Dr. R. Norris helped with some of the surgical operations. The experiments involving gas-liquid chromatography and thin-layer chromatography were done in conjunction with Dr. F. Adams. Mr. A. Hill cut and stained the histological sections required. With these facilities the experimental work described in this thesis was carried out by myself.

THE METABOLISM OF FAT IN THE RUMINANT ANIMAL

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

Trevor James Heath

The Department of Experimental Pathology
John Curtin School of Medical Research
Australian National University
Canberra, A.C.T.

June, 1963.
Dr. B. Morris helped with some of the surgical operations. The experiments involving gas-liquid chromatography and thin-layer chromatography were done in collaboration with Mr. E.P. Adams. Mr. R. Hill cut and stained the histological sections required. With these exceptions, the experimental work described in this thesis was carried out by myself.

6 June 1963

[Signature]
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 1.</td>
<td></td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Early studies on fat absorption</td>
<td>1</td>
</tr>
<tr>
<td>The effect of the ruminal micro-organisms on the dietary fat in ruminants</td>
<td>7</td>
</tr>
<tr>
<td>The lipases secreted into the gastro-intestinal tract</td>
<td>9</td>
</tr>
<tr>
<td>The extent of hydrolysis of triglycerides during digestion</td>
<td>10</td>
</tr>
<tr>
<td>The absorption of fat into the cells of the intestinal mucosa</td>
<td>13</td>
</tr>
<tr>
<td>The esterification of absorbed fatty acids in the intestinal mucosa</td>
<td>17</td>
</tr>
<tr>
<td>The chylomicron</td>
<td>18</td>
</tr>
<tr>
<td>Changes in the intestinal lymph during fat absorption</td>
<td>19</td>
</tr>
<tr>
<td>The role of intestinal lymph in the transport of absorbed fatty acids</td>
<td>20</td>
</tr>
<tr>
<td>CHAPTER 2.</td>
<td></td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>26</td>
</tr>
<tr>
<td>General methods</td>
<td>29</td>
</tr>
<tr>
<td>Surgical techniques</td>
<td>33</td>
</tr>
<tr>
<td>Analytical methods</td>
<td>43</td>
</tr>
<tr>
<td>Biochemical analyses</td>
<td>54</td>
</tr>
<tr>
<td>CHAPTER 3.</td>
<td></td>
</tr>
<tr>
<td>THE ABSORPTION OF FAT FROM THE GUT INTO THE INTESTINAL LYMPHATICS</td>
<td>59</td>
</tr>
</tbody>
</table>
The effect of chronic lymph drainage on the plasma protein level of the blood ........................................ 59

The lipid content of sheep lymph ........................................ 60

The effect of feeding milk on the flow and composition of the intestinal lymph in lambs ...................... 60

The effect of feeding fat on the flow and composition of intestinal lymph in sheep ........................................ 62

The absorption of $^{14}$C tripalmitin in lambs and in sheep ........................................ 64

The distribution of activity between the lipid constituents of the lymph during the absorption of $^{14}$C tripalmitin ........................................ 67

Summary ........................................ 68

CHAPTER 4.

THE ROLE OF BILE AND PANCREATIC JUICE IN THE ABSORPTION OF FAT IN SHEEP AND LAMBS ...................... 70

The hydrolysis of triglycerides by sheep pancreatic juice in vitro ........................................ 71

The uptake and esterification of free fatty acids by segments of intestinal wall in vitro ...................... 73

The rate of flow and composition of bile and pancreatic juice in sheep and lambs ...................... 76

The effect of deprivation of bile or pancreatic juice on the lipid content of the intestinal lymph ........................................ 78

The absorption of fat in the absence of bile or pancreatic juice ........................................ 79

Recovery of $^{14}$C tripalmitin fed to ewes and lambs deprived of bile or pancreatic juice ...................... 81

Summary ........................................ 83

CHAPTER 5.

THE HYDROGENATION OF UNSATURATED FATTY ACIDS IN THE RUMEN ........................................ 85
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>ANASTOMOTIC CONNECTIONS BETWEEN THE LYMPHATIC AND VENOUS SYSTEMS IN THE THORAX AND ABDOMEN OF SHEEP</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Is the intestinal lymph collected quantitatively from a cannula in the thoracic duct?</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>The events following obstruction to flow through the thoracic duct</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>The transfer of lymph to the blood within lymph nodes</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>The development of direct connections between the thoracic duct and hemiazygos vein in sheep</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>Summary</td>
<td>105</td>
</tr>
<tr>
<td>7</td>
<td>DISCUSSION</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>The hydrogenation of the unsaturated fatty acids of the diet</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>The role of lipases and bile in the digestion of fat</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>The intestinal lymph and fat absorption</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>The quantitative importance of the lymphatic system in the transport of absorbed fat</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>General summary</td>
<td>134</td>
</tr>
</tbody>
</table>
The gut has been modified and populations of micro-organisms capable of digesting the cellulose have become established. In different species, these modifications have occurred in different regions of the gut. In reptiles and some ungulates such as the horse, cellulose is digested by micro-organisms in the large intestine. In cattle, sheep and goats however, micro-organisms digest the cellulose in a large, well-defined system of fore-stomachs, the rumen and reticulum. Another specialised compartment, the omasum, reduces the size of the food particles and absorbs water from the digesta before it passes into the abomasum or glandular stomach.

During the first few weeks of life, the fore-stomachs are undeveloped and the alimentary canal resembles that of animals with simple stomachs. As the animal grows and eats progressively larger amounts of grass, the fore-stomachs increase in size and development and a microbial population becomes established. Thus a comparison between certain aspects of digestion in the young and adult animal may provide information concerning the role of the rumen in these digestive processes.

This thesis is concerned with the digestion and absorption of dietary fat in sheep and lambs. Soaking lambs ingest large amounts...
PREFACE.

Many species of animals live on diets which are almost entirely of plant origin. These herbivorous animals consume large amounts of cellulose, even though cellulose-digesting enzymes are not produced by the glands of the alimentary canal. In these animals the gut has been modified and populations of micro-organisms capable of digesting the cellulose have become established. In different species, these modifications have occurred in different regions of the gut. In rodents and some ungulates such as the horse, cellulose is digested by micro-organisms in the large intestine; in cattle, sheep and goats however, micro-organisms digest the cellulose in a large, well-defined system of fore-stomachs, the rumen and reticulum. Another specialised compartment, the omasum, reduces the size of the food particles and absorbs water from the digesta before it passes into the abomasum or glandular stomach.

During the first few weeks of life, the fore-stomachs are undeveloped and the alimentary canal resembles that of animals with simple stomachs. As the animal grows and eats progressively larger amounts of grass, the fore-stomachs increase in size and development and a microbial population becomes established. Thus a comparison between certain aspects of digestion in the young and adult ruminant may provide information concerning the role of the rumen in these digestive processes.

This thesis is concerned with the digestion and absorption of dietary fat in sheep and lambs. Suckling lambs ingest large amounts
of milk fat, which pass through the closed oesophageal groove into the abomasum and small intestine. Grazing sheep may also ingest considerable amounts of fat, and up to 30-40 grams of plant lipid may pass into the rumen each day. There is some evidence that this fat may be modified in the rumen before it passes into the small intestine. Although the lipids of pasture grasses contain a high level of linoleic and linolenic acids, these polyunsaturated fatty acids are in low concentration in the fat depots of sheep. It is therefore of interest to study the way in which the modifications which have occurred during the development of the ruminant gut are reflected in the processes which occur during the passage of fat through the gut and its subsequent absorption from the intestine.

Early studies on fat absorption:

During the development of the current views on fat absorption in all species of animals, the lymphatic system has figured most prominently. Than the Italian anatomist, Caspar Asellius in 1632 was
CHAPTER 1.

INTRODUCTION.

The grass which sheep eat passes into the rumen and reticulum. In these fore-stomachs, the bacteria and protozoa convert the cellulose and other carbohydrates into short-chain fatty acids, principally acetic and propionic. The proteins of the grass are also broken down by the micro-organisms with the liberation of short-chain fatty acids. These small molecules are absorbed through the epithelial lining of the rumen into the portal blood and provide some of the energy for metabolic activity.

Although most of the grasses and concentrates of vegetable origin which comprise the diet of sheep contain 2-6 percent of their dry weight of esterified long-chain fatty acids (cf. Hilditch, 1956), very little is known of the absorption of these fatty acids from the lumen of the gut. It is likely however, that some of the details of fat absorption in animals with simple stomachs will also relate to the ruminant animal. For this reason, the literature pertaining to fat digestion and absorption in both ruminant and non-ruminant animals will be reviewed.

Early studies on fat absorption.

During the development of the current views on fat absorption in all species of animals, the lymphatic system has figured most prominently. When the Italian anatomist, Gaspar Asellius in 1622 was
demonstrating the movements of the diaphragm in a well-fed dog, he
"suddenly beheld a great number of cords as it were, exceedingly thin
and beautifully white, scattered over the whole of the mesentery and
intestine, starting from almost innumerable beginnings". When Asellius
out one of these "cords", he saw "a white liquid like milk or cream
forthwith gush out". When the experiment was repeated using an animal
with an empty stomach, Asellius failed to locate the vessels, but he
was again successful when he dissected a dog a few hours after it was fed.

Thus the mesenteric lymphatics were discovered. Asellius
believed however, that these vessels carried material to the liver to be
"concocted" into blood. This hypothesis was proved incorrect some
thirty years later, following the discovery of the thoracic duct by
Pecquet (1651), van Horne (1652) and Rudbeck (1653) working independently
in different parts of Europe. The direction of flow and the destination
of the chyle was determined when Pecquet (1651) in a dog and Rudbeck
(1653) in a calf found that chyle flowed "From the guts to a certain
RECEPTACLE of that bigness, which would fill up the interstitium between
the Lumbar Muscles at least in Beasts" (Pecquet, 1651) and from this
"receptacle" through the "Milkie Veins" in the thorax to enter the
great veins in the neck.

Most workers at this time believed that these vessels carried
nourishment from the gut, but William Harvey did not concur with this
view. In 1652, shortly after he had perused Pecquet's findings, he
wrote to Robert Morison in Paris, stating inter alia that "those white
threads very like spiders' webs have definitely not been instituted for
the transport of nourishment. Nor should the fluid discernible within them be called by the name "Chyle". But rather it is the mesenteric vessels which are destined for that function". Harvey's main objection to the absorptive role ascribed to these "lacteal veins" by Pecquet was that "all blood-carrying animals which require nourishment are provided with mesenteric veins, but only a few with lacteal veins, and the provision of these is inconstant". Harvey's views were not shared by other workers, and his objections were largely over-ruled by the demonstration of "lacteals" in birds, fish and the turtle (Bartholin, 1652; Hewson, 1768; 1769 a, b).

Towards the end of the eighteenth century, it was believed (Hewson, 1774; Cruikshank, 1786) that the lacteals opened into the intestinal lumen by "Patulous orifices" at the apices of the villi which had been described by Lieberkuhn in 1745. Hewson (1774) was of the opinion that these orifices acted as capillary tubes "attracting" the intestinal contents to the first pair of valves, whence the chyle was moved along by peristaltic contractions of the lymphatic walls and by the movements of the adjacent arteries. In accordance with the view that the lymphatics opened directly into the gut lumen, William and John Hunter concluded from their experiments on sheep and other animals that the lacteals were responsible for all the absorption from the gut, and that the veins played no part in this process. Thus all of the material absorbed from the intestine was thought to pass into the open ends of the lacteals (Hunter, W., 1784). As may be expected, these authors did not distinguish between the chyme in the lumen of the gut, and the chyle
in the lacteals. John Hunter (1786) recorded that "the chyle is compounded of the gastric juice and digestible substances when properly converted", and Ross (1844) noted that "the stomach (has been styled) the organ of chymification; the duodenum the organ of chylification".

These theories were shortly to be disproved. Although Hewson (1774) supported Lieberkuhn's view on the termination of the lacteals, he described the injection of mercury into the lacteals of the villi, but made no mention of the escape of mercury through these "patulous orifices". A similar observation led Muller (1838) to conclude that the villi are not perforated at their extremity; this was confirmed histologically by Goodsir (1842). Gulliver (1846) asserted that the "radical extremities of the lacteals form loops, or closed passages in the villi".

The hypothesis that the substances in the lumen of the intestine are absorbed solely into the lymphatic system was not supported by the experiments of Magendie (1821) on the absorption of poisons, nor by those of Tiedemann and Gmelin (1827). When Ross (1844) analysed the results of Tiedemann and Gmelin, he concluded "that the lacteals absorb fatty matter alone of the usual solid matters entering into the composition of chyle" and "that the radicles of the portal vein absorb the products of the vegetable aliment, together with the reduced albumen that has not entered into combination with the oil".

It is interesting to note that Tiedemann and Gmelin (1827) ligated the bile duct and found no "oily matter" in the lacteals. Ross
(1844) suggested that "the oily matter is either formed from choleic acid or from the action of this principle on our aliment", but of the two alternatives he preferred the former.

The realization that selective absorption of fat into the lacteals did occur, and that the chyle in these lacteals was quite distinct from the chyme in the lumen of the intestine, was followed by experiments and speculations on the composition of this chyle. The presence of "chyle-globules" in the chyle was recorded in the early 1840's and Ross (1844) believed that they were "formed of a nucleus of oily matter and an envelope of albumen". Ross thought that these globules were formed during digestion.

That the milkiness of chyle was due to fat was evident to Claude Bernard (1846). When Bernard was doing post-mortem examinations on some rabbits which had been trained to eat meat, he noticed that the lacteals near the pylorus were clear but those further down the intestine were filled with milky chyle. Bernard recalled that in dogs the milky chyle appears in the lacteals very close to the pylorus, so he examined the pancreatic ducts in dogs and rabbits. He found that in rabbits, the pancreatic ducts entered the duodenum about 30-50 cm. lower than in the dog, and deduced that pancreatic juice must be responsible for rendering neutral fats into an adsorbable form (Bernard, 1865).

Prior to Bernard's observations, digestion was regarded as the province of the stomach (cf. Gastiglioni, 1947), and little was understood concerning the role of the pancreas. Carpenter (1842) in his text-book "Human Physiology" summarized the views on pancreatic digestion held at that time: "The chyle is mingled in the duodenum with the biliary and
pancreatic secretions, which effect an immediate alteration both in its sensible and physical properties".

Methods for collecting pancreatic juice had been described (by de Graaf, 1664, and Magendie, 1817), and Bernard studied the effect on various food constituents of crushed pancreatic tissue and of pancreatic juice. He found that when mixed with neutral fat, these extracts caused splitting of the fat to acid and glycerine (Bernard, 1849). During the next few decades, it became evident that "the splitting of fat is a most important preliminary step in fat digestion" (Rachford, 1891) and by this time, the formation of emulsions of fatty acids in alkaline media had been documented (cf. Rachford, 1891).

Although it was recognised that bile was necessary in fat absorption, its role was unknown until Rachford (1891) discovered that the lipolytic action of pancreatic juice was enhanced in the presence of bile. Rachford also claimed to have demonstrated that bile would prevent the formation of emulsions of fatty acid soaps. This was contrary to the prevailing opinion which held that bile could produce emulsions of fat and Moore and Rockwood (1897) showed that in addition to stabilising emulsions, the bile constituents tended to render fatty acids soluble in water.

During this period, the opinions of workers were divided on the question of whether the fat was absorbed as an emulsion or a solution, but it was generally agreed that at least some of the neutral fat was hydrolysed prior to absorption and that the absorbed free fatty acids were largely synthesized to neutral fat before they appeared in the
lymph (cf. Moore and Rockwood, 1897; Bloor, 1922).

In one of the earliest quantitative studies on fat absorption, Munk and Rosenstein (1891) collected chyle from a fistula in the leg of a girl suffering from elephantiasis. When the lipid composition of this chyle was measured during the absorption of a fatty meal, it was not possible to recover more than 60 percent of the fat which was fed. Almost all of the lipid which appeared in the lymph could be extracted into ether, and consisted predominantly of triglyceride with a small amount of cholesterol and phospholipid. These findings, in spite of the obvious technical shortcomings of the experiment, came to exert a profound influence on the subsequent interpretation of the physiology of fat absorption.

Since the beginning of the twentieth century, the investigations into the absorption of fat from the gut have been concerned with the processes occurring in the lumen of the gut and the mucosal cells, the nature of the absorbed fatty acids and the pathway by which the absorbed fat is carried to the bloodstream. These processes as they occur in ruminant and non-ruminant animals will be discussed separately.

The effect of the ruminal micro-organisms on the dietary fat in ruminants.

Most of the plant materials which are eaten by ruminant animals contain about 4-6 percent of their dry weight of lipids; glycerides comprise 1.5 - 4 percent and phosphatides and salts of phosphatidic acid, 0.5 - 1.0 percent (Hilditch, 1956). The lipids extracted from these plants contain a very high proportion of unsaturated fatty acids (Smith and Chibnall, 1932), including at least 30-40 percent of linolenic acid,
while stearic acid comprises less than 3 percent of the total fatty acids (Shorland, 1944; Jasperson and Burke, unpublished; quoted by Hilditch, 1956; Garton, 1960 b).

The hydrolysis of triglycerides and phospholipids by ruminal micro-organisms has been demonstrated in in vitro experiments (Garton, Hobson and Lough, 1958; Dawson, 1959; Garton, Lough and Vioque, 1959; 1961), and a high level of free fatty acids is present in the ruminal fluid (Garton, 1960 a). These findings are thought to be of significance in relation to the normal processes of fat digestion in the ruminant.

Reiser (1951) incubated linseed oil with sheep rumen contents and found that the proportion of linolenic acid in the oil decreased. He ascribed this to hydrogenation of the fatty acids of the linseed oil by the ruminal micro-organisms. Further in vitro experiments by Shorland, Weenink and Johns (1955), Shorland, Weenink, Johns and McDonald (1957), Garton, Hobson and Lough (1958) and Garton, Lough and Vioque (1959; 1961) have confirmed that hydrogenation of unsaturated fatty acids will take place in the presence of ruminal micro-organisms in vitro.

Other investigators have demonstrated that the degree of unsaturation of the fatty acids in the ruminant fat depots is much lower than in the dietary lipids (Reiser and Reddy, 1956; Tove and Matrone, 1962). The content of unsaturated fatty acids in the fat depots will increase however if the rumen is bypassed and repeated feeds of unsaturated fatty acids are given into the abomasum (Ogilvie, McClymont and Shorland, 1961).

When the body fats were compared with the dietary lipids, other changes were noted. Although unsaturated fatty acids in the
dietary lipids are thought to be exclusively cis-isomers, up to 10 percent of the total fatty acids in the ruminant fat depots, milk fat and rumen contents are trans-acids (Swern, Knight and Eddy, 1952; Cornwell, Backderf, Wilson and Brown, 1953; Hartman, Shorland and McDonald, 1954; 1955). In addition to these geometric isomers, positional isomers have also been detected in lipids of ruminant origin, and samples of trans-octadecenoic acid with the double bond in the 9-10, 10-11 and 11-12 positions have been described (Bertram, 1928; Gupta, Hilditch, Paul and Shrivastava, 1950; Swern, Knight and Eddy, 1952). Conjugated dienoic acids have also been detected in the milk fat of cows (Hoflund, Holmberg and Sellmann, 1955) and they were formed in in vitro experiments in which polyunsaturated fatty acids were incubated with sheep rumen contents (Shorland, Weenink, Johns and McDonald, 1957). These conjugated fatty acids, which contain two double bonds separated by one single bond, are resistant to further hydrogenation in the rumen (Shorland, Weenink, Johns and McDonald, 1957).

All these data provide indirect evidence that substantial modifications occur in the dietary lipids during their digestion, and that this probably occurs in the rumen. The crucial experiments demonstrating that these changes occur between the time the fat enters the gut and is absorbed from it, have not however been done.

The lipases secreted into the gastro-intestinal tract.

It is well known that in monogastric animals, triglycerides are partially or completely hydrolysed by pancreatic lipase before they are absorbed into the cells of the intestinal mucosa. Although lipolytic
enzymes have been demonstrated in the saliva of calves (Young, Ramsey and Wise, 1960; Ramsey, Young and Wise, 1960), and in the gastric and intestinal secretions of other animals (DiNella, Meng and Park, 1960; Desnuelle, 1961), their role in fat digestion is unknown.

Since the discovery by Bernard (1849) that pancreatic juice from dogs and rabbits would hydrolyse triglycerides, many of the factors affecting the action of pancreatic lipase in monogastric animals have been investigated. Few observations have been made on pancreatic juice from ruminant animals, but a lipase has been identified in the pancreatic juice of sheep (Hill, 1961). In addition to lipase, phospholipases which catalyse the hydrolysis of the fatty acid ester bonds of glycerophosphatides, have been demonstrated in ruminant pancreatic tissue (Shapiro, 1952; 1953; Rimon and Shapiro, 1959). It is also interesting to note that the toxin of Clostridium perfringens, a common inhabitant of the intestine of sheep, contains a phospholipase D which catalyses the removal of the phosphate and choline groups from the choline phosphatides (Macfarlane and Knight, 1941).

**The extent of hydrolysis of triglycerides during digestion.**

During the last sixty or seventy years, the extent to which the dietary triglycerides are hydrolysed in the gut prior to absorption has been argued at length, and only since isotopically labelled fats have been used has any agreement been reached. In 1900, Pfluger advanced the hypothesis that fats are completely hydrolysed before they are absorbed. This stimulated a burst of experimental activity and many publications appeared to support or refute the concept of total hydrolysis (cf.
Whitehead, 1909a and b; Mendel, 1909). The validity of the hypothesis was questioned by Mellanby (1927) who claimed that in cats, hydrolysis of fat prior to absorption was not necessary. Verzar and McDougall (1936) discounted Mellanby's work, and found that much of the fat in the intestine of cats was present as non-esterified fatty acids. These authors believed that "fat is absorbed only after being split up in the intestine by lipase", but conceded that it was difficult to prove that all of the ingested fat had been hydrolysed.

