein in fraction 3 had virtually disappeared. The changes in total lipid concentration in the 3 ultracentrifugal fractions from calf 5 during bile deprivation are shown in Table 19. It may be seen that there was a substantial decrease in lipid in fraction 3 after 11 hr. bile deprivation to a value which represented 23% of that obtained shortly after bile fistulation or 13-34% of that from non-fistulated calves. During the same period, the lipid in fraction 2 decreased by approximately 50%.

The output of phospholipid in bile immediately prior to first feeding was 0.07 and 0.10 g./hr. for the 2 calves. These values were considerably less than the estimated decrease in output of lipid in lymph (0.23 g./

TABLE 20. COMPARISON OF THE FATTY ACID COMPOSITION OF TRIGLYCERIDE FROM LIPOPROTEIN FRACTIONS OF INTESTINAL LYMPH WITH THAT OF BILIARY LIPID FROM AN UNFED, NEWBORN CALF.

The samples were collected from Calf 5 shortly after bile fistulation.

TABLE 20

Fatty Acid	Bile Total Lipid	Intestinal Lymph		
		Fraction 3	Fraction 2	Fraction 1
12:0	--	0.1	trace	--
14:0	0.1	2.9	2.5	2.0
15:0	0.3	1.3	0.9	0.6
16:0	39.6	38.6	47.1	39.8
16:1, 17:0	11.4	11.1	10.9	12.6
18:Br	1.8	1.0	0.9	1.8
18:0	8.3	7.2	9.6	8.9
18:1	35.5	35.5	27.4	31.4
18:2	2.8	1.2	0.6	1.7
18:3	0.2	0.4	0.1	1.3
Unknown	--	0.7	--	--

hr.) which occurred as a result of prolonged bile deprivation. It is important to note, however, that the values obtained for lipid output in bile were almost certainly less than those from the intact animal since it is known that interruption of the entero-hepatic circulation of bile salts results in a rapid reduction of lipid output in bile (Heath and Hill, 1969).

Fatty Acid Analysis.

The fatty acids of bile lipid and of triglyceride in the three lipoprotein fractions from the intestinal lymph of calf 5 were analysed and the results are shown in Table 20. There was a general resemblance in fatty acid composition between biliary lipid and the triglyceride of the lipoproteins, the predominant fatty acids being 16:0 and 18:1 with smaller proportions of 14:0, 18:0 and the C_{18} polyunsaturates. Appreciable levels of odd carbon number (C_{15} and C_{17}) fatty acids were also detected in lipids from the newborn, unsuckled calf. It is of interest that the proportions of 16:0 and 18:1 in the triglyceride of fractions 1 and 3 were closer to those in biliary lipid than to those in fraction 2.

DISCUSSION

The output of TEFA in hepatic lymph from calves in the present experiments was insignificant compared with the considerable output in intestinal lymph which

was approximately 50% of that reported previously in thoracic duct lymph from comparable calves (Shannon and Lascelles, 1969a). This probably represented an underestimate of the contribution of lipid in lymph from the intestines since the operative trauma involved in collecting intestinal lymph was considerably greater than that for thoracic duct lymph.

Most of the lipid in the intestinal lymph was carried in fractions 2 and 3 of the ultracentrifugal analysis. It is considered that fraction 3 isolated from calf lymph is similar to VLDL isolated from human serum (Wurm and Straus, 1968) in view of its density ($D < 1.005$), lipid composition (Table 17).and protein content. The identities of fractions 1 and 2, however, are less certain although the lipid composition of fraction 2 (Table 17) is similar to that usually reported for LDL (Alaupovic, et al., 1966).