Frazer (1938; 1940) considered that the "lipolytic hypothesis" as developed by Bloor (1922; 1939) and Verzar and McDougall (1936) had not been adequately proved and suggested as an alternative the so-called "partition hypothesis". Frazer's hypothesis, which was an extension of a hypothesis originally proposed by Brücke (1855), was based on the results of experiments in which fats and fatty acids labelled with Sudan IV were fed to rats. The number of chylomicrons in the portal and systemic blood were counted, and histological examinations made of various tissues. It was proposed that neutral fat may be absorbed from the gut into the intestinal lymphatics without prior hydrolysis, and that the fatty acids which were liberated during hydrolysis would pass directly to the liver in the portal blood (Frazer, 1943a, b). In later experiments, Frazer and Sammons (1945) examined the hydrolysis of olive oil by pancreatic lipase, and the lipid composition of the intestinal contents during fat digestion. They concluded that "fatty acids and di- and monoglycerides are the only products of hydrolysis of olive oil during the first 5 hr. of digestion", and (Frazer, 1946) "it
is obvious that a significant proportion of the fat must be absorbed as unhydrolysed or partially hydrolysed triglycerides 

Experiments using fats labelled with isotopic-tracers yielded the first quantitative information on the form in which dietary triglycerides are absorbed. Using deuterium-labelled fats, Bernhard, Wagner and Ritzel (1952) calculated that 24-53 percent of the fat fed to rats had been completely hydrolysed before it appeared in the thoracic duct lymph, and Blomstrand, Borgstrom and Dahlback (1959) estimated that in man 1/5 and 1/2 is completely hydrolysed. These values however, represent not only the extent of hydrolysis occurring in the intestinal lumen, but any loss of glycerol occurring during the passage of fat through the mucosal cells (Blomstrand, Borgstrom and Dahlback, 1959; Borgstrom, 1960 a).

When Bergstrom, Borgstrom, Tryding and Westoo (1954) discovered that the glyceride ester bonds of 2,2 dimethyl (long-chain fatty acids) were resistant to the action of pancreatic lipase, they provided a tool to measure the extent of hydrolysis taking place in the intestinal lumen. Borgstrom, Tryding and Westoo (1957) fed humans with glycerides containing traces of 1-\(^{14}\)C, 2,2 dimethyl (long-chain fatty acids), and measured the concentration of labelled acid in the neutral fat fraction of the intestinal contents. They calculated that about 40 percent of the fed glycerides were completely hydrolysed to glycerol and fatty acids, while the remaining glycerides were partially hydrolysed with a fatty acid deficit of about 40 percent. About 10 percent of the ingested triglycerides were absorbed intact. From other data, Reiser and Dieckert (1956) estimated that in rats about 3 percent of the triglycerides were absorbed intact, but in these calculations the synthesis of new glyceride
ester bonds in the gut lumen was ignored (cf. Borgstrom, 1952 e; 1954 c; 1955) and it is likely that this estimate may be too low.

Thus it is probable that in monogastric animals at least, more than half of the dietary glycerides are absorbed after hydrolysis to fatty acids, but that more than half of the glycerol of the fed glycerides is absorbed attached to one or more fatty acids (Borgstrom, 1960 a). This incomplete hydrolysis is probably the result of the strong specificity shown by the lipase for the fatty acids esterified to the primary hydroxyl groups of the glycerol molecule (cf. Desnuelle, 1961).

The absorption of fat into the cells of the intestinal mucosa.

During the latter years of the eighteenth century it was believed that all of the intestinal contents including fat were absorbed through the open ends of the lacteals (cf. Hewson, 1774). When it was shown that the lacteals form closed loops within the villi (cf. Gulliver, 1846), and that fat was preferentially absorbed into these lacteals, various hypotheses supported by morphological evidence (cf. Eimer, 1869; Baker, 1951) were advanced to explain the passage of the fat into the mucosal cells.

It was suggested (cf. Verzar and McDougall, 1936) that fats may be hydrolysed and the fatty acids absorbed either after solution in the "lipoids" of the mucosal cell walls, or as water-soluble sodium soaps. By the end of last century, many workers agreed that unhydrolysed fat may be absorbed as a fine emulsion, and the assistance of such factors as amoeboid protrusions from epithelial cells, and phagocytosis by
leucocytes were suggested to explain the entry of these fat particles into the intestinal cells (cf. Verzar and McDougall, 1936; Deuel, 1955). These theories were largely superceded by the "lipolytic hypothesis" (Bloor, 1922; 1939; Verzar and McDougall, 1936) which proposed that the fatty acids liberated by lipolysis were combined with bile acids to form water-soluble, diffusible complexes. It was thought that these complexes were broken down in the mucosal cells, and that the bile acids remained adsorbed to the cells. If the bile acids acted as a transport vehicle in this way, they could effectively "dissolve" much more fatty acid than was possible in in vitro experiments (Verzar and McDougall, 1936). Verzar and McDougall (1936) scorned any suggestion that fat may be absorbed in particulate form, and stated "it is amazing how some writers on fat absorption still keep to the old idea of fats being absorbed as an emulsion".

When Frazer (1938; 1940) suggested that some fat may be absorbed in an unhydrolysed form, he agreed that it was unlikely that the particles in an emulsion of neutral fat would be fine enough to pass through the cell membrane and suggested that the neutral fat may enter the cells in a "state of molecular division". Frazer, Schulman and Stewart (1944) however, could not produce any evidence to support the hypothesis that neutral fat could be dispersed in a state of molecular division under the conditions operating in the intestine, but they found that triglycerides formed a stable emulsion in the presence of bile salts, free fatty acids and monoglycerides. The particles in this emulsion were less than 0.5 µ in diameter and were negatively charged (Frazer,
With this evidence, Frazer (1946; 1948) modified his previous hypothesis and suggested that these stabilised particles of neutral fat may be absorbed through the fine canals which Baker (1942) claimed to have demonstrated with the light microscope. These canals were thought to penetrate the free or brush border of the mucosal cells. This view was strongly contested by Verzar (1948) who claimed that absorption of particulate fat did not occur. Subsequent investigations using the electron microscope have failed to reveal the canals described by Baker (1942) or any other discontinuities large enough to allow the passage of emulsion particles (Borgstrom, 1962).

In vivo and in vitro systems have been used to investigate the physical state of the lipids during digestion, following the realization that dietary fat is absorbed mainly as free fatty acids and partial glycerides (cf. Bernhard, Wagner and Ritzel, 1952; Borgstrom, Tryding and Westoo, 1957). When bile salts are in low concentration, they act as surface-active agents and will emulsify oils in water. With increasing concentrations of bile salts, a point is reached at which the molecules of bile salts aggregate spontaneously and form stable micelles up to 100 Å in diameter. In these micelles, some substances which are not normally soluble in water, can be held in aqueous solution (cf. Hartley, 1955; Borgstrom, 1962; Hofmann, 1961). Under the conditions normally present in the lumen of the small intestine, micellar solutions of conjugated bile salts and/or lyssolecithin may occur (Hofmann, 1961) and free fatty acids and monoglycerides are rendered soluble (Hofmann and Borgstrom, 1962). If the intestinal contents collected from humans during digestion of a
fatty meal are partitioned in an ultracentrifuge, the clear infranatant layer obtained contains a high concentration of lipids, presumably in micellar solution (Dowse, Saunders and Schofield, 1956; Borgstrom, 1960b). Borgstrom (1962) and Hofmann and Borgstrom (1962) suggested that the products of hydrolysis may be absorbed from the gut in this form. As the content of bile acids in the intestine is insufficient to allow for absorption of the bile acids with the lipids, Borgstrom (1962) suggested that the bile acids presumably act as transport vehicles for the lipids, and are themselves absorbed lower down in the intestine (cf. Verzar and McDougall, 1936).

When Palay and Karlin (1959) studied the intestinal mucosal cells of rats during the early stages of fat absorption, they were able to demonstrate droplets of fat up to 65 mµ. in diameter at the surface of the cells between the microvilli, and within pinocytotic vesicles in the immediately subjacent terminal web. They suggested that fat droplets which are not filtered out by the terminal web may enter the epithelial cells by pinocytosis at the bases of the intermicrovillous spaces; this was confirmed by Millington, Forbes, Finean and Frazer (1962). In an alternative hypothesis, Lacy and Taylor (1962) suggested that most of the lipid entered the mucosal cells through the microvilli in a form which approached molecular dimensions. Some of the lipid was thought to coalesce in the microvilli to form discrete particles just above the terminal web. Fat droplets, surrounded by a capsule of endoplasmic reticulum, pass through the cells to the extracellular spaces at the sides of the cells (Palay and Karlin, 1959) then pass through
the lamina propria to enter the lacteals through open junctions between the endothelial cells (Casley-Smith, 1962).

**The esterification of absorbed fatty acids in the intestinal mucosa.**

It has been generally agreed (Verzar and McDougall, 1936; Hubbscher and Clark, 1961) that irrespective of the form in which the fat enters the cells of the intestinal mucosa, the long-chain fatty acids are esterified to form chylomicron triglycerides before they appear in the intestinal lymph.

Phospholipids have been regarded as important in the esterification of absorbed fatty acids (Sinclair, 1929; Verzar and McDougall, 1936), but for many years their role in this process remained obscure. It had been demonstrated that free glycerol was not the precursor of glyceride glycerol (Bernhard, Wagner and Ritzel, 1952; Reiser, Bryson, Carr and Kuiken, 1952), although recent in vitro experiments suggest that free glycerol can be incorporated into glyceride glycerol in the intestinal mucosa (Saunders and Dawson, 1962). The origin of the glycerol required for the esterification of the absorbed fatty acids was not known until Weiss and Kennedy (1956) using liver preparations demonstrated that 1,2 diglycerides formed from L-α-glycerophosphate through a phosphatidic acid intermediate (Weiss, Smith and Kennedy, 1956) were precursors in the synthesis of triglycerides and glycerophosphatides. The esterification of L-α-glycerophosphate to form phosphatidic acid, and the formation of triglyceride from 1,2 diglyceride, were dependent on coenzyme A, and lecithin was formed from the 1,2 diglyceride and cytidine diphosphate choline (Kennedy, 1957). Kennedy proposed that a similar mechanism may operate in the intestinal mucosa and suggested that 1,2 diglyceride may also be formed from 2-monoglyceride in a coenzyme A dependent reaction. In 1959, Buell and Reiser demonstrated conclusively that in the intestinal mucosa, glyceride
Glycerol is derived from L-α-glycerophosphate, and Johnston and Beardon (1960) found that when segments of intestine were incubated with fatty acids and \(^{32}\text{P}\), the specific activity of the phosphatidic acids in the segments at the end of the experiments was very high. Dawson and Isselbacher (1960 a) using homogenates of rat and human intestinal mucosa demonstrated that the incorporation of palmitic acid \(^{14}\text{C}\) into neutral fat was dependent on coenzyme A, adenosine triphosphate and magnesium ions. Dawson and Isselbacher (1960 a) found an appreciable quantity of labelled fatty acids in monoglycerides, but suggested that this may be the result of lipolysis. The experiments of DiNella, Meng and Park (1960) suggest that a lipase may be present in the intestinal cells. In the system used by Dawson and Isselbacher (1960 a), short-chain fatty acids were not incorporated into triglycerides, and the esterification of palmitate was facilitated by the addition of taurocholate to the incubation medium. Final confirmation of the Kennedy (1957) hypothesis was provided by Hubscher and Clark (1961) who described a series of energy dependent reactions for the esterification of L-α-glycerophosphate and of monoglyceride to form triglycerides in the intestinal mucosal cells.

The chylomicron.

The chylomicrons are a family of light-scattering particles up to about 1 μ in diameter which form the disperse phase of the emulsion which is chyle (Gage and Fish, 1924; Elkes, Frazer and Stewart, 1939). These chylomicrons can be isolated from intestinal lymph by centrifugation (Robinson, 1955; Hillyard, Chaikoff, Enteman and Reinhardt, 1958;
Bragdon, 1958), or by flocculation with protamine sulphate (Brown, 1953) or toluidine blue (Laurell, 1953).

Laurell (1953) found that chylomicrons from rat chyle contained 86 percent neutral lipids, with 8.5 percent phospholipid, 1.8 percent cholesterol and about 2 percent protein. Bragdon (1958) confirmed that chylomicrons contained a small amount (0.5 percent) of protein, but Robinson (1955) could not detect any protein in chylomicrons after repeated washing in saline. It was thought that chylomicrons may be stabilized by a surface film of protein, following studies in which they were shown to flocculate at a pH within the range of the isoelectric points of serum albumin and serum globulin (Ludlum, Taft and Nugent, 1931). Their behaviour on free electrophoresis and with protein precipitants also suggested that protein was involved in their composition (Ludlum, Taft and Nugent, 1931; Elkes, Frazer and Stewart, 1939). Phospholipids have also been shown to be important in maintaining the stability of chylomicrons. When chylomicrons, or artificial emulsions stabilized with phospholipids, were incubated with a phospholipase, the stability of the emulsions was destroyed (Elkes and Frazer, 1943; Frazer, 1949; Robinson, 1955). Robinson (1955) concluded that the stability of the chylomicrons was dependent on a surface layer of phospholipid.

Changes in the intestinal lymph during fat absorption.

During the absorption of fat from the gut, the concentration of lipids in the intestinal lymph increases markedly. The experiments of Munk and Rosenstein (1891) who collected lymph from a chylous fistula
in the leg of a girl, suggested that almost all of the lipid in the intestinal lymph during fat absorption was triglyceride, with small amounts of cholesterol and phospholipid.

Subsequent investigations on chyle obtained from thoracic duct fistulae in unanaesthetised animals of various non-ruminant species, have confirmed that a major increase in the lymph lipid concentration occurs during the absorption of fat whether fed as triglycerides or as free fatty acids. The major part of this increase occurs in the triglyceride fraction (Bollman, Flock, Cain and Grindlay, 1950; Borgstrom, 1952 d; Morris, 1954). Phospholipids are synthesized in the cells of the intestinal mucosa during fat absorption, and the concentration of phospholipids in the intestinal lymph increases with the increase in total lipids (Bollman, Flock, Cain and Grindlay, 1950; Borgstrom, 1952 d; Morris, 1954). An increase has also been noted in the cholesterol concentration (Brockett, Spiers and Himwich, 1934; Bollman and Flock, 1951; Morris, 1954), and Borgstrom and Tryding (1956) found that some non-esterified fatty acids also appear in the intestinal lymph during fat absorption, but the lymph protein concentration does not change significantly (Borgstrom and Laurell, 1953; Simmonds, 1954; 1955 b).

**The role of intestinal lymph in the transport of absorbed fatty acids.**

The lymphatic system has been regarded as an important pathway for the transport of absorbed fatty acids since it was first discovered by Asellus, but little quantitative information was available until comparatively recently. Some data were obtained from humans with chylous
fistulae and from anaesthetised animals, but these methods have many short-comings. Munk and Rosenstein (1891) were able to recover only 60 percent of the absorbed fat from their patient with a chylous fistula, but it was not possible to ascertain whether the remaining 40 percent was absorbed directly into the portal blood, or was absorbed into the intestinal lymph and did not appear at the fistula. The recovery of fat in the lymph of dogs, cats and rabbits during anaesthesia is low (Eckstein, 1925; Little and Robinson, 1941; Bergstrom, Borgstrom, Carlestone and Rottenberg, 1950; Morris, 1954), but Simmonds (1957) found that anaesthesia did not significantly alter the pattern of fat absorption into the lymph of rats. The effect of anaesthesia on the processes of absorption however, has not been evaluated.

Frazer’s partition hypothesis attempted to define the role of the lymphatic system in fat absorption by proposing that the fat which was not hydrolysed prior to absorption (about 60 percent of the absorbed fat; Frazer, 1946) appeared in the lymph, while the free fatty acids were absorbed directly into the portal blood (Frazer, 1938; 1940; 1943 a; b; 1946; 1946).

More reliable estimates were made possible by the development of methods for the collection of lymph from unanaesthetised animals (Bollman, Cain and Grindlay, 1948) and by the general availability of isotopically labelled lipids. Bloom, Chaikoff, Reinhardt, Entenman and Dauben (1950) fed $^{14}$C labelled palmitic acid to rats and recovered 58-92 percent of the absorbed radioactivity in the lymph. Borgstrom (1952 c; d) showed that there was no major difference in the recovery of
radioactivity in the lymph of rats when labelled fatty acids were fed as triglycerides or as free fatty acids. Simmonds (1955 a) found that when oleic acid labelled with Sudan IV was given to rats, the amount of Sudan IV and of fat recovered in the intestinal lymph was of the same order as when dyed olive oil was fed. A number of workers have confirmed these results using saturated and unsaturated fatty acids with more than 14 carbon atoms, and agree that about 70-90 percent of the fatty acids which are absorbed, appear in the lymph draining the intestines (Bergstrom and Borgstrom, 1955; Borgstrom, 1960 a). More than 90 percent of the radioactivity in the lymph was generally present as neutral fat, and 2-6 percent as phospholipid (Bloom, Chaikoff, Reinhardt and Dauben, 1951; Bergstrom, Blomstrand and Borgstrom, 1954; Blomstrand, 1954). When Borgstrom (1954 c) fed rats with trace amounts of 14C stearic acid in corn oil, 88 percent of the labelled fatty acids in the lymph were carried by glycerides, 11 percent by phospholipids, and 2 percent by cholesterol esters. Up to 6 percent of the labelled fatty acids in the lymph may be present as free acids (Borgstrom and Tryding, 1956).

In all of these experiments, a variable amount of the absorbed fatty acids did not appear in the intestinal lymph, but at least some of these fatty acids had reached the blood. When rats with thoracic duct fistulae were killed one day after a radioactive feed, up to 1 percent of the absorbed radioactivity could be recovered from the liver (Bloom, Chaikoff, Reinhardt, Entenman and Dauben, 1950; Bloom, Chaikoff, Reinhardt and Dauben, 1951). From these results it is likely that a considerable
amount of the activity which had not been recovered in the lymph had been oxidised before the rats were killed. This was confirmed by Bergstrom, Blomstrand and Borgstrom (1954), and Blomstrand (1954) who found in rats that up to 6 percent of the absorbed activity was recovered in the expired breath as $^{14}\text{CO}_2$, 24 hours after a radioactive fat meal.

Two routes may be suggested for the entry of these fatty acids into the blood. They may be absorbed directly into the portal blood, or they may be absorbed into the lacteals but drain into the blood through anastomotic connections between the lymphatics and veins distal to the site of cannulation. It has been shown that fatty acids with less than 14 carbon atoms are recovered in the lymph in yields which decrease with the length of the carbon chain, and fatty acids with 10 carbons or less are absorbed preferentially into the portal blood (Kiyasu, Bloom and Chaikoff, 1952). In this connection, it is interesting to note that short-chain fatty acids are not esterified in the intestinal mucosa (Dawson and Isselbacher, 1960 a). However, there is no evidence to suggest that fatty acids with more than 14 carbon atoms are absorbed directly into the blood. Similarly, although anastomoses between the lymphatic and venous systems have been demonstrated in some rats (Job, 1918; Threefoot, Kent and Hatchett, 1963), cattle (Baum, 1911) and monkeys (Silvester, 1912), their significance remains unknown.

The current views on fat digestion and absorption in non-ruminants can be summarized as follows. In the small intestine, the dietary triglycerides in fine particulate form are hydrolysed and are absorbed mainly as free fatty acids and monoglycerides which may be incorporated
into soluble micelles with bile salts. The physical form of the fat entering the mucosal cells is not known. Most of the absorbed fatty acids and monoglycerides are esterified in the mucosal cells and appear in the intestinal lymph as chylomicron triglycerides. Fatty acids with less than 10 carbon atoms are absorbed directly into the portal blood.

During the absorption of fat from the gut, the flow rate of the intestinal lymph increases with the increase in the concentration of lipids in the lymph. About 70-90 percent of the fat absorbed from the gut appears in the intestinal lymph; about 90 percent is present as triglyceride, with small amounts of phospholipid, cholesterol ester and free fatty acid.

The fate of the fat which is absorbed from the gut, but does not appear in the intestinal lymph is not known, but it is likely that it reaches the blood directly or through lymphatic-venous anastomoses.

In ruminants the situation is less clear. During the passage of the dietary fats through the rumen, the highly unsaturated fatty acids may be hydrogenated with the formation of geometric and positional isomers, and some of the dietary triglycerides may be hydrolysed by the ruminal micro-organisms.

Little information is available about the digestion and absorption of lipids in the intestinal tract, and the role of the lymphatic system in fat absorption in ruminants has been neglected. The blood lipids have been measured during fat absorption in cattle (Aylward and Blackwood, 1936; Maynard and McCay, 1929), but it was not possible to
demonstrate an alimentary lipaemia. Aylward, Blackwood and Smith (1937) fed cows with iodinated fats, and found that the level of iodinated lipid in the blood increased to a maximum at 1.5-2 days, and declined slowly during the next 3 days.

Some measure of the efficiency of digestion of lipids in ruminants has been obtained from digestibility trials, in which "ether extractable matter" of the food and faeces are compared. Results from these trials suggest that about three-quarters of the lipid is digested in adult ruminants, and slightly more in young animals (Garton, 1960 a). However these trials yield little information of value, from the standpoint of the physiology of fat digestion. The measurement of the faecal lipids would include lipids derived from the digestive tract, intestinal bacteria and from the bile.

The experiments described in this thesis were done in an attempt to provide information on the role of the lymphatic system in the transport of fat from the gut of sheep, and to assess the part played by the bile, pancreatic juice and ruminal micro-organisms in the digestion and absorption of fat. The different aspects of fat digestion and absorption were studied in both sheep and lambs, and an attempt made to relate the development of the ruminant forestomachs to the changes which occur in these processes as the animal matures.
CHAPTER 2.

MATERIALS AND METHODS.

Experimental animals. Merino ewes and lambs were used in most of the experimental procedures described. Crossbred lambs were used in the experiments described in Chapter 5. The ages of ewes, which varied from two-tooth to broken-mouth, were estimated to be between 1½-6 years. The ewes were housed indoors in metabolism cages (Fig. 1) and were fed on lucerne chaff and a mixture of oaten hay, grain oats and lucerne hay. During some of the experiments, the ewes were allowed only lucerne chaff. The lucerne hay and lucerne chaff contained 6 percent of moisture and 2.8 percent of glyceride fat (including 0.8 percent of phospholipids and traces of diglycerides), and the grain oats contained 11 percent of moisture and 6.5 percent of glyceride fat (including 0.3 percent of phospholipid). Water and a salt lick were freely available.

The lambs used were 2-4 weeks old and were being reared by their mothers. They were housed indoors but were not restrained. In some experiments they were allowed to continue suckling, but in other experiments they were separated from their mothers and bottle-fed with a mixture of 2 parts of whole cow's milk and one part of water.

Anticoagulants. Heparin was used throughout, either as a dry powder (Pularin; Evans Medical Ltd., 125 units per mg., or Purified Dried Extract of Lung; Boots Pure Drug Co. Ltd., 121 units per mg.) or as a sterile solution (Pularin; Evans Medical Ltd., 1000 units per ml.).
Left figure: Close-up showing fistulae of the intestinal and hepatic lymph ducts in a sheep during the absorption of fat. When 20 ml. of olive oil were injected into the abomasum through an indwelling tube, the intestinal lymph (left) became milky, but the hepatic lymph (centre) remained clear.