Virtual disappearance of VLDL from intestinal lymph following bile deprivation suggests that most of the VLDL, comprising about 40% of the total lipid in the lymph, is derived from absorption of biliary lipid. That the output of TEFA in intestinal lymph was scarcely reduced after 20-23 hr. starvation (calves 4 and 10) shows that the effects of bile deprivation (calves 5 and 6) could not be attributed to starvation. Nevertheless the possibility still remains that the absence of bile salts

in bile fistulated calves prevented the absorption of endogenous lipid derived from some other source. In the mature animal shed epithelial cells probably contribute the main additional source of endogenous lipid entering the gut. However, in the newborn, unsuckled ruminant, lipid of this origin is probably minimal since it has recently been shown that rapid turnover of intestinal epithelium does not begin until shortly after birth (Smeaton, 1969). Thus it seems reasonable to conclude that the majority of VLDL in the lymph is derived from bile. Indeed, similar conclusions have been drawn by others working with mature, fat-deprived rats (Baxter, 1966; Shrivastava, et al., 1967).

It is of interest that the absorption of bile lipid resulted in the production of VLDL rather than chylomicrons. Inspection of the ultracentrifugal fractions obtained from samples collected after feeding the calves suggested that the VLDL and chylomicrons formed a continuous spectrum of lipoproteins rather than distinct classes. Indeed, Windmueller and Levy (1968) have recently proposed that lymph VLDL is a carrier for transporting triglyceride from the intestine and that, during the absorption of a fat meal, this carrier incorporates larger amounts of lipid to become chylomicrons. The present observations in the newborn calf support this hypothesis.

Approximately 50% of the lipoprotein in intestinal lymph was carried in LDL (fraction 2) and since the concentration of lipid in LDL from lymph was about 3 times the comparable values for blood serum, it is clear that most of the lipid in this lipoprotein fraction is synthesised in the intestinal wall. The results of the bile drainage experiments indicated that bile lipid contributed up to 50% of the fatty acids for LDL synthesis. It is suggested that the remainder of the LDL fatty acid is derived from blood FFA (Havel and Goldfien, 1961) and/or by de novo synthesis from small molecular weight precursors (Franks, et al., 1966).

Attention is drawn to the high LDL content of intestinal lymph of newborn calves compared with that in the thoracic duct lymph of mature, fat-deprived rats (Baxter, 1966). In this connection it is of interest to report that the lipid output in thoracic duct lymph of 3 day old calves in the post-absorptive state was considerably less than that in newborn, unsuckled calves, although there was little change in the output of VLDL. It would appear, therefore, that fat synthetic activity of the foetus, which derives most of its nourishment from carbohydrate, continues after birth at least until the animal is fed and that the ingestion of a fat-rich diet depresses de novo synthesis of long chain fatty acids (cf. Hanson and Ballard, 1968). In this context the new-

born calf is not entirely comparable with the older starved animal.

Attention is drawn to the high levels of FFA in the blood serum of newborn, unsuckled calves reported here (Table 16), which were similar to those observed in comparable, unoperated calves by Shannon and Lascelles (1966). Increased levels of FFA are commonly observed in blood plasma of young mammals during the 24 hr. after birth and it has been suggested that this is, at least in part, a reaction to cold stress (Hull, 1969). During exposure to a cold environment, the levels of VLDL in rat serum drop precipitously and it is considered that this indicates an important role for this lipoprotein as a readily available energy source (Radomski, 1966). In the present study, VLDL was virtually absent from blood serum, indicating a rapid utilization of this lipoprotein by the body tissues of the newborn calves.

The fatty acid composition of bile and lymph lipids (Table 20) resembles that previously reported for adipose tissue lipid from foetal calves (Cumont, 1968) and blood plasma lipids from newborn, unsuckled calves (Leat, 1966). The relatively high proportion of odd carbon number and branched chain fatty acids found in the present study support the suggestion that significant amounts of FFA may be transferred from maternal blood into the foetus in ruminants (Van Duyne, Parker, Havel and Holm, 1960; Leat, 1966).

CHAPTER 8.

GENERAL DISCUSSION

Use of the Lymph Collection Technique in Studies of Fat Absorption.

The interpretation of experiments involving lymph collection depends to a considerable extent on the maintenance of the experimental animals in a physiologically normal state throughout the study. Continuous drainage of lymph results in considerable loss of blood plasma protein and electrolytes with consequent loss of body weight and changes in lymph formation (Morris, 1956; Woolley and Simmonds, 1959; Smeaton, Cole, Simpson-Morgan and Morris, 1969). In smaller experimental animals (e.g. rats, rabbits and lambs) it is not possible to return lymph continuously to the bloodstream due to the small flow. However, lymph may be recirculated satisfactorily by way of lymphatico-venous shunts in the bovine and the animals are thereby maintained in a physiologically normal condition. Thus, the milk production of lactating cows (Chapters 3 and 4) returned to normal (Hartmann and Lascelles, 1966) and young calves (Chapter 6) grew satisfactorily (Shannon and Lascelles, 1967) after the establishment of thoracic duct-venous shunts.

During the first 24 hr. after surgery in rats the flow of lymph is depressed, probably due to a reduction in capillary filtration area (Woolley and Simmonds, 1959).

Furthermore, the output of endogenous lipid is depressed during the first day after the operation (Baxter, 1966; Boucrot and Clément, 1968) as is the flow of bile and output of biliary lipid (Shrivastava, et al., 1967). During bile drainage the absorption of fat into lymph is depressed when rats are fed within 24 hr. of surgery compared with feeding 48 hr. after the operation (Morgan, 1966). In cows (Hartmann and Lascelles, 1966) and milk fed calves (Shannon and Lascelles, 1967) it was found that during anaesthesia the flow of lymph and output of lipid from the thoracic duct was low. Fat absorption was also depressed and did not return to normal until 24 hr. and 2-3 days after surgery in calves and cows, respectively. It is evident from these considerations that the predominant effects of anaesthesia and operative trauma are a depression of lymph formation and lipoprotein secretion. Samples of lymph from milk fed calves and cows used in the present studies (Chapters 3, 4 and 6) were obtained after clinical recovery from the operation. Newborn calves (Chapter 7) recovered quickly under the conditions of anaesthesia employed in that they were able to stand shortly after the operation and to drink 6-12 hr. later (cf. McCrady, et al., 1964). Nevertheless, residual effects from operative trauma cannot be excluded. For this reason it was concluded that the values presented for the concentration and output of lipoprotein in the

intestinal lymph of newborn calves represented underestimates (Chapter 7).

From results obtained using week-old calves, it was calculated that dietary milk fat and milk replacer lipid were absorbed to the extent of 83% and 50%, respectively, by way of the lymph (Chapter 6). These values included any endogenous lipid in lymph. An estimate of the endogenous lipid transported in thoracic duct lymph may be obtained from the data of Shannon and Lascelles (1969b) who fed skimmed milk to calves. When values for neutral lipid are calculated and corrected to an average molecular weight of 260 g. it may be seen that these calves transported approximately 3.5 g./12 hr. neutral lipid (fatty acid) in lymph during skimmed milk feeding. This figure is probably not a valid estimate of the endogenous contribution to lymph lipid during absorption of fat from whole milk due to the difference in lymph formation (Shannon and Lascelles, 1969b) but may be used to correct values obtained during milk replacer feeding (Chapter 6). When this is done the value calculated for recovery of milk replacer lipid in lymph is reduced from 50% to 45%.

Transfer of Dietary Lipid into Milk Fat.

The results reported in this thesis (Chapters 3, 4 and 5) are consistent with current concepts of the origin of milk lipid (Linzell, 1968; Jones, 1969). Thus,