Right figure: The collection of lymph from a hepatic lymph duct fistula in a sheep.
Antibiotics. Those used were streptomycin sulphate (Glaxo-Allenburys), crystalline penicillin G (Commonwealth Serum Laboratories), and a suspension of benethamine penicillin (500,000 units), procaine penicillin G (250,000 units) and sodium penicillin G (500,000 units) (Triplopen; Glaxo-Allenburys).

Radioactive isotopes were obtained from the Radiochemical Centre, Amersham.

Glyceryl tri-1-14C palmitate had a specific activity of 1 millicurie per millimole. 200 microcuries of the 14C tripalmitin were added to 10 ml. of olive oil and stored in a sealed bottle.

1-14C palmitic acid had a specific activity of 2.6 millicuries per millimole. It was prepared as a complex with albumin before use. 4 microcuries of labelled palmitic acid were added to 40 mg. of recrystallised palmitic acid and the fatty acid neutralised using 3.6 ml. of 0.1 N-NaOH. The solution of soaps was then heated slightly and 20 ml. of a 10 percent solution of bovine albumin (Fraction V; Armour Ltd.) was added. The palmitate-albumin solution was filtered through sintered glass and stored in the refrigerator. It was used within 2 days of preparation.

131I human serum albumin had a specific activity of 12-30 microcuries per milligram of albumin and was dissolved in sterile 0.9 percent NaCl solution. Each batch of 200 microcuries was diluted with 5 ml. of sterile 0.9 percent NaCl solution before use.

Radio-opaque contrast media used were mercury, a 25 percent and a 50
percent sterile solution of 2,4,6 triiodo acetylamino benzoic acid (Diaginol; May and Baker), and a 42.5 percent sterile solution of the diethanolamine salt of diodone (Arteriodione; May and Baker).

The buffer solutions used were:

**Sørensen's citrate** buffers, which were made up by adding different volumes of reagent A (21.006 grams of citric acid and 200 ml. of N-NaOH diluted to 1 litre with distilled water) to 0.1 N-HCl to a final volume of 100 ml. For buffer solutions of pH 3.0 and pH 4.2, 40.4 and 60.6 ml. respectively of reagent A were used.

**Sørensen's phosphate** buffers, which were made up using different volumes of 0.067 M-KH$_2$PO$_4$ (9.078 grams dissolved in distilled water and diluted to 1 litre). These were made up to 100 ml. using 0.067 M-Na$_2$HPO$_4$ • 2H$_2$O (11.876 grams dissolved in distilled water and diluted to 1 litre). For a buffer solution of pH 5.4, 96.9 ml. of the KH$_2$PO$_4$ solution were used; for pH 6.0, 88 ml.; for pH 6.8, 50 ml.; for pH 7.2, 28 ml. and for a buffer solution of pH 8, 5.5 ml. of the KH$_2$PO$_4$ solution were used.

**Krebs-Ringer phosphate buffer.** A phosphate buffer solution of pH 7.4 was made up by adding 20 ml. N-HCl to an aqueous solution of 17.8 grams of Na$_2$HPO$_4$ • 2H$_2$O, and diluting to a final volume of 1 litre with distilled water. To 210 ml. of this solution was added 1,000 ml. of 0.9 percent NaCl solution, 40 ml. of 1.15 percent KCl solution, 30 ml. of 0.61 percent CaCl$_2$ solution and 10 ml. of 3.82 percent MgSO$_4$ solution. The final pH was 7.1.
N-ethylmorpholine buffer (0.1 M) was made up by dissolving 1.152 grams of N-ethylmorpholine in distilled water, adjusting the pH to 7.0 with N-HCl, and diluting to a final volume of 100 ml.

Tris buffer was prepared as a 0.1 M solution by dissolving 12.1 grams of tris (hydroxymethyl) aminomethane (Sigma 7-9; The Sigma Chemical Company) in water and diluting to 1 litre. The final pH was 8.8.

Carbonate buffer (pH 10.0) was made up by mixing 6 parts of 0.2 M-Na₂CO₃ solution (2.12 percent w/v in distilled water) with 4 parts of 0.2 M-NaHCO₃ solution (1.68 percent w/v in distilled water).

The pH of the buffer solutions was checked using a Radiometer Titrator.

Camulæae of polyethylene (Polythene; Allen and Hanburys or Intramedic; Clay Adams) and polyvinyl chloride (Transflex; Minnesota Mining and Manufacturing Company or Portex; Portland Plastics Ltd.) of various sizes were used during the different surgical procedures.

General Methods.

Fat feeding. In experiments to test the effect of feeding fat on the flow and composition of intestinal lymph in sheep, 25 ml. of maize oil or 25 ml. of olive oil was injected through indwelling polythene tubes into the abomasum of ewes. In one experiment, 50 ml. of maize oil was fed to a ewe through an oesophageal tube. Radioactive fat was injected into the gut at various sites through indwelling tubes. 10 or 20 microcuries of ¹⁴C tripalmitin in olive oil was injected into either the abomasum or duodenum of ewes and lambs, and 60 microcuries of ¹⁴C tripalmitin in olive oil was injected into the rumen of adult sheep.
The injections of radioactive fat were followed by an injection of an equivalent volume of non-radioactive olive oil. The lambs had been starved for at least 6 hours, but were given a drink of 200-250 ml. of diluted cow's milk after the radioactive fat. All the preparations of fat were warmed to 37°C before they were injected.

Collection of samples.

Blood samples were obtained from the external jugular vein in the ewes and lambs, and the plasma immediately separated from the cells by centrifugation.

Lymph, bile and pancreatic juice samples were collected into plastic bottles strapped to the flanks of the ewes or into glass containers attached to the sides of the metabolism cages (Fig. 1). Samples of lymph, bile and pancreatic juice were collected from lambs into test-tubes supported in plastic containers strapped to the flanks of the lambs (Fig. 2). Where necessary, these fluids were collected into tubes suspended in a freezing mixture of salt and ice or acetone and dry-ice, and were immediately transferred to a sub-zero refrigerator.

Samples of faeces which were free from contamination by urine, were collected from the ewes into containers placed below the metabolism cages, and from the lambs into napkins tied around the buttocks (Fig. 2).

Recirculation of lymph was employed in some instances to avoid the loss of body weight which follows continued drainage of plasma proteins. A Polythene cannula (external diameter 1.5 mm.) was placed in the
Figure 2. A three-week-old lamb with an intestinal lymphatic fistula and an indwelling tube in the abomasum. The lamb was given a drink of milk and fatty chyle is collecting into a test-tube immersed in ice. The napkin tied around the buttocks was used to collect the faeces. The photograph was taken on the fourth day after the operation.
jugular vein, and a smaller Transflex tube was joined to the lymph cannula and passed through the jugular cannula into the right atrium. When clot formation was prevented by the slow infusion of a heparin solution into the lymph cannula, the slight negative pressure in the right atrium was sufficient to maintain the flow of lymph. In some experiments, the lymph was collected into chilled containers with antibiotics and heparin, and was later returned through the jugular vein cannula using a peristaltic type slow-infusion pump (Sigmamotor; Middleport, N.Y.). As injections of heparin are followed by the appearance of clearing factor lipase in the circulation, at least 24 hours elapsed between the end of each period of recirculation and the beginning of the next experiment.

Recirculation of bile or pancreatic juice was necessary in some experiments in order to study the effect of deprivation of one or other secretion on fat absorption. The bile or pancreatic juice flowed into a small reservoir, and was returned to the duodenum through an indwelling tube, using a peristaltic type pump (Sigmamotor).

Histological methods. Segments of intestinal wall were fixed in formal saline, and sections were cut from paraffin blocks. The sections were stained with haematoxylin and eosin.

Statistical methods. Routine statistical procedures were used as described by Mather (1951). The terms used in the analyses are defined:
The mean of a series of observations \( x_1, x_2, x_3 \ldots \ldots \ldots x_n \) is given by \( \bar{x} = \frac{1}{n} S(x) \), where "n" is the number of observations and \( S(x) \) is the sum of these observations.

The variance of the distribution of the mean \( (\bar{x}) \) of "n" observations is given as \( \frac{1}{n} \) th the variance of a single observation \( (x) \).

\[
V_{\bar{x}} = \frac{V_x}{n} \quad \text{where} \quad V_x = \frac{S(x - \bar{x})^2}{n - 1}
\]

The standard deviation (Sd) of the distribution considered is the square root of the sum of the squares of the deviations from the mean divided by the number of degrees of freedom, "N".

\[
Sd.x = \sqrt{\frac{S(x - \bar{x})^2}{N}} = \sqrt{V_x}
\]

The standard error of the mean was calculated as the square root of \( \frac{1}{n} \) th of the variance of the distribution of single observations:

\[
S.E.\bar{x} = \sqrt{\frac{V_x}{n}} = \sqrt{V_{\bar{x}}}
\]

The significance of the differences between means was calculated from the estimates of the variance of this difference \( (V\delta = V_{\bar{x}} + V_Y) \) and its standard deviation \( (Sd.\delta = \sqrt{V\delta}) \). The significance of the ratio of the difference between the means of the observations to the estimated
deviation of this difference was given by the "t" test.

Calculation of correlation. Interclass correlation was estimated by the correlation coefficient "r". From two series of estimates, this was given by the calculation of the covariance of "x" and "y" and the estimates of their standard deviations. "r" was derived from the expression:

$$ r = \frac{S[(x - \bar{x})(y - \bar{y})]}{\sqrt{S(x - \bar{x})^2 S(y - \bar{y})^2}} $$

Surgical Techniques.

Anaesthesia was induced in the ewes and lambs with intravenous injections of thiopentone sodium (Pentothal; Abbott) or pentobarbitone sodium (Nembutal; Abbott), and a Magill's tube was passed into the trachea. Surgical anaesthesia was usually maintained using cyclopropane in a closed circuit, but serial injections of Nembutal were used in some cases. The animals usually recovered rapidly, and were able to stand 2-4 hours after the end of the operation. The ewes and lambs were all starved for 12-24 hours before the beginning of the operations.

Blood transfusions were given to any of the ewes and lambs suffering from post-operative shock. Fresh whole sheep's blood (200-500 ml.) or sheep plasma (100-250 ml.) was given to the ewes by slow intravenous injection, and sheep's blood (50 ml.) was given to the lambs. No attempt was made to cross-match the donor and recipient blood.

Cannulation of the jugular vein was sometimes necessary to allow the
taking of frequent blood samples. A short length of the jugular vein in the middle of the neck was dissected free from surrounding tissues, and the blood flow was occluded with two pairs of modified bowel clamps. A sterile Polythene cannula (external diameter 3 mm.) was introduced into the vein through a small hole between the bowel clamps, and was held in place with a purse-string suture. The cannula was occluded with a nylon stilette (diameter 2 mm.), and the bowel clamps removed. The skin and subcutaneous tissues were closed with a single layer of sutures.

The collection of lymph from unanaesthetised sheep. In order to study the role of the lymphatic system in the absorption of fat, it was necessary to obtain animal preparations from which lymph could be collected over relatively long periods of time under physiological conditions. To this end, chronic fistulae of the thoracic, intestinal and hepatic ducts were established using methods described by Lascelles and Morris (1961) and by Heath and Morris (1962).

Thoracic duct fistula. The thoracic duct of sheep passes through the thorax on the dorsal surface of the aorta, and lies on the right side of the hemiazygos vein, ventral to the sympathetic trunk. It is usually a single duct although in some animals two or more ducts may be present.

An incision was made over the line of the eighth rib on the right side and was continued through the cutaneous, latissimus dorsi
and ventral serrate muscles to the external surface of the rib. At the dorsal end of the incision, it was necessary to cut through part of the levatores costarum muscles and the lateral edge of the longissimus dorsi muscle. The rib was cut through about 2-3 cm. lateral to the costo-vertebral joint, and about 15 cm. of rib was removed. Care was taken to avoid damage to the intercostal vessels located under the posterior edge of the rib. The pleura was incised and manual inflation of the lungs commenced. The edges of the incision were retracted, and the diaphragmatic lobe of the right lung was packed off anteriorly. The thoracic duct lymph of sheep usually contains red blood cells (Heath, Lascelles and Morris, 1962), and the duct can be seen as a pink coloured vessel lying under the pleura on the dorso-lateral surface of the aorta. The pleura over the duct was incised, and the thoracic duct with any accessory ducts was tied off anteriorly. The operation at this stage is shown in Fig. 3. When the exposed surface of the duct was dissected free from the surrounding fat, a thread was passed around the duct posterior to the tied ligature and left loose. A length of Transflex tubing (external diameter 2.2 mm.), that had been sterilized in 70 percent alcohol and washed through with a sterile solution of heparin (about 100 units per ml.), was used for the cannulation. The cannula was connected to a trochar and led into the thorax through the dorsal end of the sixth intercostal space. An opening was made in the duct between the two ties and the bevelled end of the cannula was passed into the duct to lie beyond the caudal ligature. The ligature was tied securely, and
Figure 3. The dissection which exposes the thoracic duct in the chest.

A  Thoracic duct  F  Diaphragmatic lobe of lung
B  Aorta  G  Oesophagus
C  Posterior mediastinal lymph node  H  Caudal vena cava
D  Haemolymph nodes  K  Phrenic vessels
E  Intercostal vessels  L  Diaphragm
two stay sutures were placed around the cannula to anchor it to the tissues dorsal to the duct. In some sheep, the thoracic duct contracted after it had been cut, and in these cases it was necessary to introduce a small metal seeker into the opening to facilitate the entry of the cannula.

The thoracic cavity was closed with two layers of sutures in the muscle, and the skin incision was closed with Michel clips or with interrupted silk sutures.

**Intestinal duct fistula.** The intestinal lymph duct of sheep drains the lymph from the foreestomachs, abomasum, small intestine, colon and caecum. The tributaries from the stomachs and those from the intestines unite, and proceed dorsally within the substance of the pancreas to emerge just ventral to the caudal vena cava (Figs. 4 and 5). The intestinal and hepatic ducts pass across the caudal vena cava and join the lumbar trunk to form the cysterna chyli. Many variations are possible in the arrangement of the terminal part of the intestinal duct which may be paired or multiple and may receive branches from the hepatic duct. The variations seen in the intestinal and hepatic ducts in some of the experimental sheep are shown in Fig. 4.

The abdominal cavity was opened through an incision on the right side just caudal to and following the line of the last rib. The intestines were packed off ventrally and the caudate lobe of the liver was retracted anteriorly. The right kidney was separated from its attachment to the caudal end of the pancreas and was retracted dorsally. When the peritoneum was cut along the ventral surface of the caudal
Some variations noted in the arrangement of the intestinal and liver lymph ducts in sheep and lambs. The intestinal ducts (I) emerge from the substance of the pancreas (P) and may join the efferent vessels (L) from the hepatic lymph node (N) caudal to the hepatic artery (A); the lymph ducts then pass to the left of the caudal vena cava (CVC) near the renal veins (LRV, RRV).
Figure 5. The dissection of the hepatic and intestinal lymph ducts.

A  Intestinal lymph duct
B  Fat overlying right kidney
C  Retracted edge of pancreas
D  Caudal vena cava
E  Hepatic lymph ducts
F  Hepatic artery
G  Caudate lobe of liver
H  Hepatic lymph node
K  Portal vein
L  Hepatic lymph node
M  Small intestine
N  Dorsal surface of pancreas
vena cava and the pancreas retracted latero-ventrally, the intestinal duct was visible on the dorsal border of the pancreas (Fig. 5). A ligature was tied around the intestinal duct as far dorsally as possible, usually just under the ventral border of the caudal vena cava, and the pancreatic tissue was dissected away from the duct for a distance of about 2-3 cm. A Transflex cannula (external diameter 2.2 mm.) was passed into the duct and tied in place. In order to prevent kinking of the duct, the cannula was carried around the caudal vena cava in the line of the original duct, before being led out through the dorsal end of the original incision. Fig. 1 shows sheep with fistulae of the intestinal and hepatic ducts, and Fig. 2 shows intestinal lymph being collected from a lamb.

Hepatic duct fistula. The hepatic lymph duct is formed from efferent lymphatics which run from the hepatic lymph nodes. The duct runs posteriorly, then bends dorsally to run parallel to the intestinal duct. Some of the hepatic lymph nodes are covered by the pancreas, but usually at least one large node is visible near the surface of the pancreas alongside the portal vein. The efferent ducts from these nodes run across the dorsal surface of the pancreas to join either the main hepatic lymph duct or the intestinal duct.

The same skin incision and approach was used for both the hepatic and intestinal ducts and both these ducts were cannulated at the one operation. The pancreas was separated from the ventral surface of the caudal vena cava and the main hepatic duct was visible running posteriorly across the dorsal surface of the pancreas near the hepatic
artery. A ligature was placed around the dorsal extremity of the hepatic trunk and any accessory lymphatics were tied off. The exposed surface of the hepatic duct was dissected free from peritoneum and surrounding tissue for about 1 cm. The duct was cut open and a Transflex cannula (external diameter 1.3 mm.) was placed in the duct and tied in position. The cannula was held in place with stay sutures and was led out through the original incision.

Although the methods described were used in most of the operations, in some cases variations in the arrangement of the different lymphatics required slight changes in technique.

Fistulae of the common bile duct were established to collect bile, or bile and pancreatic juice from ewes and lambs. The surgical approach was similar to that used for the intestinal lymph duct, which was also cannulated during these operations.

To collect bile, the lymph fistula was established first, then the right lateral lobe of the liver was retracted anteriorly to expose the confluence of the hepatic and cystic ducts to form the common bile duct near the porta of the liver. The peritoneum over the origin of the common bile duct was incised, and two silk loops about 1 cm. apart were placed around the duct and left loose. When traction was applied to these loops the flow of bile was obstructed, and a Transflex cannula (external diameter 2.2 mm.) was inserted into the duct through a small hole between the two loops. The loops were tied firmly to fix the cannula in position and to prevent the escape of pancreatic juice into the peritoneal cavity.
To collect pancreatic juice, lymph and bile fistulae were established, then a second Transflex cannula was placed in the common bile duct 1–2 cm. before it entered the duodenal wall. All the pancreatic juice drains into the common bile duct in sheep (Taylor, 1960).

The sheep deprived of bile and pancreatic juice showed clinically normal ruminal and intestinal movements, and passed normal amounts of faeces during the experiments.

Gut fistulae were established at various sites at the same time as the lymphatic ducts were cannulated. When a thoracic duct fistula was established, an incision was made through the right abdominal wall parallel to and just behind the last rib, but when fistulae of the intestinal and hepatic ducts were established, a separate incision was not required. Rubber ended Polythene tubes (external diameter 2.0 mm.) were introduced into the lumen of the gut through small incisions in the wall, and were held in place with purse-string sutures. In both ewes and lambs tubes were placed in the abomasum and duodenum, and into the right face of the dorsal sac of the rumen.

In some experiments it was necessary to collect contents from the abomasum or small intestine for pH measurement. An incision, parallel to the last rib, was made in the mid-flank region on the right side, and the abomasum or part of the small intestine was exteriorized. A piece of Portex tubing (external diameter 13.0 mm.) had been prepared with a groove encircling the tube, 2 mm. from one end. The grooved end of the tube was inserted through an incision in
the wall of the gut, and was held in place with a purse-string suture tied over the groove. The tube was led out to the exterior through a stab wound in the flank, and the free end was occluded with a rubber stopper. When the stopper was removed, food material was forced through the tube by movements of the gut, and samples were collected under paraffin oil.

**Lymphangiography.**

In an effort to identify the route by which lymph drained to the bloodstream after the thoracic duct was obstructed, lymphangiography was used in conjunction with radio-isotopic techniques. The thoracic ducts of the sheep were tied off in the chest, or thoracic duct fistulae were established and subsequently occluded. Before, and at intervals after the thoracic duct was occluded in each sheep, $^{131}I$ labelled human serum albumin was injected through a mesenteric lymphatic and the level of radioactivity in the thoracic duct lymph or in the blood draining from the lymph nodes in the abdomen, was measured. Radio-opaque contrast media were also injected into a mesenteric lymphatic, and the sequence of events followed radiologically.

Direct observations were made of lymphatics filled with radio-opaque contrast media using an Image Intensifier-X-Ray unit (Siemens). A tube voltage of 78 kilovolts and a current of 2-3 milliamps were used, and electronic amplification of the X-Ray image made visual examination possible without serious radiation hazard.

At intervals during the injection of a radio-opaque contrast medium, X-Ray plates of non-screen film (Kodirex; Kodak) were exposed
without using a grid. A current of 24 milliamps and a voltage of 53-60 kilovolts was applied for 4-8 seconds, during which time the respiratory movements of the sheep were stopped by increasing the resistance in the closed circuit.

The exposed plates were immersed in developer (D 19; Kodak) for 5 minutes at 68°C, then washed in running water. They were fixed in Kodak Acid Fixer (containing 2.5 percent of Kodak Hardener) for 10 minutes, then washed in running water and dried.

**Intralymphatic injection techniques.** Injections of contrast medium and of $^{131}$I labelled albumin were made into the efferent ducts from the lymph nodes which lie within the mesentery, about 5 cm. from the wall of the jejunum and ileum. These efferent ducts converge to form one or two main collecting ducts, which pass dorsally along the course of the anterior mesenteric artery to join the collecting ducts from the caecum and colon near the root of the mesentery.

An incision 15 cm. long was made in the right mid-flank region parallel to and about 10 cm. caudal to the last rib, and extending dorsally to within 5 cm. of the lateral border of the longissimus dorsi muscle. The muscle fibres were parted along their length and an incision was made in the peritoneum. The jejunum and ileum were removed from the abdominal cavity and covered with moist sterile towels. An efferent duct from one of the mesenteric lymph nodes was dissected free from the surrounding tissue for about 1 cm. and a loop of silk was placed around the duct about 2 cm. from the lymph node. A Polythene cannula (external diameter 1.1 mm.) was introduced into the duct between
the lymph node and the loop and tied in place. The position of cannula was checked with an injection of Evans Blue dye. Injections of $^{131}$I labelled albumin and contrast medium were made through the cannula during the period of anaesthesia, and when the injections were completed the cannula was generally removed and the duct tied off. In some sheep this procedure was repeated two or three times, but in other sheep it was not possible to pass a cannula into the mesenteric lymphatics. In these cases injections were made into the lumbar trunk which lies dorsal to and slightly to the left of the caudal vena cava. In one sheep (43) the mesenteric cannula was allowed to remain in position, and experiments on the unanaesthetised sheep were done at daily intervals for 5 days.

Before each experiment was started, the position of the cannula was checked radiologically following the injection of Diaginol contrast medium.