it has been found that C_4-C_{14} fatty acids in milk fat are synthesized de novo within the mammary gland from blood acetate and β-hydroxybutyrate, C_{18} fatty acids are derived from plasma lipids and 16:0 is derived from both sources (Folley and McNaught, 1961; Annison, Linzell, Fazakerley and Nichols, 1967; Linzell, Annison, Fazakerley and Leng, 1967; Gerson, Shorland, Wilson and Reid, 1968). The mammary gland removes chylomicrons and low density ("β") lipoproteins from the circulation and utilizes the triglyceride fatty acids of these lipoproteins for synthesis of milk fat (Barry, Bartley, Linzell and Robinson, 1963; Lascelles, Hardwick, Linzell and Mepham, 1964; Glascock, et al., 1966). Experiments in this laboratory have shown that the only plasma lipids taken up by the lactating mammary gland of the cow are triglyceride and FFA (Hartmann and Lascelles, 1964). The uptake of FFA is of little quantitative significance but may assume a more important role when the availability of other substrates is restricted. There exists, however, an equilibrium between plasma FFA and mammary lipid (Annison, et al., 1967) so that circulating FFA may exert a greater influence on the composition of milk fat than its uptake would indicate.

The increased unsaturation of milk fat following the feeding of linseed oil (Chapter 5) is thus consistent with the changes observed in chylomicron triglyceride

following oil feeding (Chapter 4). On this basis it may be assumed that the increase in iodine value of milk fat was largely due to greater proportions of 18:1 transported in thoracic duct lymph.

As discussed in Chapter 5, it would appear that, at low levels of lipid intake, dietary supplementation with fat increases the yield of milk and fat (cf. Storry, et al., 1968) whereas high fat intakes suppress secretion of milk and milk fat. However, between these extremes comparatively large changes in lipid intake give rise to little change in the yield of milk or fat. Thus, despite the ingestion of quantities of lipid (Chapter 5) that would be expected to double the amount of chylomicron triglyceride available to the cow over at least a 12 hr. period (Hartmann, et al., 1966), the yield of milk and fat showed no response to oil feeding. In this connection, it has been found that ingestion of unsaturated oils decreased the molar ratio of acetate to propionate in the rumen (Shaw and Ensor, 1959), probably by interfering with the fermentation of fibrous material (Robertson and Hawke, 1964). This would be expected to depress the secretion of milk fat (see Van Soest, 1963). Thus, the failure of oil feeding to increase the yield of milk fat was probably due to a concomitant depression of de novo synthesis of fatty acid by the mammary gland.

Commercial Aspects of Feeding Oil to Dairy Cows.

The kinetics of the absorption, transport and incorporation of dietary lipid into milk fat were used in Chapter 5 to produce a significant increase in the iodine value of milk fat without the necessity for daily administration of oil. It is felt that this may represent a commercially feasible method of exercising some control over butter spreadability. More direct approaches to this problem are available, including blending of vegetable oils with butter (Black, 1968) fractionation of butter oil into hard, medium and soft fractions with recombination in ratios which produce butter of any desired consistency (Dixon and Maitland, 1969; Baker, 1969) and feeding of oil-casein particles protected from the action of rumen microorganisms by formaldehyde treatment (Mills, et al., 1970). However, the findings of Chapter 5 may prove of value since the other methods require costly manufacturing processes. One readily apparent application of this work is the control of seasonal variations in butter hardness (Dixon, 1964) at the farm level, which would return a saving in the cost of manufacture at the factory level.

Effects of Ingested Oil on Rumen Microorganisms.

The chemical sequences involved in the hydrogenation by rumen microorganisms of safflower oil (Chapter 4) and

linseed oil (Chapter 5) may differ since the major fatty acids in these two oils are linoleate and linolenate, respectively (see Chapter 1, Fig. 2). The observed overloading of the capacity of the rumen microorganisms to hydrogenate lipid (Chapter 4) may be purely a mass action effect. However, it has been found that fatty acids are relatively toxic to certain species of bacteria, especially those involved in methane production (Czerkawski, Blaxter and Wainman, 1966; Demeyer and Henderickx, 1967). Thus the effect of single, large doses of vegetable oils may be attributable, at least in part, to selective toxicity to the microorganisms involved in hydrogenation. Since more than one microorganism is required for the complete hydrogenation of 18:2 or 18:3 (Kepler, et al., 1966), the selective reduction in the population of any one of these organisms would be expected to accentuate the accumulation of intermediates in the hydrogenation sequence, giving rise to patterns similar to that reported in Chapter 4 and by Czerkawski (1967b).

Inhibition of methane production after administration of unsaturated fatty acids to sheep resulted in enhanced energy retention (Czerkawski, et al., 1966). It would be expected that the periodic feeding of linseed oil as reported in Chapter 5 would exert a similar beneficial effect on energy utilization by the lactating cow.

Selective Incorporation of Fatty Acids into Lymph Lipids.

The high level of stearic acid in lymph triglyceride from the cow (Table 3) and the similarity between lymph triglyceride and dietary lipid in calves (Tables 11 and 13) provided no evidence for selective malabsorption of stearic acid. Furthermore, this data suggested that, as in monogastric species (Whyte, et al., 1963; Blomstrand, et al., 1964), the incorporation of absorbed fatty acids into lymph triglyceride of the bovine is relatively non-specific. Conclusions regarding specificity for particular fatty acids in the formation of lymph phospholipid and cholesterol ester were not as readily drawn from similar data (Chapters 3 and 6). High levels of 18:2 were found in the phospholipids of cow lymph but the levels of 18:0 were lower than in lymph triglyceride (Table 4). It is probable, therefore, that formation of lymph phospholipid displays a similar specificity for 18:2 in the cow as it does in the rat (Whyte, et al., 1963). The failure to observe specificity for 18:0 in phospholipid synthesis may have resulted from a large endogenous contribution of other fatty acids or to the high proportions of 18:0 present in absorbed lipid resulting in saturating levels of substrate, thus eliminating competition with triglyceride formation. The fatty acid composition of lymph phospholipid from calves, on the other hand, was consistent with selective incorpora-

tion of both 18:0 and 18:2 (Table 10).

Lymph cholesterol ester could not have been derived entirely from the capillary filtrate in cows (Table 4), but showed no evidence of specificities for fatty acids similar to those observed in rats (Karmen, et al., 1963). In the calf, the cholesterol ester of lymph reflected the composition of lymph triglyceride (Table 10). These results may be explained by assuming that cholesterol ester synthesis within the intestine of the bovine is non-specific with regard to substrate fatty acid but that the relative contribution from the capillary filtrate is greater in the cow than in the calf. Further examination of this phenomenon using radioactively labelled fatty acids is warranted in view of the apparent difference between the bovine and monogastric species.

The above considerations emphasise that conclusions drawn from fatty acid composition data with respect to the synthesis of lymph phospholipid and cholesterol ester must remain tentative. In this connection, the observation that changes in the composition of absorbed fatty acid resulted in only minor changes in the composition of lymph phospholipid from both cows (Table 6) and calves (Table 13) is consistent with an overwhelming endogenous contribution to this lipid fraction.

Milk Replacer Lipids in the Nutrition of the Young.

The substitution of mothers milk with various synthetic preparations is becoming increasingly important in the rearing of dairy calves (cf. Raven and Robinson, 1964) and infants (Davies, 1969). Highly variable results have been obtained with milk replacers for calves ranging from fatal (Radcliffe and White, 1968) to highly satisfactory (Raven and Robinson, 1964) and paediatricians have expressed concern at the consequences of replacing breast milk with artificial diets (Davies, 1969; Corden, 1969). Poor results obtained with some milk replacers are undoubtedly due to failure to take adequate precautions in the formulation of these products. Amongst the factors determining the extent of utilization of fat in artificial calf diets are the levels of vitamin D (Stokes, 1969), vitamin E (Adams, et al., 1959) and minerals (Holt, et al., 1935), the particle size of the fat droplets (Raven and Robinson, 1964), and the extent of denaturation of whey proteins from heat treatment during manufacture (Shillam and Roy, 1963). In addition, it has been found that the positional distribution of fatty acids in the dietary triglyceride influences digestive utilization, lipids with 16:0 predominantly in the 2-position being best absorbed by both rats (Tomarelli, et al., 1968) and infants (Filer, et al.,

1969). It would be of interest to repeat these latter experiments in calves since the positional distribution of 16:0 in bovine milk fat varies with the molecular weight of the triglyceride, 16:0 being preferentially esterified in the 2-position of high molecular weight triglycerides and in the 1,3-positions of low molecular weight triglycerides (Dimick, McCarthy and Patton, 1965).