Collection of venous blood draining from lymph nodes. In an attempt to determine whether lymph may reach the blood within lymph nodes following obstruction of the thoracic duct, $^{131}$I labelled albumin was injected into a mesenteric lymphatic and venous blood collected from abdominal lymph nodes. The radioactivity in this blood was compared with the level of radioactivity in the circulating blood. Blood was collected from the following lymph nodes:

An hepatic node, which lies on the portal vein near the porta of the liver. Samples of blood were aspirated almost simultaneously from needles placed in the portal vein 1 cm. cranial to, and 1 cm. caudal
to the node.

The right renal node, which lies at the base of the right renal vein. The node was isolated by extraperitoneal dissection, and the efferent vein was exposed. The vein was incised and blood samples aspirated using a Pasteur pipette.

A node lying on the right face of the caudal vena cava at the level of the fourth lumbar vertebra, and connected to the intestinal trunk by a long (15 cm.), caudally directed lymphatic. The efferent vein was ligated and incised, and blood samples were aspirated using a Pasteur pipette.

**Analytical Methods.**

**Lipid extraction.** Lipids were extracted from some samples of lymph and plasma using the method of Bloor (1914). Usually 0.1-0.5 ml. of lymph or plasma was extracted in 10 ml. of boiling 3:1 ethanol-diethyl-ether mixture. The extract was allowed to cool, and the precipitated protein was removed by filtration. Larger volumes of lymph were extracted by boiling in 20-30 volumes of 2:1 chloroform-methanol mixture. The extract was allowed to stand for 2-4 hours before an equal volume of water was added carefully over the surface (Albrink, 1959). When this two-phase system was allowed to stand overnight, the methanol and most of the water-soluble salts migrated into the upper aqueous phase, which was discarded. The lower phase, containing the lipids dissolved in chloroform, was filtered to remove the precipitated protein.
During the extraction of $^{14}C$ labelled lipids from faeces, all of the faeces passed during an experiment were dried in an oven at 110-120°C, for more than four hours. The dried faeces were weighed, then ground up in a grister and weighed samples of about 10 grams were added to about 200 ml. of 2:1 chloroform-methanol in a tared flask. The extraction mixture was boiled for 10-15 minutes, allowed to stand for 2-4 hours, then weighed and filtered. The weight of the chloroform-methanol extract was determined by difference, and the specific gravity of the extract was calculated from the weight of a known volume of filtrate. The volume of extract containing the lipids extracted from a known weight of faeces was calculated from the expression: \[
\text{Volume} = \frac{\text{Total weight of extract}}{\text{Specific gravity of extract}}.
\]
Small portions of the extract (usually 0.2 ml.) were spread onto Polythene planchets and the amount of radioactivity was measured.

Segments of intestinal wall were frozen with liquid air, then ground up with sand using a mortar and pestle. The lipids were extracted with boiling 2:1 chloroform-methanol mixture and the extract was allowed to stand for 2-4 hours before the non-lipid material was removed by filtration.

Silicic acid column chromatography was used to obtain pure samples of cholesterol esters and total phospholipids. The columns had an internal diameter of 1.0-1.8 cm. and contained 1-12 grams of activated silicic acid. The silicic acid was 100 mesh size (Baker) and was activated by heating in an oven at 110°C, for 12 hours. One gram of silicic acid was used for each 5-15 mg. of total lipid. The eluting
solvents were redistilled before use.

Cholesterol esters were separated using a modification of the method of Barron and Hanahan (1958). The silicic acid was activated by heating, then washed in succession with diethyl ether, 15 percent benzene in hexane and n-hexane in a Buchner funnel. About 10 ml. of each solvent was used for each gram of silicic acid. The lipid extracts were dehydrated over anhydrous sodium sulphate and the solvent was evaporated. The lipids were then dissolved in about 5 ml. of hexane and added to the surface of the column. Any hydrocarbons and pigments present were eluted with 3 ml. of hexane per gram of silicic acid, and the cholesterol esters were eluted with 10 ml. of 15 percent benzene in hexane per gram of silicic acid.

Phospholipids were separated from other lipid components by a modification of the method of Borgstrom (1952 a). The lipid extracts were dehydrated, then taken to dryness and the lipids redissolved in 3-5 ml. of chloroform before being added to the column. The cholesterol esters, free fatty acids and glycerides were eluted together with 30 ml. of chloroform per gram of silicic acid, and the phospholipids were eluted with 15-20 ml. of methanol per gram of silicic acid.

The efficacy of the columns was checked using thin layer chromatography and radio-isotopes. When $^{14}$C labelled glycerides and free fatty acids were added to the columns, no radioactivity appeared in the cholesterol ester or phospholipid fractions, which contained no contaminants when examined on thin layer silicic acid chromatograms.
Separation of free fatty acids and neutral fat using alkaline ethanol (Borgstrom, 1952 b). The phospholipids were removed from a lipid extract using silicic acid chromatography, and the chloroform eluate was taken to dryness. The lipids were dissolved in about 30 ml. of petroleum ether and transferred to a 125 ml. separating funnel containing 50 ml. of alkaline ethanol (500 ml. of ethanol, 50 ml. of N-sodium hydroxide and 10 ml. of a 0.1 percent solution of thymol blue in ethanol were diluted to 1 litre with distilled water). The neutral fat was extracted into the petroleum ether phase by shaking, and the two phases were allowed to separate. The alcoholic lower layer was transferred to a second separating funnel containing 30 ml. petroleum ether, shaken up and then transferred to a third separating funnel. The alcoholic solution was acidified with concentrated hydrochloric acid, and the free fatty acids were extracted into petroleum ether. To obtain quantitative extraction of the fatty acids, the alcoholic solution was extracted once more with petroleum ether in a fourth separating funnel. Four other quantities of 50 ml. of alkaline ethanol were then passed through the four separating funnels in the same way. The petroleum ether in the first two separating funnels contained the triglycerides, and the free fatty acids were in the petroleum ether in separating funnels 3 and 4. When the method was examined using $^{14}$C palmitic acid, 99 percent of the radioactivity was recovered in separating funnels 3 and 4.

Thin layer chromatography (Vogel, Doizaki and Zieve, 1962) was used to separate the classes of phospholipids present in an extract of bile
lipids, and to check the purity of various lipid fractions.

A slurry was made from 30 grams of a mixture of silicic acid and plaster of Paris (Kieselgel G; Merck and Co.) by the addition of 60 ml. of water. A layer of this slurry, 0.2 mm. thick was applied to the surface of glass plates each 20 cm. square, using a spreader designed for this purpose (C. Desaga; Heidelberg). The plates were activated at 110°C for 30 min. then stored in a dessicator over dried silica gel.

To separate the different phospholipid classes, 1-5 mg. of the mixed phospholipids were applied at a series of points along one side of each plate, and reference compounds were applied at other points. The plates were placed upright in a chromatography tank containing a mixture of 80 parts of chloroform, 25 parts of methanol and 3 parts of water to a depth of about 1 cm. After 60 minutes, the plates were removed, then air dried and sprayed with a 0.2 percent solution of 2′7′ dichlorofluorescein in ethanol. The fluorescent spots were identified under ultraviolet light. In some cases the plates were treated with:

Ninhydrin to identify the amino phosphatides, or

Dragendorff reagent (KBI₄ in aqueous solution) to identify the choline phosphatides (Bregoff, Roberts and Delwiche, 1953), or

Iodine vapour which stains all the lipid-containing spots a brown colour, or

30 percent sulphuric acid to identify the cholesterol esters.
The plates were then heated at $120^\circ C$ for 5 minutes and the spots containing cholesterol esters were coloured pink.

To check the purity of samples of methyl esters of fatty acids and of fractions eluted from silicic acid columns, plates were prepared as before, but they were developed for 30 min. in a solvent system containing 90 parts of petroleum ether, 10 parts of diethyl ether and 1 part of glacial acetic acid. Reference compounds were used to identify the various spots. Reference samples of lysophosphatidyl choline were prepared from rat liver lipids by silicic acid chromatography. All the other lipid classes were eluted using the solvent system described by Getz, Bartley, Stirpe, Notton and Renshaw (1961) and the lysophosphatidyl choline was eluted with methanol.

Gas-liquid partition chromatography. A Model 154 D Vapor Fractometer (Perkin-Elmer Corporation) fitted with a hydrogen flame ionization detector was used to analyse mixtures of methyl esters of long-chain fatty acids. The methyl esters were separated on either a stainless steel column 2 metres long, which was packed with the succinate polyester of diethylene glycol (molecular weight 4,000) supported on ground up diatomaceous earth, or on a capillary (Golay) column, 150 feet long and with an internal diameter of 0.01 inches, which was lined with Apiezon L grease.

When fatty acid methyl esters are injected into the Vapor Fractometer through a self-sealing silicone septum, they are immediately vaporized on a flash heater and carried through the instrument in a
stream of nitrogen (Fig. 6). With the Golay column in place, the flow through the column is very small (1/2 to 2 ml. per min.) due to the high resistance to flow. In order to ensure negligible vapor spread at the head of the column, it is necessary that the total load of esters and solvent entering the column be very small (of the order of 1 microgram). The difficulties involved in injecting such a small load have been overcome by the presence in the nitrogen stream of a stream splitter, which ensured that under the operating conditions used, only 0.3-0.4 percent of the injected load entered the column and that the remainder passed to the atmosphere through a restriction.

As the sample is carried from the stream splitter through the column, its components are separated according to their respective affinities for the Apiezon L grease. Thus the shorter chain acids, having a lower affinity for this stationary phase, pass through more rapidly, while the longer chain acids are retained on the column for a longer time. On emerging from the column, each component flows into the detector where it is ionized by a hydrogen flame. The ionization current is picked up by a charged electrode located close to the flame, and is monitored by an amplifier. A signal from the amplifier drives the pen of a standard stripchart recorder (Leeds and Northrup) to produce in graphic form a quantitative description of the sample.

The Golay column was operated at a temperature of 220°C. and nitrogen was admitted at pressures which varied from 10-18 pounds per square inch (p.s.i.); a pressure of 15 p.s.i. was generally used. Hydrogen was introduced into the detector at a pressure of 9 p.s.i. and the detector was purged with air at 27 p.s.i. The sample,
Complete transesterification requires an acid medium and completely anhydrous conditions (Staffel, Chu and Akers, 1939). Dry hydrogen chloride gas, produced by adding concentrated hydrogen chloride into concentrated sulphuric acid, was bubbled into an aqueous alcoholic methanal (Vogel, 1989) to produce a solution of 5 percent by weight.

**Figure 6.** Schematic representation of the Perkin-Elmer Vapor Fractometer fitted with a capillary column and flame ionization detector. The instrument is described fully in the text.
which varied in volume from 1-3 microlitres, was injected into the sample injection block with a Hamilton Microliter syringe.

With the packed column in place, the resistance to flow through the column is much smaller and the total load of esters (0.1-0.5 microlitres) which was injected was carried over the column. However under the conditions used, only about 3 percent of the column effluent flowed through the detector, while the remainder was allowed to flow out into the atmosphere.

The packed column was operated at 194°C and nitrogen was admitted at 10 p.s.i. Air was admitted to the detector at 27 p.s.i. and hydrogen at 24 p.s.i.

Methyl esters of fatty acids were prepared by transesterification in an acid medium, or by alkaline hydrolysis followed by esterification. Complete transesterification requires an acid medium and completely anhydrous conditions (Stoffel, Chu and Ahrens, 1959). Dry hydrogen chloride gas, produced by adding concentrated hydrogen chloride into concentrated sulphuric acid, was bubbled into magnesium dried methanol (Vogel, 1956) to produce a solution of 5 percent by weight of hydrogen chloride in methanol.

A lipid extract containing 5-30 mg. of total lipid, was dried over anhydrous sodium sulphate for 2-4 hours, then filtered and taken to dryness in a 50 ml. flask. 1 ml. of 5 percent hydrogen chloride in methanol was added to the flask for each 2 mg. of phospholipid or glyceride. Four times this amount was added if cholesterol esters were present. 1 ml. of a solution of 0.1 percent (w/v) hydroquinone
in ethanol was added to each flask to prevent oxidation of the fatty acids (Bottcher, Woodford, Boelsma-van Houte and van Gent, 1959). A water condenser fitted with a calcium chloride moisture trap was fitted to each flask, and the contents refluxed for 10 hours.

When samples containing sphingomyelin or cholesterol esters were refluxed for shorter periods and then examined with thin-layer chromatography, it was found that quantitative esterification had not occurred.

The esters were extracted into hexane, then dried over sodium sulphate and stored in the cold. Some samples of esters prepared in this way were contaminated with cholesterol or plant pigments. These were removed by chromatography on Florisil (The Floridin Company; Tallahassee, Florida) by a modification of the method of Kishimoto and Radin (1959). A solution of esters in hexane was added to the surface of a column containing 5 grams of Florisil, and 100 ml. of hexane were passed over the column. More than 97 percent of the esters were recovered in the hexane eluate, but the contaminants remained adsorbed to the column.

Some of the lipid samples were hydrolysed with alkaline ethanol, then esterified using a solution of boron trifluoride in methanol. The solvent was evaporated from an extract containing about 40 mg. of total lipid. 10 ml. of a 2 percent solution of potassium hydroxide in ethanol and 1 ml. of hydroquinone solution were added to the flask containing the lipids, and the mixture was allowed to reflux for 1 hour. Most of the alcohol was removed by evaporation, then 10 ml.
of water was added and the non-saponifiable material was extracted into hexane. The solution of soaps was acidified, and hexane was used to extract the fatty acids.

The solution of fatty acids in hexane was dried over sodium sulphate and the hexane was removed by evaporation. The fatty acids were esterified (Metcalfe and Schmitz, 1961) by refluxing for 10 minutes with a 12.5 percent (w/v) solution of boron trifluoride in methanol (Applied Science Laboratories; State College, Pa.). The methyl esters were extracted into hexane, then dried over sodium sulphate and stored in the cold. Samples of methyl esters were examined using thin-layer chromatography and were found to contain no contaminating lipid.

Portions of fatty acid mixtures were esterified both with boron trifluoride in methanol, and with hydrogen chloride in methanol, and the fatty acid composition of each sample of esters was measured. There were no consistent differences between the results obtained with the two methods.

Identification of the fatty acids. Initially samples of standard fatty acid esters (Applied Science Laboratories; State College, Pa.) were separated on each column to identify their positions on the chromatogram. When the temperature of the column and the flow rate of nitrogen were kept constant, the various esters left the column at a characteristic time. Some of the peaks obtained during the experimental runs were identified by comparing their retention times with those of the standard esters. Peaks with a retention time which did not correspond to that of any of the standard esters were identified (James, 1960) using the relationship:
Retention time of substance $R_o(CH_2)_xY$

\[
\frac{\text{Retention time of substance } R_o(CH_2)_xY}{\text{Retention time of substance } R_o(CH_2)_{x-1}Y} = K \quad \text{(for a homologous series of compounds)}
\]

The retention time of each known peak was measured and the retention time relative to that of methyl palmitate was calculated. The logarithm of this relative retention time was plotted against the number of carbon atoms in the molecule and a series of parallel lines was obtained for each column, representing the saturated acids, monoenes, dienes and trienes respectively (Fig. 7).

Quantitative determination of the fatty acids. An expression for the areas of the peaks was derived from the product of the height and the width of the peak at half height (Cremer and Muller, 1950), assuming a roughly Gaussian shape. The total area of the chromatogram was calculated in this way, and the individual fatty acids were expressed as a percentage of the total area.

The method was checked by comparing the composition estimated in this way with the composition determined by weighing out the individual standard esters obtained from Applied Science Laboratories (Table 1).

**Table 1.** The composition of a standard mixture of fatty acid methyl esters, estimated by weighing, and using the Vapor Fractometer.

<table>
<thead>
<tr>
<th>Methyl ester</th>
<th>p.c. of total area (Golay column)</th>
<th>p.c. of total area (packed column)</th>
<th>p.c. of total weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>14.8</td>
<td>11.9</td>
<td>12.1</td>
</tr>
<tr>
<td>Palmitate</td>
<td>21.0</td>
<td>22.9</td>
<td>21.9</td>
</tr>
<tr>
<td>Palmitoleate</td>
<td>6.2</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Stearate</td>
<td>24.0</td>
<td>24.9</td>
<td>24.9</td>
</tr>
<tr>
<td>Oleate</td>
<td>34.1</td>
<td>34.0</td>
<td>34.1</td>
</tr>
</tbody>
</table>
Diethylene glycol succinate packed column

Apiezon capillary (Golay) column

The identification of peaks obtained with the Vapor Fractometer. A direct relation is shown between the log of the retention time of the fatty acid methyl esters (relative to methyl palmitate) and the carbon chain length for different degrees of unsaturation.

- a Saturated acids
- b Monoenoic acids
- c Dienoic acids
- d Trienoic acids

- a Saturated acids
- b Monoenoic acids
- c Dienoic and trienoic acids
Biochemical analyses.

Non-esterified fatty acids were determined by the titrimetric method of Dole (1956). 1 ml. of plasma or lymph was shaken up with 5 ml. of an extraction mixture containing 40 parts of isopropyl alcohol, 10 parts of heptane and 1 part of N-sulphuric acid, and allowed to stand for 10 minutes. The non-esterified fatty acids were extracted into heptane by adding 2 ml. of heptane and 3 ml. of water and allowing the mixture to stand for 2 minutes. One ml. of the heptane phase was titrated with 0.01 N-sodium hydroxide using 1 ml. of thymol blue solution (0.01 percent w/v in 90 percent alcohol) as indicator. The two phases were mixed by a stream of nitrogen which entered at the base of the titration vessel (Fig. 8). The titrations were done in duplicate and the results between duplicates agreed to within ± 2.5 percent. When $^{14}C$ palmitic acid was added to plasma samples which were extracted using this method, 99-101 percent of the radioactivity was recovered in the heptane phase. At intervals during each series of titrations, estimations were carried out on standard solutions of palmitic acid in heptane, and blank titrations were done on 1 ml. of heptane and 1 ml. of indicator. The results obtained with the palmitic acid standards agreed to within 2.5 percent, and the blank titrations were negligible.

Total esterified fatty acids (T.E.F.A.) were estimated by the method of Stern and Shapiro (1953). The fatty acid esters were extracted from samples of lymph (0.02-0.2 ml.) and plasma (0.2 ml.) into 10 ml.
Figure 8. The apparatus for measuring non-esterified fatty acids.

The fatty acids in heptane solution were added to an alcoholic solution of thymol blue and the two phases mixed with a stream of nitrogen which entered through a small hole in the bottom of the titration vessel. 0.01 N-sodium hydroxide was added from an "Agla" micrometer syringe.
of boiling 3:1 ethanol-diethyl ether mixture. The proteins were removed by filtration, and 3 ml. of filtrate was reacted for 20 minutes with hydroxylamine hydrochloride in alkaline solution. The reaction mixture was acidified, and the colour developed with ferric chloride. The colour intensity at 540 μ. was measured immediately, as the colour is relatively unstable (Fig. 9). Solutions containing 1, 2, 4 and 6 microequivalents of triacetin and of maize oil triglycerides were used to obtain a standard curve, and it was found that there was no significant difference between the colour developed with equivalent amounts of these glycerides.

Phospholipids were determined by the method of Zilversmit and Davis (1950). The phospholipids in 0.2 ml. samples of lymph and plasma were extracted into 10 ml. of boiling 3:1 ethanol-diethyl ether mixture, or were precipitated with the proteins using trichloracetic acid. The organic matter was digested in boiling perchloric acid which was allowed to cool and then diluted with water. A blue colour was developed by adding ammonium molybdate and a reducing solution, and the tubes were allowed to stand for 20 minutes before the colour intensity was measured at 810 μ. Solutions containing 10, 20, 30, 40 and 50 μg. of phosphorus were used to construct a standard curve (Fig. 10).

Total cholesterol was estimated by the method of Rappaport and Eichhorn (1960). 0.1 ml. of plasma or lymph was added to 0.6 ml. of a 12 percent solution of sulphisalicylic acid in glacial acetic acid, and the Liebermann-Burchard colour developed by the addition of 1.5 ml. of acetic anhydride and 0.2 ml. of concentrated sulphuric acid. The tubes were
Figure 9. 

(1) The calibration line for the estimation of total esterified fatty acids by the method of Stern and Shapiro (1953).

(2) The colour complex formed during T.E.F.A. estimation:
   Left: Change in optical density with time.
   Right: Absorption spectrum.
Figure 10. (1) The calibration line for the estimation of lipid phosphorus by the method of Zilversmit and Davis (1950). (2) The blue colour developed during phosphorus estimation: Left: Change in optical density with time. Right: Absorption spectrum.
shaken vigorously then allowed to stand for 10 minutes when the colour intensity was measured at 540 m\(\mu\). A standard curve was derived using varying amounts of cholesterol in glacial acetic acid (Fig. 11).

**Total protein** was measured by the biuret method of Gornall, Bardawill and David (1949). Four ml. of a biuret reagent containing cupric sulphate, sodium potassium tartrate and sodium hydroxide were added to tubes containing 0.05 ml. of lymph or plasma and 0.95 ml. of a saturated solution of sodium sulphate. The tubes were allowed to stand for 30 minutes and the colour intensity was measured at 540 m\(\mu\). The high concentration of fat in some samples of intestinal lymph caused a turbidity to develop in the reaction mixture which was clarified by shaking with diethyl ether. A calibration curve was obtained from solutions containing various concentrations of bovine serum albumin (Fraction V; Armour Ltd., England) (Fig. 12).

The **total glycerol** concentration in various lipid fractions was measured by the method of Carlson and Wadstrom (1959). The glycerides were hydrolysed using alkaline ethanol, and the free glycerol and fatty acids extracted into petroleum ether. 0.7 M-sulphuric acid was shaken up with the petroleum ether phase to extract the glycerol, which was then oxidised to formaldehyde and the colour developed by boiling for 30 minutes in the dark with a strongly acid solution of chromotrophic acid. The tubes were allowed to cool, and the colour intensity was measured at 570 m\(\mu\). Standard solutions of tripalmitin in chloroform were used to construct a standard curve (Fig. 13).
**Figure 11.** (1) The calibration line for the estimation of cholesterol by the method of Rappaport and Eichhorn (1960).

(2) The colour developed during the Lieberman-Burchard reaction:
- Left: Change in optical density with time.
- Right: Absorption spectrum.
Figure 12. (1) The calibration line for the estimation of protein by the method of Gornall, Bardawill and David (1949).

(2) The colour of the biuret solution:
Left: Change in optical density with time.
Right: Absorption spectrum.
Figure 13. (1) The calibration line for the estimation of glycerol by the method of Carlson and Wadstrom (1959).

(2) The colour developed during the chromotropic acid reaction:

Left: Change in optical density with time after the reaction mixture was removed from the boiling water-bath.

Right: Absorption spectrum.
Radioactivity assay. Samples of lymph and plasma containing $^{14}\text{C}$ were spread onto Polythene planchets 2 square cm. in area; those containing $^{131}\text{I}$ were spread onto metal planchets 3.1 square cm. in area. A volume of plating fluid (0.1 ml.) containing 2 percent of glycerol, 0.2 percent trichloracetic acid, 1 percent of tragacanth and 0.01 percent of Teepol was added to the planchets and mixed with the samples. Extracts containing lipids labelled with $^{14}\text{C}$ were spread directly onto Polythene planchets. All the planchets were covered with a disc of lens tissue and dried in an oven at 37°C or under an infra-red lamp before the activity was measured using a thin mica end-window G-M tube. When the counting rates in replicate samples were measured over 1,000 counts, the results agreed within 5 percent.

Construction of a self-absorption curve for $^{14}\text{C}$ in tripalmitin. 2 µc. of $^{14}\text{C}$ tripalmitin was dissolved in 20 ml. of ether, and 1 ml. of this extract was added to increasing amounts of non-radioactive olive oil in a series of tubes, and ethanol was added to a volume of 3 ml. Samples of 0.05 ml. from each tube were plated onto tared Polythene planchets which were dried and weighed before the counting rate was measured. The logarithm of the observed activity with the indicated mass to the activity at zero mass, was directly related to the sample mass within the range used (0.5-8 mg./sq. cm.). The regression coefficient relating $\log \frac{N \times 100}{N_0}$ to sample mass was calculated for the two GM tubes used:

Phillips tube:

$$\log \frac{N \times 100}{N_0} = 2.00 - 0.013 \, (\text{Sample Mass in mg./sq. cm.})$$
Figure 14. The self-absorption curves used to correct the counting rates of $^{14}$C containing lipids measured with (a) Tracerlab and (b) Phillips thin mica end-window GM tubes.
Tracerlab tube:

$$\log \frac{N \times 100}{N_0} = 2.00 - 0.012$$ (Sample Mass in mg./sq. cm.)

Using these relationships (Fig. 14), the observed activity in each sample containing $^{14}C$ labelled lipids was corrected for self-absorption to zero mass.

The effect of chronic lymph fistula on the plasma protein level of the blood.

In order to ascertain whether the sheep and lambs used in these experiments were suffering any appreciable degree of hypoproteinemia, the concentration of plasma proteins in the blood was measured at regular intervals. It was found that the sheep with chronic lymph fistula lost an average of 70 to 90 grams of plasma protein each day, but the plasma protein level in the blood decreased by only 10-20 percent. One sheep lost over 400 grams of protein during 14 days.
CHAPTER 3.

THE ABSORPTION OF FAT FROM THE GUT INTO THE INTESTINAL LYMPHATICS.

In the initial studies on the absorption of fat, estimates were made of the amount of dietary lipids carried in the lymph draining from the intestines of sheep. An attempt was then made to ascertain whether the changes which occur in the anatomy of the gut of lambs during their growth and development are accompanied by changes in the pattern of fat absorption. To this end, fat was injected into the abomasum of sheep and lambs and the rate of flow of the intestinal lymph and its lipid composition was measured. The quantitative importance of the lymphatic pathway in fat absorption in sheep and lambs was studied by measuring the levels of radioactivity in the lymph and faeces after \(^{14}C\) tripalmitin was injected into the gut at various sites.

The effect of chronic lymph drainage on the plasma protein level of the blood.

In order to ascertain whether the ewes and lambs used in these experiments were suffering any appreciable degree of hypoproteinemia, the concentration of plasma proteins in the blood was measured at regular intervals. It was found that the sheep with thoracic duct fistulae lost an average of 78 \(\pm\) 20 grams of plasma protein each day, but the plasma protein level in the blood decreased by only 10-30 percent. One sheep lost over 400 grams of protein during 14 days.
drainage from hepatic and intestinal lymph fistulae, but the plasma protein concentration in the blood decreased by only 30 percent and returned to the original level within five days after the lymph flow from the cannula had stopped (Fig. 15). Although the sheep and lambs did lose some weight during the experimental periods, they remained clinically normal throughout.

The lipid content of sheep lymph.

Lymph was collected for 12 hour periods from sheep with fistulae of the intestinal, hepatic and thoracic ducts. The concentration of total esterified fatty acids in the lymph and in samples of plasma was measured, and the absolute output of esterified fat in the lymph was calculated (Table 2). Corrections were made for the contribution of lipoprotein, bile and bacterial lipids, and it was estimated that about 5-8 grams of dietary lipid were being absorbed each day into the lymph of the sheep with intestinal lymph duct fistulae. It is likely that under some conditions this figure may be much higher and in one sheep it was estimated that 12 grams of dietary lipid were collected in the intestinal lymph during a 10 hour period (cf. Lascelles and Morris, 1961).

The effect of feeding milk on the flow and composition of the intestinal lymph in lambs.

Three lambs with intestinal lymphatic fistulae were starved for 8 hours and then given a drink of 200-250 ml. of diluted cow’s milk which contained 6-10 grams of fat. The concentration of fat in the lymph increased rapidly and reached a maximum about 6 hours after the
Figure 15. The concentration of protein in the plasma of a sheep during the drainage of hepatic and intestinal lymph. The sheep lost over 400 grams of plasma protein during the two weeks before the intestinal and hepatic fistulae stopped flowing at "i" and "l" respectively. During this period, the plasma protein concentration in the blood fell by about 30 percent, but returned to the pre-operative level 5 days after the lymph drainage had stopped.
Table 2. The rate of flow of lymph and the lipid composition of lymph and plasma samples from sheep fed on lucerne hay and lucerne chaff (2.8 percent fat) and grain oats (6.5 percent fat).

<table>
<thead>
<tr>
<th></th>
<th>Number of sheep</th>
<th>Flow rate ml/min. S.E</th>
<th>T.E.F.A. conc. µEq./ml. S.E.</th>
<th>T.E.F.A. output µEq./min. S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal Lymph</td>
<td>4</td>
<td>0.47 ±0.067</td>
<td>44 ±2.0</td>
<td>22 ±2.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>4</td>
<td>3.7 ±0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic duct Lymph</td>
<td>4</td>
<td>2.0 ±0.28</td>
<td>7.9 ±1.4</td>
<td>19.4 ±5.0</td>
</tr>
<tr>
<td>Plasma</td>
<td>4</td>
<td>1.6 ±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Lymph</td>
<td>4</td>
<td>0.11 ±0.056</td>
<td>2.3 ±0.43</td>
<td>0.18 ±0.034</td>
</tr>
<tr>
<td>Plasma</td>
<td>4</td>
<td>2.8 ±0.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fat was given (Fig. 16). It appeared that fat absorption in these lambs continued over a period of at least 12 hours as the concentration of fat in the lymph had not returned to the prefeeding level within this time. The regressions relating phospholipid concentration to total esterified fatty acid concentration were significant in each experiment \((p < 0.05-0.001)\). The total cholesterol concentration was significantly correlated \((p < 0.02)\) with the concentration of total esterified fatty acids in two of the three experiments.

The lymph flow increased rapidly and doubled during the first two hours after the milk was given. This increase in flow was associated with a fall in the protein concentration in the lymph (Fig. 16), but the protein output increased. It was thought likely
Figure 16. The flow rate of intestinal lymph, and the concentration and output of protein and total esterified fatty acids in the lymph of a three-week-old lamb given a drink of 200 ml. of milk.
that the absorption of water directly from the gut into the intestinal lymphatics contributed to the fall in protein concentration in the lymph (Korner, Morris and Courtice, 1954; Simmonds, 1954).

The effect of feeding fat on the flow and composition of intestinal lymph in sheep.

When 25 ml of olive oil was injected into the abomasum of sheep, the changes in lymph flow rate and fat concentration followed one another closely. However, the pattern differed greatly from that seen in lambs. The concentration of fat in the lymph decreased during the first two hours after the injection of the fat (Fig. 17), but after the second hour it increased rapidly and reached a maximum at about 3-5 hours. The concentration then decreased, but rose again to a second peak at 5-8 hours, then declined and approached the pre-feeding level at about 24 hours.

The lymph flow rate also decreased during the first two hours following the injection of the fat, but then increased to reach a maximum about 1 hour after the fat concentration reached its peak. The flow decreased during the next 1-2 hours, increased to a second peak between 5-8 hours, then gradually returned to the prefeeding level during the next 18 hours. The maximum rate of flow was an average of 50 percent higher than the rate of flow before the fat meal. Although this response in lymph flow occurred when either maize oil or olive oil was injected into the abomasum, it was best seen when maize oil was given. When a smaller volume (2 ml.) of olive oil was injected into the abomasum or duodenum, the response was essentially
Figure 17. Changes in the flow rate and total esterified fatty acid concentration of intestinal lymph in an adult sheep after the injection of 25 ml. of olive oil into the abomasum.
the same, but the lymph flow returned to the prefeeding level within 8 hours. However, when 4 ml of olive oil was injected into the rumen, no significant changes occurred in the lymph flow or fat concentration. When the fat was injected into the abomasum or duodenum, the output of fat in the lymph followed closely the pattern of lymph flow. Although the rate of lymph flow varied during the absorption of fat, the concentration of protein remained relatively constant. The output of protein thus varied with the flow rate of the lymph (Table 3).

Table 3. Changes in the rate of flow and the concentration and output of protein and total esterified fatty acids in the intestinal lymph of a sheep after 2 ml of olive oil was injected into the duodenum or abomasum.

<table>
<thead>
<tr>
<th>Site of injection</th>
<th>Time (min.)</th>
<th>Lymph flow (ml/min.)</th>
<th>Protein conc. (g percent)</th>
<th>Protein output (mg/minute)</th>
<th>Fat conc. (µEq/ml)</th>
<th>Fat output (µEq/minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>10</td>
<td>0.61</td>
<td>3.0</td>
<td>16.3</td>
<td>45</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.30</td>
<td>3.0</td>
<td>9.0</td>
<td>39</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.66</td>
<td>2.7</td>
<td>23.2</td>
<td>72</td>
<td>62</td>
</tr>
<tr>
<td>Abomasum</td>
<td>30</td>
<td>0.38</td>
<td>2.8</td>
<td>10.6</td>
<td>48</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0.17</td>
<td>3.4</td>
<td>5.8</td>
<td>47</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>0.75</td>
<td>2.5</td>
<td>18.8</td>
<td>69</td>
<td>52</td>
</tr>
</tbody>
</table>

During the absorption of fat, the concentration of phospholipids, non-esterified fatty acids and total cholesterol in the intestinal lymph was always higher than their concentration in the hepatic lymph or the
plasma (Fig. 18). The concentration of esterified fatty acids in the lymph during fat absorption was significantly correlated with the concentration of phospholipids in the lymph \( (p < .01) \), and during the period of maximum fat absorption, the phospholipid concentration in the intestinal lymph increased by up to 110 percent. The regression of non-esterified fatty acid concentration on total esterified fatty acid concentration in the lymph was significant \( (p < .01) \) in the experiments with maize oil, but was not significant in the experiments with olive oil. The concentration of cholesterol in the intestinal lymph was significantly correlated with the total fat concentration in the lymph of one sheep which received injections of olive oil and maize oil into the abomasum, 24 hours apart.

The absorption of fat from the gut had no effect on the rate of flow or composition of liver lymph. In sheep with intestinal or thoracic duct fistulae, no consistent changes occurred in the concentration of lipids in the plasma during fat absorption.

The absorption of \(^{14}C\) tripalmitin in lambs and in sheep.

When 20 \( \mu \)c. of \(^{14}C\) tripalmitin in 2 ml. of olive oil was injected into the abomasum or duodenum of lambs, radioactivity was present in the intestinal lymph at the end of the first hour and increased to a maximum by the end of the third hour (Fig. 19). The recoveries of labelled fat in the lymph and faeces of the lambs are given in Table 4. In one experiment in which a lamb was given \(^{14}C\) tripalmitin through an oesophageal tube, radioactivity did not appear in the intestinal lymph until 6 hours after the fat was given. The peak
Figure 18. The concentration of phospholipids and non-esterified fatty acids in the intestinal lymph (○), hepatic lymph (▲) and plasma (△) of a sheep during fat absorption.
Figure 19. The average output and the cumulative percentage recovery of radioactivity in the intestinal lymph of a three-week-old lamb after the injection of 20 μc of $^{14}$C tripalmitin into the abomasum.

- Output of radioactivity.
- Cumulative percentage of radioactivity recovered in lymph.
Table 4. The recovery of $^{14}\text{C}$ tripalmitin in the lymph and faeces of adult sheep and young lambs. The radioactive fat was injected into the rumen, abomasum or duodenum in different experiments and the lymph collected from the intestinal duct or the thoracic duct.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Lymphatic cannulated</th>
<th>Site of injection</th>
<th>Percent of injected fat recovered in</th>
<th>Percent of injected fat absorbed</th>
<th>Percent of injected fat recovered in faeces and lymph</th>
<th>Percent of absorbed activity recovered in lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep 2</td>
<td>Intestinal duct</td>
<td>Abomasum</td>
<td>64</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sheep 3</td>
<td>Intestinal duct</td>
<td>Abomasum</td>
<td>43</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>Sheep 4</td>
<td>Intestinal duct</td>
<td>Abomasum</td>
<td>11</td>
<td>36</td>
<td>64</td>
<td>47</td>
</tr>
<tr>
<td>Sheep 4</td>
<td>Intestinal duct</td>
<td>Duodenum</td>
<td>22</td>
<td>29</td>
<td>71</td>
<td>51</td>
</tr>
<tr>
<td>Sheep 4</td>
<td>Intestinal duct</td>
<td>Abomasum</td>
<td>34</td>
<td>32</td>
<td>68</td>
<td>66</td>
</tr>
<tr>
<td>Sheep 4</td>
<td>Intestinal duct</td>
<td>Duodenum</td>
<td>18</td>
<td>71</td>
<td>29</td>
<td>89</td>
</tr>
<tr>
<td>Sheep 5</td>
<td>Thoracic duct</td>
<td>Duodenum</td>
<td>21</td>
<td>55</td>
<td>45</td>
<td>75</td>
</tr>
<tr>
<td>Sheep 8</td>
<td>Thoracic duct</td>
<td>Duodenum</td>
<td>44</td>
<td>42</td>
<td>58</td>
<td>86</td>
</tr>
<tr>
<td>Sheep 9</td>
<td>Thoracic duct</td>
<td>Duodenum</td>
<td>29</td>
<td>12</td>
<td>88</td>
<td>41</td>
</tr>
<tr>
<td>Sheep 12</td>
<td>Thoracic duct</td>
<td>Duodenum</td>
<td>25</td>
<td>45</td>
<td>55</td>
<td>70</td>
</tr>
<tr>
<td>Sheep 13</td>
<td>Thoracic duct</td>
<td>Abomasum</td>
<td>38</td>
<td>49</td>
<td>51</td>
<td>87</td>
</tr>
<tr>
<td>Sheep 14</td>
<td>Thoracic duct</td>
<td>Rumen</td>
<td>36</td>
<td>10</td>
<td>90</td>
<td>46</td>
</tr>
<tr>
<td>Sheep 15</td>
<td>Thoracic duct</td>
<td>Rumen</td>
<td>67</td>
<td>10</td>
<td>90</td>
<td>77</td>
</tr>
<tr>
<td>Lamb 1</td>
<td>Intestinal duct</td>
<td>Oesophageal tube</td>
<td>38</td>
<td>6</td>
<td>94</td>
<td>44</td>
</tr>
<tr>
<td>Lamb 3</td>
<td>Intestinal duct</td>
<td>Abomasum</td>
<td>91</td>
<td>10</td>
<td>90</td>
<td>101</td>
</tr>
<tr>
<td>Lamb 5</td>
<td>Intestinal duct</td>
<td>Duodenum</td>
<td>53</td>
<td>40</td>
<td>60</td>
<td>93</td>
</tr>
<tr>
<td>Lamb 5</td>
<td>Intestinal duct</td>
<td>Duodenum</td>
<td>46</td>
<td>15</td>
<td>85</td>
<td>61</td>
</tr>
</tbody>
</table>

x Lymph clotted in the cannula before absorption was complete.
of absorption occurred at 17 hours, and radioactivity remained in the lymph for 54 hours. The pattern of fat absorption thus resembled that seen when fat was injected into the rumen of adult sheep (Fig. 20).

When 20 µc. of 14C tripalmitin dissolved in 2 ml. of olive oil was injected into the abomasum or duodenum of sheep with intestinal lymphatic fistulae, an average of 59 percent of the radioactivity was absorbed from the gut; of this absorbed activity an average of 48 percent was recovered in the intestinal lymph. Radioactivity was present in the lymph by the end of the first hour (Fig. 21), and most of the labelled fat which was absorbed into the lymph was recovered in the first 6 hours. In one sheep (sheep 4), four experiments were carried out in which the labelled fat was given alternately into the abomasum or duodenum over a period of 14 days. In each experiment, less than 90 percent of the labelled fat which was injected into the abomasum or duodenum was recovered from the intestinal lymph and faeces (Table 4).

It was thought possible that some of the lymph draining from the intestines may have entered the thoracic duct through other lymph channels and escaped collection. In an attempt to collect all the lymph draining from the intestines, the thoracic duct was cannulated in 5 sheep and the absorption of 14C tripalmitin studied. Between 21 and 44 percent of the labelled fat which was fed was recovered in the thoracic duct lymph. This was not significantly different from the recovery in the intestinal lymph (Table 4). A variable percentage of fat was not absorbed and appeared in the faeces, but again not more than 90 percent of the total radioactivity was recovered in the intestinal lymph and faeces (Table 4).
Figure 20. The average output of radioactivity per minute in the lymph from the intestines of an adult sheep and a three-week-old lamb. The sheep was given 60 µc of \(^{14}C\) tripalmitin into the rumen, and the lamb 20 µc by an oesophageal tube.

- ▲ Adult sheep.
- △ Young lamb.
The absorption of radioactive fat injected into the sheep was studied in two sheep with thoracic duct fistulae. In these experiments following the injection of C14 palmitic acid into the abomasum there was a rapid increase in radioactivity in the lymph. Figure 21 shows the appearance of radioactivity in the lymph from the intestines of a sheep after C14 palmitin dissolved in olive oil was injected into the abomasum. The percentage of the total esterified fatty acids in the lymph which was derived from the injected fat is shown, together with the cumulative percentage recovery of labelled fat in the lymph.

**Figure 21.** The appearance of radioactivity in the lymph from the intestines of a sheep after C14 palmitin dissolved in olive oil was injected into the abomasum. The percentage of the total esterified fatty acids in the lymph which was derived from the injected fat is shown, together with the cumulative percentage recovery of labelled fat in the lymph.

- **Radioactivity in lymph in counts/min./ml.** \( \times 10^{-3} 
- **Lymph fat specific activity**
- **Injected fat specific activity** \( \times 100 
- **Cumulative percentage recovery of labelled fatty acids.**
The absorption of radioactive fat injected into the rumen was studied in two sheep with thoracic duct fistulae. In these experiments following the injection of the labelled fat, no radioactivity appeared in the lymph for the first three hours. From the third hour, the radioactivity increased to a maximum at about 22 hours (cf. Fig. 20), and there was still activity present in the thoracic duct lymph of one of the sheep 86 hours after giving the fat. A total of 90 percent of the radioactive fat which was injected into the rumen was absorbed from the gut (Table 4). When the lymph clotted in the cannula of the other sheep at the beginning of the fourth day of the experiment, 36 percent of the radioactive fat which was fed had been recovered in the lymph.

The distribution of activity between the lipid constituents of the lymph during the absorption of \( ^{14}C \) tripalmitin.

When \( ^{14}C \) tripalmitin was injected into the abomasum or duodenum of sheep, more than 90 percent of the activity in the lymph was present in the glyceride fraction. An average of 1.2 percent of the total activity was recovered in the phospholipid fraction and an average of 3.6 percent was present as non-esterified fatty acids. In two experiments, no activity was detected in the lymph phospholipids. In experiments in which \( ^{14}C \) tripalmitin was injected into the rumen, the lymph phospholipids contained an average of 5.9 percent of the total activity in the lymph, and the non-esterified fatty acids contained an average of 4.5 percent. In experiments with lambs, an average of 0.6 percent of the total activity in the lymph was recovered in the phospholipids and 1.1 percent in the non-esterified fatty acids. The remainder of the activity was present as glycerides.

As none of the sheep or lambs was in a post-absorptive state
when the radioactive meal was given, the radioactive fat which was absorbed into the lymph was always diluted with exogenous fat. An approximate estimate of the percentage of the total esterified fatty acids in the lymph which was derived from the injected fat was obtained by comparing the specific activity of the fat in the lymph with that of the fat which was fed. When 20 µc. of $^{14}$C tripalmitin in 2 ml. of olive oil was injected into the abomasum of sheep and lambs and the absorption of radioactivity into the lymph was studied, it was found that during the period when the concentration of radioactivity in the lymph was highest up to 40 percent of the fat in the intestinal lymph of sheep (Fig. 21) and up to 51 percent of the fat in the intestinal lymph of lambs, came from the injected $^{14}$C tripalmitin.

**Summary**

Fistulae of the intestinal or thoracic lymph ducts were established in young lambs and adult sheep and the effect of feeding milk and fat on the rate of flow and composition of the lymph was studied. Studies were also made of the role of the lymphatic system in the absorption of $^{14}$C tripalmitin dissolved in olive oil and injected into the gut at various sites.

When lambs were given a drink of milk, the intestinal lymph flow increased and reached a peak between 2-3 hours while the concentration of fat in the lymph increased to a peak at 5-8 hours. There was a significant correlation between the concentration of total esterified fatty acids in the intestinal lymph of lambs during fat absorption and the concentration of phospholipids in the lymph.
When sheep with intestinal lymphatic fistulae were fed on lucerne chaff and grain oats, about 5-8 grams of dietary fat were absorbed into the lymph each day. When the sheep received an injection of 25 ml. of olive oil or maize oil into the abomasum, the lymph flow and fat concentration decreased during the next hour. The flow rate increased as fat absorption proceeded, and two peaks of both flow rate and fat concentration occurred at 3-5 hours and 5-8 hours following the injection of fat. During the absorption of fat, the concentration of total esterified fatty acids in the lymph was significantly correlated with the concentration of phospholipids.

When $^{14}$C tripalmitin was injected into the abomasum or duodenum of lambs, most of the absorbed activity was recovered in the lymph. In adult sheep, significantly less of the labelled fat which was absorbed was recovered in the lymph. When labelled fat was given into the rumen, absorption was slow and occurred over a period of days. In both sheep and lambs during the absorption of labelled fat, more than 90 percent of the radioactivity in the lipids of the intestinal lymph was in the form of glycerides. Small amounts of activity appeared in the lymph phospholipids and non-esterified fatty acids. Due to the long period over which a meal of radioactive fat is absorbed when it is given into the rumen, it appears that the absorption of dietary fat is usually a continuous process in sheep.