In the present study, the recovery of dietary lipid in lymph was reduced during feeding of milk replacer (Chapter 6). Vitamin levels specified by the manufacturer were adequate and the fat mixture used was homogenised with lecithin so that vitamin status and state of emulsification would not appear to have been involved in the poor absorption of fat. The extent of heat treatment during manufacture could not be determined and since the fat mixture contained both lard and vegetable fats, the proportion of 16:0 in the 2-position could not be predicted (cf. Tomarelli, et al., 1968) although it is probable that neither of these factors accounted for the observed depression in lymph formation (Table 12). It has been claimed that the digestive utilization of fat in artificial diets is low in neonatal calves (Radostits and Bell, 1968) and infants (Widdowson, 1965) and increases with age, but reports on this subject are conflicting (Thieulin, 1968). It may be concluded that correctly formulated milk replacers are well digested by the very

young. In this regard, it has been found in this laboratory that the utilization of Denkavit lipid by calves is independent of age (Hartmann and White, personal communication). The evidence in the present experiments (Chapter 6) indicated that oxidation of lipid caused poor absorption of milk replacer lipid. It would appear that this was a batch effect and would not, therefore, be expected to be consistent. In this respect, it is suggested that the problem may profitably be examined by measuring the output of lipid in lymph after feeding fresh milk replacer oxidized under controlled conditions. Such experiments may also be expected to yield valuable information on the effect of oxidation products on lymph formation. These findings emphasise the importance of strict control of manufacturing and storage conditions in the production of synthetic milks for the nutrition of the young.

Lipoprotein Secretion by the Intestine of the Newborn.

Chapter 7 is of particular interest in that it shows in the calf that the mechanisms for fat absorption are present and functional at birth. Fat absorption is of obvious importance to the survival of the animal. Thus, in the newborn lamb the predominant source of energy is fat rather than glycogen despite the fact that the lamb is born with only minimal amounts of fat in the body (Alexander, 1962). Visual observation during surgery

suggested that the fat content of the newborn calf is also low. This contrasts with the human and guinea pig in which the young are born with high levels of fat in the total body tissues (Widdowson, 1950).

The foetal guinea pig and rabbit accumulate fat in the liver during late pregnancy (Dawkins, 1966; Roux, 1966) which suggests that this may represent a readily mobilised store of energy to meet stress demands at birth. In order to determine whether a similar situation exists in the lamb, an experiment was conducted in which pregnant ewes were sacrificed at intervals of 24, 16 and 8 days before term and the foetuses removed. It was found that the levels of lipid in the liver and intestine did not differ significantly between the three age groups. The liver contained (mean ±SE) 2.17±0.07 and the small intestine 1.55±0.06 (n=13)g. lipid/100g. fresh tissue. The accumulation of lipid in foetal liver during late pregnancy appears, therefore, to be restricted to certain species.

The results obtained with foetal lambs cannot necessarily be extrapolated to calves since there are marked differences between the two species e.g. in response to cold stress. Thus non-shivering thermogenesis does not occur in the calf (Jenkinson, Noble and Thompson, 1968) whereas the newborn lamb possesses a well developed non-shivering thermogenic mechanism

(Thompson and Jenkinson, 1969). This is correlated with the absence of brown adipose tissue in the calf (Jenkinson, et al., 1968) compared with almost total dominance of multivacuolar cells in adipose tissue of newborn lambs (Thompson and Jenkinson, 1969) and with the presence of a relatively immature adrenal medulla in the calf compared with the mature medulla of the lamb at birth (Comline and Silver, 1966).