The lymphatic system appears to play an important part in the absorption of long-chain fatty acids from the gut of sheep and lambs. However a significant amount of the absorbed $^{14}$C tripalmitin was not recovered in either the intestinal or thoracic duct lymph in adult sheep.
In monogastric animals, the dietary triglycerides are largely hydrolysed in the lumen of the gut by pancreatic lipase and the products of hydrolysis are converted into a stable physical form in the presence of bile. These processes are important preliminaries to fat absorption and in dogs and rats deprived of bile or pancreatic juice fat absorption is significantly reduced (Annegers, 1954). Although lipolytic activity has been demonstrated in the ruminal contents and pancreatic juice of sheep (Garton, Hobson and Lough, 1958; Hill, 1961) the role of hydrolysis in the absorption of triglycerides in ruminants has not been studied.

In an attempt to ascertain the importance of bile and pancreatic juice in the absorption of fat in sheep and lambs, both in vitro and in vivo techniques were used. In vitro experiments were designed to measure the hydrolytic activity of sheep pancreatic juice in the presence and absence of bile and of calcium ions, and in media with different hydrogen ion concentrations. The rate of flow and the composition of bile and pancreatic juice were measured in sheep and lambs under several experimental conditions. Experiments were carried out in vivo to study the effect of continued deprivation of bile and pancreatic juice on the content of lipids in the intestinal lymph, and on the lymphatic absorption of 14C tripalmitin from the gut of adult sheep and young lambs.
The hydrolysis of triglycerides by sheep pancreatic juice in vitro.

To test the effect of bile on the rate of hydrolysis of triglycerides at pH 6.1, reaction mixtures containing 0.1 ml. of freshly collected sheep pancreatic juice, 0.5 ml. of fresh sheep bile, 0.5 ml. of a stabilized cotton-seed oil emulsion (Lipomul IV; The Upjohn Company, Kalamazoo, Michigan) and 10.9 ml. of Sörensen's phosphate buffer (pH 6.1) were incubated at 37°C. for periods of up to 2 hours. In control reactions, the bile was replaced by 0.5 ml. of buffer.

The effect of calcium ions on the rate of hydrolysis of triglycerides was studied by incubating 0.1 ml. of pancreatic juice with 0.5 ml. of Lipomul, 0.5 ml. of 0.3 M-calcium chloride solution and 10.9 ml. of 0.1 M N-ethylmorpholine (pH 7.0). In control reactions, the calcium chloride solution was replaced by 0.5 ml. buffer.

The effect of pH on the rate of hydrolysis was studied by incubating 1.0 ml. of pancreatic juice with 0.5 ml. of Lipomul and 10 ml. of buffer solutions of varying pH. The buffers used were citrate (pH 2.2 - pH 4.2), phosphate (pH 5.4 - pH 8.0), tris (pH 8.6) and carbonate (pH 9.7). Incubations were carried out at 37°C. for 30 minutes.

In each experiment, at the end of the incubation period, the hydrolysis was stopped by the addition of about 0.1 ml. of concentrated sulphuric acid. In order to extract the free fatty acids liberated by the hydrolysis, 1 ml. of the reaction mixture was shaken up with 5 ml. of a mixture of 40 parts of isopropyl alcohol, 10 parts of heptane and
1 part of $N\cdot H_2SO_4$, and the free fatty acids were extracted into heptane (Dole, 1956). The levels of free fatty acids were determined by titration with 0.02 N-sodium hydroxide in a two-phase system using thymol blue (0.02 percent w/v in alcohol) as indicator.

The lipids in the reaction mixture were removed by washing three times with diethyl ether, then the ether was washed twice with about 4 ml. of 0.7 M-sulphuric acid to extract any glycerol. The sulphuric acid washings were pooled with the extracted reaction mixture, and portions taken for glycerol analysis. All estimations were done in duplicate and control estimations were done at zero time.

When Lipomul was incubated with sheep pancreatic juice, hydrolysis of the triglycerides proceeded rapidly (Fig. 22). However the percentage of fatty acids liberated during the first 30 minutes was more than doubled by the addition of bile or calcium ions. Negligible amounts of free glycerol appeared during the first 30-60 minutes of incubation showing that most of the fatty acids were derived from the hydrolysis of triglycerides and diglycerides during this period. Free glycerol appeared subsequently as the reaction proceeded. In samples incubated for 2 hours, the production of glycerol was increased by the addition of calcium ions, but the addition of bile was without effect.

When Lipomul was incubated with pancreatic juice in media of different pH, it was found that hydrolysis proceeded most rapidly at a pH of about 7.8 but appreciable hydrolysis occurred at lower pH values (Fig. 23). Measurements were made of the pH of the intestinal contents of several sheep and it was found that at a distance of 10 cm. distal
Figure 22. The effect of bile and of calcium ions on the liberation of fatty acids and glycerol by sheep pancreatic lipase from Lipomul. 330 µEq. of triglycerides were incubated with 0.1 ml. of fresh pancreatic juice in a total volume of 12 ml.

Upper figure: The rate of liberation of fatty acids (▲) and glycerol (●) in the presence of 0.5 ml. of fresh sheep bile is compared with the rate of liberation of fatty acids (▲) and glycerol (○) in the absence of bile.

Lower figure: The rate of liberation of fatty acids (▲) and glycerol (●) in the presence of 0.5 ml. of 0.3 M-calcium chloride solution is compared with the rate of liberation of fatty acids (▲) and glycerol (○) in the absence of calcium ions.
Figure 23. The effect of pH on the liberation in vitro of fatty acids from Lipomul by sheep pancreatic juice. Fresh pancreatic juice (1.0 ml.) was incubated for 30 min. at 37°C, with 0.5 ml. of cotton-seed oil emulsion in 10 ml. of buffer solution. Buffers used were citrate (pH 2.2 - 4.2), phosphate (pH 5.4 - 8.0), tris buffer (pH 8.8) and carbonate (pH 9.7).
to the entrance of the common bile duct the pH was 4.2, while the contents obtained from the mid-jejunum had a pH of 7.9. Considerable hydrolysis occurred *in vitro* at ranges of pH between these two values.

The uptake and esterification of free fatty acids by segments of intestinal wall *in vitro*.

To test whether bile plays any part in promoting the uptake and esterification of free fatty acids by the gut wall, annular segments of gut were cut from the proximal jejunum and distal ileum of a normal sheep and a normal lamb, and from the proximal jejunum of a sheep and a lamb that had been deprived of bile for 22 hours. The animals were killed by cutting their throats. The segments taken from the sheep weighed $349 \pm 6.6$ mg. and those from the lambs weighed $279 \pm 13$ mg.

The segments were everted, washed in Krebs-Ringer phosphate buffer at $37^\circ C$. and transferred to incubation flasks. The segments from the normal sheep and lamb were incubated with 6 ml. of Krebs-Ringer phosphate buffer, 1 ml. of sheep bile and 1 ml. of a solution of albumin-$14^C$ palmitate complex. The segments from the sheep and lamb that had been deprived of bile, and additional segments from the normal sheep and lamb, were incubated with 7 ml. of Krebs-Ringer phosphate buffer and 1 ml. of the albumin-$14^C$ palmitate solution without added bile. Each incubation flask contained $7.7$ micro-equivalents of palmitic acid and the pH of the incubation mixture was 7.1. The incubations were carried out for 30 minutes at $37^\circ C$. in an atmosphere of 95 percent oxygen and 5 percent carbon dioxide. All incubations were done in duplicate, and each incubation was begun within 15 minutes after the
animal was killed.

At the end of the incubation period, the segments were washed immediately in buffer, blotted dry and frozen in liquid air before the lipids were extracted into a mixture of chloroform (2 parts) and methanol (1 part). The mixed lipids in the extracts were separated into free fatty acid, neutral lipid and phospholipid fractions, and the radioactivity in the total extract and in each of the fractions was measured. In preliminary studies it was found that no more than 2 percent of the labelled palmitic acid left in the incubation mixture at the end of the experiments, had been esterified. When histological sections which were prepared from some of the segments at the end of the experiments were examined, the mucous membrane appeared normal.

Table 5 shows the amount of palmitic acid taken up by the jejunal segments, and the percentage of the absorbed activity which was esterified. The segments from the normal sheep and lamb took up slightly more fatty acid than segments from the sheep and lamb which had been deprived of bile. The addition of bile to the incubation medium did not increase the amount of fatty acids taken up by the segments from the normal sheep and lamb. About the same percentage of the absorbed fatty acid was esterified by the segments from the sheep and lamb which were deprived of bile as by the segments taken from the normal sheep and lamb.

In the presence of bile, the amount of fatty acid taken up by the segments of proximal jejunum was significantly greater than the amount taken up by the segments from the distal ileum of the normal sheep.
Table 5. The uptake and esterification of free $^{14}$C palmitic acid in vitro by segments of jejunum from a normal sheep and a normal lamb and from a sheep and a lamb which had been deprived of bile for 22 hours. In half of the experiments with segments from the normal sheep and lamb, bile was added to the incubation medium. Duplicate incubations were done in each experiment, and the amount absorbed in each incubation is shown.

<table>
<thead>
<tr>
<th>Bile deprived</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile in medium</td>
</tr>
<tr>
<td>Sheep</td>
<td>Lamb</td>
</tr>
<tr>
<td>Percent absorbed</td>
<td>3.2</td>
</tr>
<tr>
<td>mµEq. fatty acid absorbed per 100 mg. segment</td>
<td>88</td>
</tr>
<tr>
<td>Percent of absorbed fat esterified</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 6. The uptake and esterification of free $^{14}$C palmitic acid in vitro by segments of jejunum and ileum from a normal sheep and a normal lamb.

<table>
<thead>
<tr>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep</td>
</tr>
<tr>
<td>Percent absorbed</td>
<td>4.5</td>
</tr>
<tr>
<td>mµEq. fatty acid absorbed per 100 mg. segment</td>
<td>103</td>
</tr>
<tr>
<td>Percent of absorbed fatty acids in Triglycerides</td>
<td>26</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>74</td>
</tr>
<tr>
<td>Free acids</td>
<td>0</td>
</tr>
</tbody>
</table>
and lamb (Table 6). In addition, the ileal segments esterified a much smaller percentage of the absorbed free fatty acid than the segments from the proximal jejunum. In all the experiments, a considerable proportion of the absorbed fatty acids was in the phospholipid fraction.

The rate of flow and composition of bile and pancreatic juice in sheep and lambs.

Bile and pancreatic juice were obtained from fistulae in 4 sheep and 4 lambs and the composition and mean rates of flow were estimated (Table 7). When the sheep and lambs were deprived of bile, the rate of flow and concentration of solid matter in the bile decreased rapidly during the next 5-12 hours (Fig. 24), but increased when bile was again returned to the duodenum. No consistent changes occurred in the bile phospholipid concentration when bile was diverted from the gut. The changes in bile flow and solid matter concentration after 25 ml. of maize oil was injected into the abomasum of sheep resembled the changes in the lymph flow and fat concentration during the absorption of a meal of fat. The bile flow decreased during the first hour after the fat was given, then increased to two or three peaks during the next 10 hours (Fig. 24). During this time, the concentration of solid matter in the bile showed a similar pattern to the flow rate, but the concentration of phospholipids did not change. No consistent changes occurred in the rate of flow or the concentration of the solid matter in the bile of lambs following a drink of milk. Similarly, in lambs, a drink of milk was not followed by consistent changes in the rate of
**Figure 24.** The composition and flow of bile from a fistula in a sheep when:

- 25 ml. maize oil was injected into the abomasum.
  - Bile was returned to the duodenum at a constant rate.
- Return of bile to the duodenum was stopped.
  - No maize oil was given during this experiment.
Table 7. Some characteristics of the bile and pancreatic juice in sheep and lambs. The means and standard errors are shown for 12 hour collection periods during the first 3 days after the fistulae were established. pH values are shown as a range.

<table>
<thead>
<tr>
<th></th>
<th>Lamb</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic juice:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate (ml./hr.)</td>
<td>2.3 ± 0.27</td>
<td>10.3 ± 1.8</td>
</tr>
<tr>
<td>Protein concentration (mg./ml.)</td>
<td>14.1 ± 0.99</td>
<td>22.0 ± 2.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 - 8.0</td>
<td>7.5 - 8.3</td>
</tr>
<tr>
<td>Bile:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow rate (ml./hr.) - normal</td>
<td>4.6 ± 0.93</td>
<td>37.1 ± 4.9</td>
</tr>
<tr>
<td>Flow rate (ml./hr.) - bile deprived</td>
<td>1.8 ± 0.32</td>
<td>17.5 ± 1.6</td>
</tr>
<tr>
<td>Solid matter (mg./ml.) - normal</td>
<td>32.9 ± 3.4</td>
<td>50.0 ± 4.1</td>
</tr>
<tr>
<td>Solid matter (mg./ml.) - bile deprived</td>
<td>21.5 ± 1.5</td>
<td>29.0 ± 5.5</td>
</tr>
<tr>
<td>Phospholipid (mg./ml.)</td>
<td>15.2 ± 3.0</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>pH</td>
<td>8.0 - 8.4</td>
<td>7.7 - 8.5</td>
</tr>
</tbody>
</table>

Flow or the concentration of total protein or of lipase in the pancreatic juice.

The phospholipids from the bile of sheep and lambs were fractionated using thin-layer chromatography, and the phosphorus content of each fraction was measured. Phosphatidyl choline comprised about 90 percent of the bile phospholipids in lambs, but sheep bile contained about equal proportions of phosphatidyl choline and lysophosphatidyl choline. In Table 8, these results are compared with an analysis carried out on bovine bile.
Table 8. The composition of the phospholipids in ruminant bile.

<table>
<thead>
<tr>
<th>Phospholipid fraction</th>
<th>Sheep 47</th>
<th>Sheep 62</th>
<th>Lambs 27/8 (pooled)</th>
<th>Lamb 30</th>
<th>Steer 1</th>
<th>Steer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidyl choline</td>
<td>35</td>
<td>49</td>
<td>90</td>
<td>95</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td>Lysophosphatidyl choline</td>
<td>39</td>
<td>45</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>--</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>8</td>
<td>--</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

The effect of deprivation of bile or pancreatic juice on the lipid content of the intestinal lymph.

The concentration of lipids in the intestinal lymph from normal sheep and lambs was always higher than the plasma concentration (cf. Chapter 3). The concentration of these lipids in the lymph of adult sheep decreased rapidly when bile was diverted from the intestine (Fig. 25). Within 6-10 hours, the concentration of total esterified fatty acids, free fatty acids, total cholesterol and phospholipids in the lymph had fallen below the plasma levels. When bile was diverted from the intestines of lambs, the concentration of lipids in the lymph also fell, but in this case they always remained higher than the plasma concentrations. The levels of esterified fatty acids in the intestinal lymph of sheep and lambs deprived of pancreatic juice for more than 12 hours were significantly reduced but they remained higher than the
Figure 25. The concentration of total esterified fatty acids (T.E.F.A.) in the intestinal lymph of a ewe and a lamb deprived of bile (▲), and a ewe and a lamb deprived of pancreatic juice (△). The plasma concentration is shown by the line P.
Table 9. The concentration of total esterified fatty acids (T.E.F.A.) in the intestinal lymph of normal sheep and sheep deprived of bile or pancreatic juice for more than 12 hours. The T.E.F.A. concentrations shown are the mean values for a 12 hour period.

<table>
<thead>
<tr>
<th>Normal Sheep</th>
<th>Lymph T.E.F.A. (µEq./ml.)</th>
<th>Deprived of pancreatic juice Sheep</th>
<th>Lymph T.E.F.A. (µEq./ml.)</th>
<th>Deprived of bile Sheep</th>
<th>T.E.F.A. (µEq./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td></td>
<td>number</td>
<td></td>
<td>number</td>
<td>Lymph</td>
</tr>
<tr>
<td>2</td>
<td>64.7</td>
<td>24</td>
<td>16.3</td>
<td>16</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>69.5</td>
<td>25</td>
<td>15.2</td>
<td>16</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>63.8</td>
<td>27</td>
<td>12.6</td>
<td>21</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>42.8</td>
<td>27</td>
<td>14.6</td>
<td>22</td>
<td>1.4</td>
</tr>
<tr>
<td>25</td>
<td>24.4</td>
<td></td>
<td></td>
<td>27</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Mean 53.4 14.7 1.5 2.3

levels in the plasma (Tables 9 and 10). The levels of esterified fatty acids in the lymph fell much more slowly in sheep and lambs deprived of pancreatic juice than in those deprived of bile.

The absorption of fat in the absence of bile or pancreatic juice.

When sheep which had been deprived of bile for more than 12 hours received an injection of 25 ml. of maize oil into the abomasum, the flow and composition of the intestinal lymph did not change. This was in contrast to the findings in normal sheep (cf. Chapter 3). Injections of 150 ml. of cow's milk into the duodenum of a sheep which had been deprived of bile did not alter the lymph flow or the concentration of total...
Table 10. The concentration of total esterified fatty acids (T.E.F.A.) in the intestinal lymph of normal lambs and those deprived of bile and/or pancreatic juice for more than 12 hours. The T.E.F.A. concentrations shown are the mean values for a 12 hour period. The lambs each had a drink of 100-200 ml of diluted cow's milk during the collection period. The mean plasma T.E.F.A. concentration was 4.1 µEq./ml.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Deprived of pancreatic juice</th>
<th>Deprived of bile</th>
<th>Deprived of bile and pancreatic juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb number</td>
<td>Lymph T.E.F.A. (µEq./ml.)</td>
<td>Lamb number</td>
<td>Lymph T.E.F.A. (µEq./ml.)</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>13</td>
<td>36.0</td>
</tr>
<tr>
<td>3</td>
<td>90.6</td>
<td>15</td>
<td>13.6</td>
</tr>
<tr>
<td>5</td>
<td>38.3</td>
<td>16</td>
<td>27.6</td>
</tr>
<tr>
<td>9</td>
<td>50.0</td>
<td>17</td>
<td>22.0</td>
</tr>
<tr>
<td>16</td>
<td>37.9</td>
<td>19</td>
<td>44.6</td>
</tr>
<tr>
<td>Mean</td>
<td>70.3</td>
<td>28.8</td>
<td>10.3</td>
</tr>
</tbody>
</table>
esterified fatty acids in the lymph. When lambs which had been deprived of bile were given a drink of milk, there was no consistent change in the lymph flow, but the total esterified fatty acid concentration in the lymph on the average, doubled, indicating that some fat absorption was occurring.

In the absence of pancreatic juice, some fat absorption occurred in the presence of bile. When 25 ml. of maize oil was injected into the abomasum of sheep deprived of pancreatic juice, the total esterified fatty acid concentration in the intestinal lymph increased but the changes in lymph flow were less well defined than in normal sheep. When lambs which were deprived of pancreatic juice were given a drink of milk, the lymph flow and total esterified fatty acid concentration increased slightly, but the extent and pattern of the increases were variable.

Recovery of \( ^{14} \text{C} \) tripalmitin fed to ewes and lambs deprived of bile or pancreatic juice.

When \( ^{14} \text{C} \) tripalmitin dissolved in olive oil was injected into the abomasum of sheep which had been deprived of bile, no radioactivity appeared in the intestinal lymph, but 80-100 percent of the fed radioactivity was recovered in the faeces (Table 11). When bile was present but pancreatic juice was absent, an average of 10 percent of the radioactive fat was recovered in the lymph. In lambs deprived of bile or pancreatic juice, most of the radioactive meal was recovered in the faeces. If bile was given into the intestines of a lamb previously deprived of bile, at the same time as the radioactive meal, fat
Table 11. Percentage of $^{14}$C tripalmitin (dissolved in olive oil and injected into the abomasum or duodenum) recovered in the intestinal lymph and in the faeces of sheep and lambs deprived of (a) bile, or (b) pancreatic juice, or (c) both. Some animals were given two lots of $^{14}$C tripalmitin under different conditions of bile and pancreatic juice. The recovery of radioactive fat in the lymph is expressed as a percentage of the radioactivity given in each experiment. The recovery of radioactivity in the faeces is expressed as a percentage of the total radioactivity given.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Treatment</th>
<th>Lymph</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb 11</td>
<td>a</td>
<td>5.5</td>
<td>84</td>
</tr>
<tr>
<td>15</td>
<td>a</td>
<td>2.5</td>
<td>79</td>
</tr>
<tr>
<td>15</td>
<td>b</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>b</td>
<td>2.1</td>
<td>88</td>
</tr>
<tr>
<td>16</td>
<td>c</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>b</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>c</td>
<td>1.0</td>
<td>Not collected</td>
</tr>
<tr>
<td>19</td>
<td>b</td>
<td>5.6</td>
<td>41</td>
</tr>
<tr>
<td>19</td>
<td>c</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Sheep 16</td>
<td>a</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>21</td>
<td>a</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>a</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>24</td>
<td>a</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>24</td>
<td>b</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>b</td>
<td>4.6</td>
<td>Not collected</td>
</tr>
<tr>
<td>27</td>
<td>b</td>
<td>6.8</td>
<td>85</td>
</tr>
<tr>
<td>27</td>
<td>b</td>
<td>5.3</td>
<td></td>
</tr>
</tbody>
</table>
Figure 26. The effect of bile on the absorption of $^{14}C$ tripalmitin injected each hour into the duodenum of a lamb with a bile fistula. For the first 6 hours bile was returned to the duodenum. Between 6 and 15 hours the bile was diverted from the gut but was returned after 15 hours.
absorption proceeded briskly. The level of radioactivity in the lymph rose and fell synchronously with the introduction of bile into the intestine (Fig. 26).

**Summary.**

In in vitro experiments, the optimum pH for the hydrolysis of triglycerides by sheep pancreatic juice was about 7.8, but appreciable hydrolysis occurred over the range of pH (4.2 - 7.9) found in the small intestine of sheep. The liberation of free fatty acids from triglycerides by sheep pancreatic lipase in vitro was increased in the presence of calcium ions and sheep bile.

Segments of jejunum taken from a normal sheep and a normal lamb absorbed free 14C palmitic acid from an incubation mixture at about the same rate as segments taken from a sheep and a lamb which had been deprived of bile. Bile did not appear to exert any effect on the esterification of the absorbed fat in these experiments. Segments of jejunum absorbed much more palmitic acid than did segments of ileum, and the esterification of the absorbed palmitic acid proceeded much more rapidly in the segments of jejunum than in those taken from the ileum.