The present experiments (Chapter 7) make it clear that the liver, while not containing an overt store of lipid at birth in most species, may be providing a functional store of readily mobilised lipid by secreting phospholipid in bile. The availability of this lipid to the animal depends on the ability of the intestine to absorb fat and to secrete VLDL into lymph. It may be noted here that the liver of the newborn rabbit apparently cannot secrete phospholipid directly into the bloodstream (Biezenski and Kimmel, 1969). The recent observation that fatty acids from VLDL triglyceride taken up by the liver are preferentially incorporated into phospholipid (Buckley, Kook and Rubinstein, 1969) together with the present results suggests that there may be some recycling of fatty acid, perhaps in the form of an intact monoglyceride moiety, between the liver and intestines by way of bile and lymph VLDL. Enterohepatic

circulation of lipid in the newborn possesses the biological advantage of providing lipid in a form readily utilized by peripheral tissues (VLDL, see Radomski, 1966) while at the same time activating the fat absorptive processes in readiness for the ingestion of large quantities of lipid. It would be of considerable interest to determine if this secretion of VLDL is functional before birth and, if so, at what stage of pregnancy it commences. In this connection the techniques described by Smeaton, et al., (1969) for the collection of lymph from foetal lambs in utero would be of value.

SUMMARY OF EXPERIMENTAL RESULTS

CHAPTER 3. Fatty Acid Composition of Lipid in the Thoracic Duct Lymph of Grazing Cows.

The fatty acid composition of lipid fractions was studied in the thoracic duct lymph of three cows grazing on pasture. One cow (a three-year-old Ayrshire cross) was dry and the other two (a five-year-old Jersey and a four-year-old Guernsey) were in the second and sixth months of lactation, respectively. The composition of triglyceride in lymph samples collected at different times from the three cows was very similar. The major fatty acids in the triglyceride were 16:0, 18:0, and 18:1 which comprised, respectively, 22, 42, and 17% of the total. Plasma triglyceride had a similar composition. The major fatty acids of lymph phospholipid from one cow were 16:0, 18:0, 18:1, and 18:2 which formed, respectively, 17, 28, 13, and 24% of the total. Plasma phospholipid had a similar composition. Lymph cholesterol ester from the same cow contained predominantly 18:0, 18:2, and 18:3, which made up 15, 24, and 16%, respectively, of the total fatty acids. The cholesterol ester of plasma contained higher proportions of 18:2 and 18:3 than that of lymph. A large number of minor components, principally branched chain and odd carbon number fatty acids, were found in approximately similar proportions in all lipid fractions.

CHAPTER 4. Effect of Feeding Safflower Oil on the Composition of Absorbed Fatty Acid in Grazing Cows.

Two lactating cows were fed a single, large dose of safflower oil. Its effect on the fatty acid composition of triglyceride and phospholipid in lymph from the thoracic duct was examined. The proportion of the fatty acids designated 18:1, 18:2, 18:3, 19:0 and 21:0 increased after oil feeding and there was a compensating decrease in the proportion of most of the other fatty acids. The hourly output of most of the fatty acids in lymph triglyceride did not change after oil feeding, whereas, the out put of 18:1, 18:2, 18:3, 19:0 and 21:0 increased three- to ten-fold. Evidence is presented that the fatty acids identified from gas chromatography as 19:0, 18:3 and 21:0 contained significant proportions, respectively, of 18:1 having the double bond near the methyl end of the carbon chain, cis, trans conjugated 18:2 and trans-trans conjugated 18:2.

The effect of oil feeding on the fatty acid composition of phospholipid in lymph from one of the cows was examined. The proportion of 18:0 and 18:3 decreased and that of 18:2 increased following oil feeding. These changes were not as marked as those observed for triglyceride.

CHAPTER 5. The Effect of the Periodic Feeding of Linseed Oil on the Production and Iodine Value of Milk Fat.

A study was made of the changes in the iodine value of milk fat from cows following the feeding of linseed oil (450 g.) at intervals of 6 days. Three cows in mid-lactation and of differing age and breed were used. The experiment consisted of a series of 3 collection periods, each of 6 days' duration, with 12 days between periods. Three doses of oil were given (6 days apart) between periods 1 and 2, the last dose of oil being given immediately before the start of period 2. The mean iodine values for the 3 periods were 35.6, 41.0 and 36.4, respectively. There was a distinct pattern of change in iodine value with time after feeding the oil, a maximum of 45.5 being attained on the second day with a subsequent decline to 38.5 on the sixth day. Analysis of variance showed that these effects were significant and due to oil feeding, whereas changes in yield and content of fat and production of milk could be accounted for by normal decline in lactation. It was concluded that the periodic feeding of a large dose of linseed oil produces a significant increase in the iodine value of milk fat and avoids the possible disadvantages of continuous fat supplementation.

CHAPTER 6. The Fatty Acid Composition of Lymph Lipids and the Feeding of Milk Replacer to Pre-ruminant Calves.

Lipids in thoracic duct and intestinal lymph from young calves fed whole milk or milk replacer were analysed. Lymph triglyceride contained C_8 and longer fatty acids, the major components being 14:0, 16:0, 18:0 and 18:1, with small amounts of branched chain and odd carbon number fatty acids. C_{10} fatty acid from milk fat was apparently absorbed entirely into lymph. The composition of lymph triglyceride closely reflected that of the diet and showed no diurnal variation when the calves were fed once daily. Triglyceride from blood plasma resembled that from lymph except for higher proportions of 18:2 and 18:3. The fatty acid composition of phospholipid and cholesterol ester from lymph and blood plasma is reported.

During feeding of milk replacer, lymph flow decreased to 72% of that during whole milk feeding. This was accompanied by a reduction in the recovery of dietary long chain fatty acid as neutral lipid in lymph (50% compared with 83%) which could not be accounted for by malabsorption of saturated fatty acids in the milk replacer. The concentration of protein in lymph and blood plasma indicated that the decrease in lymph formation was due to a reduction in blood flow through the intestinal capillaries. These effects were attributed to the presence of

a "toxic factor" in the milk replacer and it is suggested that this may have resulted from the oxidation of unsaturated fatty acids.

CHAPTER 7. The Transport of Lipoprotein in the Intestinal and Hepatic Lymph of Newborn Calves.

Experiments have been conducted to determine the origin and character of the lipid in thoracic duct lymph of newborn, unsuckled calves. This involved the collection and analysis of intestinal and hepatic lymph from 9 normal and bile fistulated calves.

The output of total esterified fatty acid (TEFA) in intestinal and hepatic lymph in unfed calves after recovery from the anaesthetic was about 0.6 and 0.025 g./hr., respectively.

Three lipoprotein fractions were obtained by ultracentrifugation of blood and lymph samples, which appeared to correspond to very low (VLDL, d 1.005), low (LDL) and high (HDL, d = 1.20) density lipoprotein of human serum. The VLDL, LDL and HDL of intestinal lymph contained approximately 40%, 50% and 10%, respectively, of the total lipid in lymph.

VLDL was not found in blood serum or hepatic lymph and it was evident that VLDL and most of the LDL in intestinal lymph were derived from sources other than the capillary filtrate.

The output of TEFA in intestinal lymph fell steadily during the course of an 11-16 hr. period of bile deprivation to values 55% of those in comparable calves without bile fistulae. This was accompanied by a virtual disappearance of VLDL and a decrease in LDL of approximately 50%. These effects could not be attributed to starvation.

There was a general resemblance in fatty acid composition between biliary lipid and the triglyceride of the lipoproteins, the predominant fatty acids being 16:0 and 18:1. The presence of appreciable levels of odd carbon number and branched chain fatty acids were consistent with the transfer of significant amounts of free fatty acid from maternal blood to the foetus.

It is concluded that most of the lipid in thoracic duct lymph of unfed, newborn calves is derived from the intestines and that the VLDL in intestinal lymph arises by absorption of biliary phospholipid.

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