The mean concentration of phospholipids in the bile from sheep was 15.2 mg./ml., and in bile from lambs, the mean concentration was 13.7 mg./ml. Phosphatidyl choline comprised about 90 percent of the total phospholipids in the bile from lambs, but in sheep bile almost equal proportions of phosphatidyl choline and lysophosphatidyl choline were present. The bile from 2 steers was examined for comparison and 90 percent of the phospholipids were found to be phosphatidyl choline.

When sheep and lambs were deprived of bile, the rate of flow
of the bile, and its content of solid matter decreased rapidly, but the concentration of phospholipids in the bile remained relatively constant. In sheep and lambs deprived of bile, the concentration of lipid in the intestinal lymph fell rapidly. In sheep, within 6-10 hours the lipid content of the intestinal lymph was less than the plasma concentration. In lambs, the lipid content of the intestinal lymph remained higher than in the plasma. In sheep and lambs deprived of pancreatic juice, the content of lipid in the intestinal lymph was much lower than in normal sheep and lambs.

The absorption of maize oil and $^{14}$C tripalmitin was significantly less than normal in sheep and lambs deprived of either bile or pancreatic juice. No $^{14}$C tripalmitin was absorbed into the lymph in sheep which were deprived of bile.
CHAPTER 5.

THE HYDROGENATION OF UNSATURATED FATTY ACIDS IN THE RUMEN.

Although the diet of ruminant animals contains a high level of polyunsaturated fatty acids, the blood lipids and depot fats of these animals are relatively saturated (cf. Garton, 1960a). Evidence is available from the analysis of the lipids of rumen contents and depot fats which suggests that the dietary fats are hydrogenated and geometric and positional isomers are formed in the rumen.

To obtain crucial evidence of the extent to which these changes affect the composition of the absorbed fat, experiments were carried out to compare the fatty acid composition of the lipids in the lymph from the intestines with the fatty acid composition of the dietary lipids. All the sheep used in these experiments had intestinal or thoracic lymph duct fistulae and were fed exclusively on lucerne chaff which was freely available to them. In some of the experiments, maize oil was given to the sheep through an oesophageal tube or through an indwelling tube in the abomasum, and the effect of these treatments on the fatty acid composition of the lymph lipids was measured. Similar experiments were carried out in young lambs with undeveloped rumens and a negligible population of ruminal micro-organisms. These lambs were 2 weeks old and had been separated from their mothers at 4 days and bottle-fed on diluted pasteurised cow's milk. The fatty acid composition of the intestinal lymph lipids was also measured in one lamb which was suckling its mother.
Experiments were also done to measure the distribution of the various fatty acids between the different classes of lipids to decide whether there was any preferential incorporation of these fatty acids into glycerides, phospholipids or cholesterol esters.

As the fatty acids with 18 carbon atoms formed the largest single group of fatty acids in the lipids examined, and as these acids reflected most accurately the changes which occurred during ruminant digestion, particular attention has been paid to the changes which occurred in the relative proportions of the C\textsubscript{18} fatty acids. The fatty acid composition of the various lipids analysed during these experiments are however, listed in Appendix Tables I, II and III.

**The fatty acid composition of the dietary and lymph lipids in sheep.**

The lipids extracted from the lucerne chaff contained 25 percent of palmitic acid and 61 percent of C\textsubscript{18} acids; 60 percent of these C\textsubscript{18} acids were trienes and the saturated and monoenoic acids together comprised only 11 percent of the C\textsubscript{18} fatty acids (Fig. 27). In the total lipids from the lymph of sheep eating this lucerne chaff, the mean concentration of palmitic acid (26 $\pm$ 1.4 percent) and C\textsubscript{18} acids (61 $\pm$ 2.2 percent) was not significantly different from the concentrations in the dietary lipids. The composition of the C\textsubscript{18} acids however, differed from those in the diet. Of the C\textsubscript{18} acids in the lymph lipids, only 7.0 $\pm$ 0.4 percent were trienes, but 78 $\pm$ 1.1 percent were saturated and monoenoic acids (Table 12).

A significant proportion of the fatty acids in the sheep lymph was found to be trans-isomers of monoenoic fatty acids. These were
The fatty acid composition of the lipids in lucerne chaff. Methyl esters of the fatty acids were separated on a column packed with the succinate polyester of diethylene glycol (upper figure) and on a capillary column lined with Apiezon L grease (lower figure). The fatty acids are designated by the number of carbon atoms, followed by the number of double bonds in the molecule; thus 18:0 represents stearic acid, 18:3 represents linolenic acid etc.
Table 12. The degree of unsaturation of the C₁₈ fatty acids in lucerne chaff and in the total lipids from the intestinal lymph of 5 sheep fed on lucerne chaff. The saturated, monoenoic, dienoic and trienoic C₁₈ acids are expressed as a percentage of the total C₁₈ fatty acids.

<table>
<thead>
<tr>
<th>C₁₈ fatty acid</th>
<th>Lucerne chaff</th>
<th>Sheep number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>36 40 48 49 54</td>
</tr>
<tr>
<td>Saturated</td>
<td>5.1</td>
<td>42 22 42 28 45</td>
</tr>
<tr>
<td>Monoenoic</td>
<td>5.5</td>
<td>39 56 35 46 33</td>
</tr>
<tr>
<td>Dienoic</td>
<td>3.0</td>
<td>13 15 15 20 15</td>
</tr>
<tr>
<td>Trienoic</td>
<td>6.0</td>
<td>6.2 7.6 8.1 5.8 7.3</td>
</tr>
</tbody>
</table>

Table 13. The occurrence of trans-isomers of fatty acids in the lymph from the intestines of sheep fed on lucerne chaff. The lymph lipids were separated into two fractions: the phospholipids contained no detectable trans-isomers and the percentage of trans-isomers in the fraction containing glycerides and cholesterol esters is shown.

<table>
<thead>
<tr>
<th>Sheep number</th>
<th>Total fatty acids</th>
<th>C₁₈ monoenes</th>
<th>C₁₆ monoenes</th>
<th>C₁₅ monoenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>10.2</td>
<td>36</td>
<td>33</td>
<td>43</td>
</tr>
<tr>
<td>54</td>
<td>9.1</td>
<td>33</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>
identified from standard reference samples of trans-isomers which when separated on the Apiezon capillary (Golay) column were found to have a slightly longer retention time than the cis-isomer.

When the methyl esters of the chaff lipids were separated on a Golay column, there was no evidence of trans-isomers of the unsaturated fatty acids. Similarly, no trans-isomers could be detected in the fatty acids in the lymph phospholipids from three sheep fed on lucerne chaff. 9.1 and 10 percent of the total fatty acids in the glyceride fraction from the lymph of two of these sheep were trans-isomers of monoenoic acids and in these sheep about one-third of the C\textsubscript{18} monoenoic, C\textsubscript{15} monoenoic acids were present as trans-isomers (Table 13, Fig. 28).

The fatty acids from cows' milk contained 11 percent of trans-acids. Of the C\textsubscript{18} monoenoic acids, 41 percent were trans-isomers. The glyceride fatty acids in the intestinal lymph of a lamb drinking this milk contained 11 percent of trans-isomers. Of the C\textsubscript{18} monoenoic acids, 37 percent were trans-isomers.

The effect of feeding maize oil on the fatty acid composition of the lymph from the intestines of sheep and lambs.

When 50 ml. of maize oil was given to a sheep through an oesophageal tube, no significant changes occurred in the degree of unsaturation of the fatty acids in the intestinal lymph (Appendix Table I). After the administration of the maize oil which contained 66 percent of C\textsubscript{18} acids, the concentration of C\textsubscript{18} acids in the total lymph
Figure 28. The fatty acid composition of the glycerides in the intestinal lymph of a sheep which was fed on lucerne chaff and given maize oil through an oesophageal tube. The fatty acid composition of the lymph glycerides before (upper figure) and after (lower figure) the maize oil was fed are shown. The methyl esters of the fatty acids were separated on a capillary column lined with Apiezon L grease.
lipids increased from 65 percent to 74 percent. Although linoleic acid comprised more than half of the $C_{18}$ fatty acids in the maize oil, the concentration of linoleic acid in the lymph decreased slightly during the absorption of the maize oil (Table 14; Fig. 28). Most of the linoleic acid which was fed did not appear in the intestinal lymph. During the absorption of the maize oil however, trans-isomers of the $C_{18}$ monoenoic acids were found in high concentration in the lymph, and amounted to 70 percent of the $C_{18}$ monoenoic acids in the lymph glyceride fraction (Appendix Table I).

When the rumen was bypassed and 25 ml. of maize oil was injected into the abomasum of sheep, the degree of unsaturation of the fatty acids in the total lymph lipids increased significantly. The concentration of the $C_{18}$ fatty acids in the total lymph lipids also increased (Fig. 29). In particular the content of linoleic acid increased from 15 percent to 40 percent in one sheep, and from 20 percent to 33 percent in a second sheep (Table 15). This increase in the concentration of unsaturated fatty acids was accompanied by a fall in the concentration of palmitic and stearic acids in the lymph.

The fatty acid composition of the intestinal lymph lipids in lambs fed on milk was very similar to the composition of the milk-fat (Fig. 30). When 10 ml. maize oil was given into the rumen however, the fatty acid composition of the lymph lipids changed (Appendix Table II). During absorption of the maize oil the concentration of linoleic acid in the total lymph lipids increased, and this increase was accompanied by a corresponding decrease in the concentration of
Figure 29. The fatty acid composition of the glycerides in the intestinal lymph of a sheep which was fed on lucerne chaff and given maize oil into the abomasum. The fatty acid composition of the lymph glycerides before (upper figure) and after (lower figure) the maize oil was fed are shown. Methyl esters of the fatty acids were separated on a packed column.
Figure 30. The fatty acid composition of the intestinal lymph glycerides of a suckling lamb (lower figure) and of the milk from its mother (upper figure). Methyl esters of the fatty acids were separated on a capillary column.
Table 14. The effect of maize oil given through an oesophageal tube on the degree of unsaturation of the C\textsubscript{18} fatty acids in the total lymph lipids of a sheep fed on lucerne chaff. The proportions of the various acids are expressed as percentages of the total C\textsubscript{18} fatty acids.

<table>
<thead>
<tr>
<th>C\textsubscript{18} fatty acid</th>
<th>Lucerne chaff</th>
<th>Maize oil</th>
<th>Lymph lipids Before maize oil</th>
<th>Lymph lipids After maize oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>5.1</td>
<td>3.2</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>Monoenoic</td>
<td>5.5</td>
<td>43</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td>Dienoic</td>
<td>30</td>
<td>53</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Trienoic</td>
<td>60</td>
<td>0.7</td>
<td>7.3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 15. The effect of maize oil given into the abomasum on the degree of unsaturation of the C\textsubscript{18} fatty acids in the total lymph lipids of two sheep fed on lucerne chaff. The proportions of the various acids are expressed as percentages of the total C\textsubscript{18} fatty acids.

<table>
<thead>
<tr>
<th>C\textsubscript{18} Fatty acid</th>
<th>Maize oil</th>
<th>Sheep 48</th>
<th>Sheep 49</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before maize oil</td>
<td>After maize oil</td>
<td>Before maize oil</td>
</tr>
<tr>
<td>Saturated</td>
<td>3.2</td>
<td>42</td>
<td>14</td>
</tr>
<tr>
<td>Monoenoic</td>
<td>43</td>
<td>35</td>
<td>42</td>
</tr>
<tr>
<td>Dienoic</td>
<td>53</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Trienoic</td>
<td>0.7</td>
<td>8.1</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Table 16. The composition of the C18 group of fatty acids in the phospholipid and glyceride fractions isolated from the lymph from the intestines of sheep and lambs. The glyceride fraction from sheep lymph contains cholesterol esters in addition to the glycerides. The proportions of the various fatty acids are expressed as the average percentages of the total C18 fatty acids in a series of 12 experiments with sheep and 4 experiments with lambs.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Sheep Glycerides</th>
<th>Sheep Phospholipids</th>
<th>Lambs Glycerides</th>
<th>Lambs Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearic</td>
<td>30.2 ± 3.2</td>
<td>30.5 ± 1.1</td>
<td>22.3 ± 1.6</td>
<td>30.1 ± 1.0</td>
</tr>
<tr>
<td>Oleic</td>
<td>48.5 ± 2.8</td>
<td>31.6 ± 1.4</td>
<td>62.4 ± 2.3</td>
<td>39.0 ± 2.1</td>
</tr>
<tr>
<td>Linoleic</td>
<td>15.6 ± 3.7</td>
<td>30.6 ± 1.8</td>
<td>12.4 ± 2.8</td>
<td>25.4 ± 2.6</td>
</tr>
<tr>
<td>Linolenic</td>
<td>5.6 ± 0.6</td>
<td>7.4 ± 0.7</td>
<td>2.9 ± 0.5</td>
<td>5.6 ± 1.0</td>
</tr>
</tbody>
</table>

x Includes trans-isomers of C18 monoenoic acids.

Oleic acid and of the shorter-chain fatty acids derived from the milk-fat (Fig. 31). During this period the content of trans-isomers in the lymph also decreased. Whereas trans-isomers comprised 11 percent of the fatty acids in the lymph glycerides collected before the maize oil was given, during absorption of the maize oil 5.9 percent of the glyceride fatty acids in the lymph were trans-isomers.

The distribution of the C18 fatty acids between the glycerides, phospholipids and cholesterol esters in the lymph.

In sheep, there did not appear to be any preferential incorporation of stearic acid or linolenic acid into any of the lipid classes. The mean proportion of linoleic acid in the phospholipids in
Figure 31. The fatty acid composition of the intestinal lymph glycerides of a milk fed lamb before (upper figure) and after (lower figure) a feed of maize oil was given into the rumen. Methyl esters of the fatty acids were separated on a packed column.
all the experiments with sheep was significantly greater ($p < 0.001$),
and the mean proportion of oleic acid in the phospholipids significantly
less ($p < 0.01$) than the mean proportion of these acids in the glyceride
fraction (Table 16) which contained both glycerides and cholesterol esters.
The glycerides and cholesterol esters from the lymph of chaff-fed sheep
however contained almost the same proportion of the various C$_{18}$ fatty
acids (Appendix Table I).

In lambs fed on milk, the proportion of oleic acid in the
lymph phospholipids was less than in the glycerides or cholesterol esters,
and the proportion of stearic, linoleic and linolenic acids was greatest
in the phospholipid fraction (Tables 16 and 17). The relative propor-
tions of these fatty acids in the different lipid fractions did not
change during the absorption of maize oil given into the rumen.

Table 17. The effect of maize oil given into the rumen of a milk-fed
lamb on the degree of unsaturation of the total lipids, and
of the glycerides, phospholipids and cholesterol esters
in the intestinal lymph. The proportions of the various
fatty acids are expressed as percentages of the total C$_{18}$
fatty acids.

<table>
<thead>
<tr>
<th>C$_{18}$ Fatty acid</th>
<th>Milk-fed</th>
<th>Maize oil to rumen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL</td>
<td>G</td>
</tr>
<tr>
<td>Saturated</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Monoenoic</td>
<td>67</td>
<td>69</td>
</tr>
<tr>
<td>Dieneic</td>
<td>9.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Trieneic</td>
<td>2.8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

TL - total lipids
G - glycerides
PL - phospholipids
CE - cholesterol esters
The concentration of each of the fatty acids (in microequivalents per ml. of lymph) was calculated from the concentration of total esterified fatty acids in the lymph and the percentage of each of these acids in the total lipid fraction. Similarly, the concentration of each of the fatty acids in the phospholipid fraction (in microequivalents per ml. of lymph) was estimated, and the percentage of each fatty acid esterified to phospholipids in the lymph was calculated (Table 16). In one lamb after a drink of milk, the concentration of triglycerides in the intestinal lymph exceeded 240 µEq./ml.; although only 4.7 percent of the total fatty acids were esterified to phospholipids, 13 percent of the linoleic acid was transported in this fraction. The concentration of total esterified fatty acids in the lymph from the sheep was relatively low (cf. Appendix Table I; Chapter 3), and it may be expected that an

**Table 16.** The percentage of the various fatty acids in lymph transported in phospholipids. The concentration of exogenous fat in the lymph of these sheep which were fed on lucerne chaff was low, and an appreciable amount of the lymph fatty acids were derived from the circulating lipoproteins. The concentration (in µEq./ml. of lymph) of each fatty acid esterified to phospholipids is expressed as a percentage of the total concentration (in µEq./ml. of lymph) of that fatty acid in the lymph.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Sheep number</th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36</td>
<td>40</td>
<td>48</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td>Palmitic</td>
<td>27</td>
<td>27</td>
<td>15</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>Stearic</td>
<td>32</td>
<td>32</td>
<td>17</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>Oleic(^x)</td>
<td>31</td>
<td>14</td>
<td>18</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>Linoleic</td>
<td>54</td>
<td>55</td>
<td>51</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>Linolenic</td>
<td>22</td>
<td>21</td>
<td>40</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>Total F.A.</td>
<td>30</td>
<td>23</td>
<td>18</td>
<td>27</td>
<td>14</td>
</tr>
</tbody>
</table>

\(^x\) Includes trans-isomers of C\(_{18}\) monoenoic acids
appreciable proportion of the fatty acids were not contained within chylomicrons but were derived from the circulating lipoproteins. In the lymph from these sheep which were fed on lucerne chaff, an average of $22.9 \pm 2.9$ percent of the total fatty acids were esterified to phospholipids, but $49.2 \pm 2.7$ percent of the linoleic acid was carried in this fraction. During the absorption of the maize oil, the proportion of the lymph fatty acids derived from the absorbed fat increased, and the increase in the concentration of total esterified fatty acids and of linoleic acid was much greater than the increase in the phospholipid concentration in the lymph (Table 19). Under these circumstances, the phospholipids carried a much smaller proportion of the lymph fatty acids. Although the concentration of linoleic acid in the phospholipid fraction did increase, the percentage of the total linoleic acid in the lymph that was esterified to phospholipids fell significantly (Table 19).

The cholesterol esters were isolated from samples of lymph taken before the injection of maize oil into the abomasum of sheep and into the rumen of lambs, and during absorption of the maize oil. The fatty acid composition of the cholesterol esters did not change significantly during this period (Tables 17 and 20). There was no evidence that any of the fatty acids were esterified preferentially to form cholesterol esters.

The fatty acid composition of the phospholipids in the bile.

Although most of the long-chain fatty acids in the lumen of the intestine are derived from the diet, a considerable amount may enter in the bile esterified to phospholipids. When the composition
Table 19. The effect of maize oil given into the abomasum on the percentage of the various fatty acids transported by phospholipids in the lymph of a sheep fed on lucerne chaff.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Total concentration (µEq./ml.)</th>
<th>Concentration of phospholipid fatty acids (µEq./ml.)</th>
<th>Percent of total phospholipid fatty acids</th>
<th>Percent carried by phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Palmitic</td>
<td>2.13</td>
<td>5.92</td>
<td>0.52</td>
<td>0.77</td>
</tr>
<tr>
<td>Stearic</td>
<td>1.35</td>
<td>2.52</td>
<td>0.49</td>
<td>0.58</td>
</tr>
<tr>
<td>Oleic\textsuperscript{x}</td>
<td>2.23</td>
<td>8.24</td>
<td>0.52</td>
<td>0.64</td>
</tr>
<tr>
<td>Linoleic</td>
<td>0.96</td>
<td>5.58</td>
<td>0.41</td>
<td>0.77</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.28</td>
<td>0.77</td>
<td>0.10</td>
<td>0.17</td>
</tr>
</tbody>
</table>

| Total fatty acids | 7.70 | 25.10 | 2.10  | 3.00  | 27    | 12    |

\textsuperscript{x} Includes trans-isomers of C\textsubscript{18} monoenoic acids

of the fatty acids in the bile phospholipids was examined, it was found that in sheep, although oleic acid was the major constituent (Table 21; Appendix Table III), relatively high proportions of palmitic acid and stearic acid were also present. In bile from lambs, the concentrations of palmitic acid and linoleic acid were higher, and the concentrations of stearic acid and oleic acid were lower than in sheep. When the fatty acids were separated on a capillary column, no trans-isomers could be detected. In some samples, one or two peaks which had a retention
Table 20. The effect of maize oil given into the abomasum on the composition of the C\textsubscript{18} fatty acids in the glycerides and cholesterol esters in the lymph from the intestines of two sheep fed on lucerne chaff. The cholesterol ester fractions from the two sheep were pooled and the composition of the pooled samples is shown. The proportions of the various acids are expressed as percentages of the total C\textsubscript{18} fatty acids.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Chaff-fed Sheep 48</th>
<th>Chaff-fed Sheep 49</th>
<th>Maize oil abomasum Sheep 48/49 (Pooled)</th>
<th>Maize oil abomasum Sheep 48/49 (Pooled)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>G</td>
<td>CE</td>
<td>CE</td>
</tr>
<tr>
<td>Stearic</td>
<td>44</td>
<td>26</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Oleic</td>
<td>37</td>
<td>52</td>
<td>52</td>
<td>43</td>
</tr>
<tr>
<td>Linoleic</td>
<td>10</td>
<td>17</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>Linolenic</td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

x Includes trans-isomers of C\textsubscript{18} monoenoic acids.

Sheep fed on this lucerne and maize chaff, however, had only a small increase in the lengths of their intestinal lymph lipids, which contained large amounts of saturated acids and cis and trans-isomers of monoenoic acids. When a rise of time slightly longer than methyl stearate on both capillary and packed columns, could not be identified.
Table 21. The major fatty acids in the bile phospholipids from sheep and lambs. The proportions of the various acids are expressed as percentages of the total fatty acids in the bile phospholipids. Smaller amounts of C₁₄ saturated, C₁₅ saturated, C₁₆ monoenoic, C₁₇ saturated and C₁₇ monoenoic acids were also present.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Sheep</th>
<th>Lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>47</td>
<td>61</td>
</tr>
<tr>
<td>Palmitic</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>Stearic</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Oleic</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>Linoleic</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Linolenic</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Summary.

The lipids from lucerne chaff contain a high proportion of linoleic and linolenic acids. Sheep fed on this lucerne chaff however, had only a small proportion of these highly unsaturated fatty acids in their intestinal lymph lipids, which contained large amounts of saturated acids and cis and trans-isomers of monoenoic acids. When a feed of maize oil, which contained about 20 grams of linoleic acid, was given to a sheep through an oesophageal tube and the lymph collected during the period of absorption, no increase occurred in the linoleic acid content of the lymph lipids but the proportion of trans-isomers of C₁₈ monoenoic acids in the lymph glyceride fractions increased significantly. When the rumen was bypassed and maize oil injected into the abomasum of sheep,
or into the undeveloped rumen of young lambs, significant increases occurred in the linoleic acid content of the lymph lipids. The proportion of trans-isomers of monoenoic acids in the lymph glycerides of a lamb fed on milk was not significantly different from the proportion of trans-isomers in the milk lipids. The proportion of trans-isomers in the lymph glycerides from this lamb decreased during the absorption of maize oil given into the rumen. It is apparent that the highly unsaturated fatty acids in the diet of adult sheep are hydrogenated with the formation of saturated acids and cis and trans-isomers of monoenoic acids during their passage through the rumen.

Of the C₁₈ fatty acids in the intestinal lymph of sheep and lambs, the cis and trans-isomers of C₁₈ monoenoic acids were in significantly higher concentration in the glyceride fraction than in the phospholipids. Linoleic acid was preferentially esterified to phospholipids. In the lymph of sheep fed on lucerne chaff, the glyceride content was relatively low and an appreciable amount of the fatty acids were derived from the circulating lipoproteins. Of the total fatty acids in this lymph, an average of 22 percent was esterified to phospholipids, but this fraction contained 49 percent of the linoleic acid. However, during the absorption of large amounts of linoleic acid, a much lower percentage of the total linoleic acid in the lymph was carried in the phospholipids. The fatty acid composition of the cholesterol esters in the lymph of sheep and lambs did not change significantly during the absorption of maize oil.
CHAPTER 6.

ANASTOMOTIC CONNECTIONS BETWEEN THE LYMPHATIC AND VENOUS SYSTEMS IN THE THORAX AND ABDOMEN OF SHEEP.

The significance of the lymphatic system in the transport of absorbed fat can only be assessed provided the lymph draining from the intestines can be collected quantitatively. In rats, during the absorption of $^{14}$C labelled fatty acids an appreciable amount of the absorbed radioactivity is not collected from a thoracic lymph duct fistula, and some of the $^{14}$C is excreted in the expired breath, or deposited in the liver (cf. Chapter 1). When $^{14}$C labelled tripalmitin was injected into the gut of sheep, some of the radioactivity which was absorbed did not appear in the lymph collected from the intestinal lymph duct or the thoracic duct. It is possible that all of the radioactive fat was absorbed into the intestinal lymph but that some of this lymph drained into the blood through connections between the lymphatics and veins in the abdomen, and thus was not collected at the lymphatic fistula.

Sheep and lambs in which the flow of lymph through the thoracic duct cannula became permanently obstructed, showed no distress and remained clinically normal. This suggested that the lymph was draining to the blood through alternative channels. An attempt was made therefore to determine whether all of the lymph draining from the intestines of sheep is collected when the thoracic duct is cannulated in the chest. The development of alternative lymphatic pathways in sheep in which the thoracic duct was occluded was studied using $^{131}$I labelled human serum.
albumin in conjunction with lymphangiographic techniques.

Is the intestinal lymph collected quantitatively from a cannula in the thoracic duct?

The thoracic ducts of 15 sheep were cannulated and at the same time, 0.5 ml. or 1 ml. of $^{131}$I human serum albumin was injected into one of the mesenteric lymphatics. The labelled albumin was washed into the lymphatic with 0.5 ml. of a solution of Evans Blue dye, and the thoracic duct lymph was collected for periods up to 1 hour. In 13 sheep, all of the radioactivity was recovered in the lymph, usually in the first 10-15 minutes. In the remaining 2 sheep, 86 percent and 85 percent of the injected radioactivity was recovered in the lymph. The lower recoveries in these two sheep may have been due to the presence in the injected albumin solution of free $^{131}$I. This could diffuse out through the walls of the lymphatics, resulting in a low recovery in the thoracic duct lymph. The levels of free $^{131}$I in the labelled albumin used were not determined, but the manufacturers stated that only negligible amounts of free iodine were present.

The events following obstruction to the flow through the thoracic duct.

When $^{131}$I labelled human serum albumin was injected into a mesenteric lymphatic of a sheep immediately after the thoracic duct was occluded, about 5 percent of the injected radioactivity appeared in the plasma during the next 40 minutes. Labelled albumin was injected into the mesenteric lymphatic on each of the following two days with similar results (Fig. 32). However when the experiment was repeated on the fifth day, the level of radioactivity in the blood increased much more
Figure 32. The development of alternate drainage for the intestinal lymph following ligation of the thoracic duct. The thoracic duct was ligated, and at the initial operation (△) and on the two subsequent days (△ and ○) jugular vein blood was collected at intervals after 0.5 ml. of a solution of $^{131}$I human serum albumin was injected into a mesenteric lymphatic. When 0.5 ml. of $^{131}$I albumin was injected on the fifth day (●), radioactivity appeared rapidly in the blood. The observed counting rate in the blood samples was corrected for radio-decay.
rapidly and reached a peak about half an hour after the injection indicating that a more direct connection had developed between the lymphatic and venous systems.

A water-soluble contrast medium (Diaginol) was injected into the mesenteric cannula in this sheep at intervals during the first 5 days, and the radiological changes noted. During the first 15 minutes after the Diaginol was injected, the edges of the lymphatics became indistinct due to extravasation of the contrast medium. Although some extravasation of the contrast medium occurred in animals with patent thoracic ducts, it occurred much more rapidly after the thoracic duct was occluded. During the first 2 days, the lymphatics appeared grossly dilated and some of the Diaginol flowed in a retrograde direction along the lumbar trunk (Fig. 33). During this period, no retrograde flow was evident along the efferent lymphatics from lymph nodes. However, when Diaginol was injected on the fifth day after the thoracic duct was occluded, retrograde flow did occur along the efferent lymphatics from one of the hepatic lymph nodes. When this node was examined using the Image Intensifier, it appeared likely that some Diaginol may be passing to the blood within the substance of the lymph node. There was no evidence of the development of new lymphatics bypassing the thoracic duct ligature. When the experiments were repeated with two other sheep, a similar pattern was observed.

From these experiments, it appeared likely that when the thoracic duct was occluded, lymph escaped through the walls of the lymphatics during the next few days. This was confirmed radiologically
Figure 33. X-ray of the caudal thorax and abdomen of a sheep 5 days after the thoracic duct was occluded at the ninth thoracic vertebra. The lymph ducts filled with Diaginol contrast medium are dilated, and the valves have become incompetent; retrograde flow has occurred along the lumbar trunk and along the efferent duct from a hepatic lymph node.
Figure 33. X-ray of the caudal thorax and abdomen of a sheep 5 days after the thoracic duct was occluded at the ninth thoracic vertebra. The lymph ducts filled with Diaginol contrast medium are dilated, and the valves have become incompetent; retrograde flow has occurred along the lumbar trunk and along the efferent duct from a hepatic lymph node.
and at post-mortem. When 2 sheep were examined radiologically 8 days after the thoracic duct was occluded, a large discrete radiotranslucent area was seen surrounding the thoracic duct caudal to the obstruction (Fig. 34). When the thoracic duct was filled with Diaginol, there was no indication that the duct had ruptured. However when the animals were examined at post-mortem on the eighth day, the fibrous tissue capsule surrounding the radiotranslucent area was found to be lined with clotted chyle although there was no sign that extravasation of lymph was still occurring. In 6 sheep examined radiologically and visually during anaesthesia, the mesenteric lymphatics were grossly dilated during the first 4-5 days after the thoracic duct was occluded. The lymphatic valves had become incompetent, and when Diaginol was injected into one of these lymphatics, it flowed in a retrograde direction along the lumbar trunk, and along some of the efferent vessels from lymph nodes (cf. Fig. 33). After 5-7 days however, the lymphatics had returned to a more normal size, suggesting that there was less resistance to flow through the lymphatics.

The transfer of lymph to the blood within lymph nodes.

Thoracic duct fistulae were established in 4 sheep and the absence of direct connections between the intestinal lymphatics and the abdominal veins was determined using $^{131}$I labelled albumin. The fistulae were occluded and 9-14 days later, labelled albumin was injected into a mesenteric lymphatic. At intervals during the next 10 minutes, samples of blood were collected from the efferent vein from the right renal lymph node or from the caudal vena cava about 1 cm cranial to
Figure 34. X-ray of the caudal thorax and abdomen of a sheep 8 days after the thoracic duct was occluded at the ninth thoracic vertebra. A large, radio-translucent sac enclosed by a fibrous capsule is visible surrounding the thoracic duct. The thoracic duct is filled with Diaginol contrast medium, and does not appear to have ruptured.
Figure 34. X-ray of the caudal thorax and abdomen of a sheep 8 days after the thoracic duct was occluded at the ninth thoracic vertebra. A large, radio-translucent sac enclosed by a fibrous capsule is visible surrounding the thoracic duct. The thoracic duct is filled with Diaginol contrast medium, and does not appear to have ruptured.
the node. The level of radioactivity in this blood was always higher than the level of radioactivity in control samples of blood collected from the jugular vein, portal vein or from the caudal vena cava 2 cm. distal to the node (Table 22). In one of the sheep, a sample of blood collected from the efferent vein 8.5 minutes after the albumin was injected, contained 7100 c.p.m./ml., whereas samples of blood collected from the jugular vein at 6.5 and 10 minutes contained 5200 and 4000 c.p.m./ml. respectively.

Table 22. The level of radioactivity in c.p.m. per ml. of plasma in samples collected from the caudal vena cava 1 cm. cranial to, and 2 cm. caudal to the right renal lymph node after I labelled albumin was injected into a mesenteric lymphatic. The thoracic duct had been occluded 9 days previously.

<table>
<thead>
<tr>
<th>Blood sample taken from</th>
<th>Time in min. after albumin injected</th>
<th>c.p.m./ml. plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial to renal node</td>
<td>3</td>
<td>2600</td>
</tr>
<tr>
<td>Caudal to renal node</td>
<td>4</td>
<td>2300</td>
</tr>
<tr>
<td>Cranial to renal node</td>
<td>4.5</td>
<td>2800</td>
</tr>
<tr>
<td>Caudal to renal node</td>
<td>5</td>
<td>2500</td>
</tr>
<tr>
<td>Cranial to renal node</td>
<td>5.5</td>
<td>2800</td>
</tr>
</tbody>
</table>

In another sheep, the labelled albumin was injected into the mesenteric lymphatic and samples of blood were collected from the efferent vein draining from a small node on the right face of the caudal vena cava. The level of radioactivity in this blood reached 5100 c.p.m./ml. within the first minute, but samples of blood collected from the jugular vein during the first 15 minutes contained less than 200 c.p.m./ml. As the
subsequent development of lymphatic-venous anastomoses was studied using these sheep, the lymph nodes were not removed for histological examination.

The development of direct connections between the thoracic duct and hemiazygos vein in sheep.

Thoracic duct fistulae were established in three sheep and the absence of direct functioning connections between the thoracic duct and hemiazygos vein was confirmed using $^{131}I$ labelled albumin and lymphangiography. The thoracic duct fistulae were occluded, and two weeks later the transfer of lymph to the blood within lymph nodes was demonstrated in two of the sheep. After 4-7 weeks, water-soluble contrast medium (Arteriodione) was injected into a mesenteric lymphatic and the flow through the lymphatics studied using the Image Intensifier. In each case the Arteriodione appeared to drain from the thoracic duct into the hemiazygos vein just cranial to the diaphragm. This was confirmed when mercury was injected into the mesenteric lymphatic in two of the sheep, and small droplets of mercury became dislodged from the thoracic duct to flow into the hemiazygos vein (Fig. 35). In one of the sheep, the mercury also filled a small vessel which passed from the thoracic duct to the caudal mediastinal lymph node. The thoracic duct of the third sheep was filled with latex by infusion through the mesenteric cannula. The sheep were killed, and the area of the thoracic duct was fixed in formol-saline and dissected. In one sheep, it appeared likely that the mercury reached the vein through a small connection under the capsule of a lymph node, but this could not be
Figure 35. X-ray showing a direct connection between the thoracic duct and hemiazygos vein in a sheep. The thoracic duct was ligated at the ninth thoracic vertebra, and 4 weeks later X-ray plates were exposed after mercury was injected into a mesenteric lymphatic.
X-ray showing a direct connection between the thoracic duct and hemiazygos vein in a sheep. The thoracic duct was ligated at the ninth thoracic vertebra, and 4 weeks later X-ray plates were exposed after mercury was injected into a mesenteric lymphatic.
Figure 36. A direct connection between the thoracic duct and hemiazygos vein in a sheep. A cannula in the thoracic duct at the ninth thoracic vertebra was occluded, and 6 weeks later the thoracic duct was filled with latex and the area dissected out.
definitely established. In the other two sheep, small vessels were seen joining the thoracic duct and the hemiazygos vein immediately caudal to the occluded thoracic duct cannula (Fig. 35).

Summary.

Thoracic duct fistulae were established in 15 sheep and $^{131}$I human serum albumin was injected into a mesenteric lymphatic. In 13 of the sheep, all of the injected radioactivity was recovered in the thoracic duct lymph, and in the remaining 2 sheep 85 percent and 86 percent was recovered.

When the thoracic duct of sheep was occluded, the abdominal lymphatics became distended during the first few days, and in some cases lymph escaped into the surrounding tissues. The valves of some lymphatics became incompetent, allowing the lymph to flow in a retrograde direction. After 1-2 weeks, the lymph flowed to the blood within the substance of lymph nodes, and after 4-7 weeks, anastomoses could be demonstrated between the thoracic duct and the hemiazygos vein.

Thus it is likely that initially most or all of the lymph from the intestines of sheep is collected from a cannula in the thoracic duct. However if the flow through the cannula is temporarily obstructed due to the formation of clots, alternative routes may develop and some of the lymph from the intestines may drain directly into the blood.
CHAPTER 7.

DISCUSSION.

When a young lamb is suckling its mother, the milk passes from the oesophagus through the closed oesophageal groove into the abomasum and duodenum. The proteins are coagulated in the abomasum and form a semi-solid curd which slows the movement of the milk through the intestine and ensures that adequate enzymic digestion occurs. In grazing sheep however, the oesophageal groove does not close and the grass passes directly into the rumen. A variety of species of bacteria and protozoa reduce the protein and carbohydrate to small molecules which are absorbed into the portal blood. It is likely that the lipids are not absorbed from the rumen, but are released slowly with the remainder of the ruminal contents and pass through the abomasum into the small intestine. The results of experiments in which radioactive fat was given into the abomasum of lambs and into the rumen of sheep, suggest that up to 80 percent or more of the dietary fat may be absorbed in both sheep and lambs, and that a large proportion of the absorbed fat appears in the intestinal lymph. Some of the processes which occur during the digestion of this fat in the lumen of the gut, and during its absorption through the mucosal cells of the intestine into the lymph, will be considered.

The hydrogenation of the unsaturated fatty acids of the diet.

Although the lipids in lucerne chaff contain a high concentration of linoleic and linolenic acids, the lipids in the intestinal lymph of sheep feeding on lucerne chaff contained only small
quantities of these highly unsaturated fatty acids; most of the linoleic and linolenic acids which entered the rumen did not appear in the lymph. When maize oil was fed into the rumen and the fatty acid composition of the lymph lipids of sheep and lambs was studied, it was concluded that considerable hydrogenation of unsaturated fatty acids with the formation of saturated and monoenoic acids, did occur in the rumen.

The effect of the ruminal contents on dietary lipids was first investigated by Reiser (1951) who incubated linseed oil in vitro with sheep rumen contents and found that a marked reduction in the linolenic acid content of the oil had occurred. Subsequently, Reiser and his colleagues fed steers on a diet containing 5 percent of cottonseed oil and found that the depot fats of the animals had a higher concentration of saturated acids and a lower content of oleic acid than the animals which received a basal diet containing low levels of fat; this finding was ascribed to hydrogenation of the unsaturated fatty acids of the cottonseed oil in the rumen (Willey, Riggs, Colby, Butler and Reiser, 1952). These observations were corroborated by Shorland, Weenink and Johns (1955), Shorland, Weenink, Johns and McDonald (1957), Garton, Hobson and Lough (1958) and Garton, Lough and Vioque (1961) who did experiments in which unsaturated fatty acids were incubated with rumen contents, and by Hoflund, Holmberg and Sellmann (1955, 1956 b), Reiser and Reddy (1956) and Tove and Matrone (1962) who found that the fats in the tissues and ruminal liquor of ruminants were much more saturated than the fatty acids which were fed. However in young ruminants, and in adult ruminants when the rumen is bypassed and unsaturated fat injected
into the duodenum, unsaturated fatty acids are incorporated into the fat depots (Hoflund, Holmberg and Sellmann, 1956 a; Ogilvie, McClymont and Shorland, 1961). From these observations, it is clear that a considerable proportion of the dietary fatty acids are modified in the rumen before they are absorbed. It is probable that at least some of the relatively saturated fatty acids that are absorbed are deposited in the fat depots and contribute to the high degree of saturation of the depot fats. Several other theories have been advanced however, to explain the high concentration of saturated fatty acids in the tissue lipids of ruminant animals. Shorland (1955) noted that when rabbits, horses and even fish were fed on grass, they incorporated the polyunsaturated fatty acids into the depot fats, but that in grass-fed sheep little or no linoleic acid appeared in the fat depots. On the basis of experiments of this type, Shorland (1955) developed a hypothesis originally suggested by Hilditch and Lovern (1936) and proposed a biochemical evolution related to the origin of the depot fats. Shorland (1955) proposed three groups of animals:

1. those which deposit exclusively exogenous fat,
2. those which deposit exogenous and endogenous fat, and
3. those which deposit exclusively endogenous fat.

Shorland concluded that fish were incapable of synthesizing fatty acids de novo and that the dietary fatty acids were deposited unchanged, but that in amphibians, reptiles and non-ruminant mammals, the fatty acids in the depots were derived partly from the diet and partly from endogenous synthesis. In ruminant animals, Shorland considered that the depot fat
"consists almost entirely of endogenous fat, with palmitic, stearic and oleic acids as major constituents". Although it appeared that this endogenous fat was derived almost entirely from acetate, Shorland believed that the presence of odd-numbered fatty acids in ox and sheep depot fat (Hansen, Shorland and Cooke, 1954) supported the view that compounds other than acetate participate in the formation of animal fats. At this time it was thought that "with the solitary exception of iso-valeric acid (found only in the depot fats of the dolphin and porpoise) the molecules of all natural straight chain glyceridic fatty acids saturated or unsaturated, contain an even number of carbon atoms" (Hilditch, 1956). In all the samples of lucerne chaff and lymph lipids examined however, fatty acids with 15 and 17 carbon atoms were present in low concentration. When fatty acids with odd numbers of carbon atoms are fed to ruminants, they are incorporated into the depot fats (Appel, Bohm, Keil and Schiller, 1947). As these fatty acids are absorbed into the lymph it is likely that the odd-numbered fatty acids found in the ox and sheep depot fats are derived at least in part from the diet.

Although the volatile fatty acids which are absorbed from the rumen will contribute to the endogenous synthesis of depot fats (Blaxter, 1961), the long-chain fatty acids which are absorbed from the gut may also be deposited in the depots. The fatty acids in the depot fats of ruminants are thus derived partly from the endogenously synthesised fat, partly from dietary fat which has been modified by the ruminal microorganisms, and partly from the dietary fatty acids which have been absorbed unchanged.
In an effort to explain the distribution of fatty acids in the glyceride molecules of natural fats, Hilditch (1956) suggested that individual triglycerides tend towards maximum heterogeneity in composition, and that triglycerides containing three molecules of a single fatty acid are not formed until the concentration of that acid comprises 60 percent of the total fatty acids. In the depot and milk fats of ruminant animals, however, the proportion of fully saturated triglycerides was much higher than would be expected if the "even distribution theory" was correct. Hilditch explained this finding by suggesting that the high levels of stearic acid in the fat depots may have arisen by the hydrogenation of oleic acid in the glycerides which had been deposited according to the even distribution theory. Although some dehydrogenation of saturated fatty acids may occur in the tissues of ruminant (Appel, Bohm, Keil and Schiller, 1947) and nonruminant animals (Bu'Lock and Ridyard, 1960), there is no evidence that hydrogenation of unsaturated fatty acids does occur in the fat depots of ruminant animals.

In addition to stearic and oleic acids, trans-isomers of monounsaturated fatty acids are formed from the dietary fatty acids before they appear in the intestinal lymph. These trans-isomers have been found previously in other ruminant tissues (Bertram, 1928; Swern, Knight and Eddy, 1952; Cornwell, Backderf, Wilson and Brown, 1953; Hartman, Shorland and McDonald, 1954; 1955), and Swern, Knight and Eddy (1952) suggested that they may be formed in situ in the fat depots by the action of oxidases. These oxidases have been found in rats,
pigs and rabbits (Bergström and Holman, 1946), but the tissues of these animals contain only trace amounts of trans-isomers (Hartman, Shorland and McDonald, 1955). On the other hand, Hartman, Shorland and McDonald (1954) suggested that the trans-isomers may be formed in the rumen during the hydrogenation of polyunsaturated fatty acids. In support of this hypothesis, Hartman, Shorland and McDonald (1954) found that whereas the dietary lipids contained negligible amounts of trans-acids, these isomers comprised about 9 percent of the total fatty acids in the ruminal content. In the experiments described in Chapter 5, trans-acids were not detected in the lipids from lucerne chaff, but the lipids from the intestinal lymph of sheep fed on this chaff contained up to 10 percent of trans-isomers of monounsaturated fatty acids. Shorland, Weenink, Johns and McDonald (1957) provided further evidence from in vitro experiments. When fatty acids with 18 carbon atoms were incubated with sheep rumen contents, the hydrogenation of unsaturated fatty acids was paralleled by the formation of trans-isomers. When linolenic acid was used, 67 percent of the fatty acids present at the end of the experiment were trans-isomers. In addition, positional isomers of linoleic acid were formed from both linoleic and linolenic acids, including isomers with conjugated double bonds (i.e. \(-\text{CH} = \text{CH} -\text{CH} = \text{CH} -\)) which were apparently resistant to further hydrogenation; this was supported by the finding that carotene, which contains conjugated double bonds, was not destroyed during the incubations. It is interesting to note that the positional and geometric isomers which are formed during microbial hydrogenation of unsaturated
fatty acids are very similar to those produced during the industrial hardening of lipids by hydrogenation (Shorland, Weenink, Johns and McDonald, 1957).

The species of micro-organisms responsible for the hydrogenation of fatty acids in the rumen are not known, and it is not known whether the hydrogenation takes place within the cells or extra-cellularly. Wright (1959; 1960) found that both bacteria and protozoa are probably important in this process. When chloroplast lipids were incubated with rumen liquor containing rumen bacteria, almost all of the polyunsaturated fatty acids were hydrogenated, but the degree of hydrogenation was much less when the fatty acids were incubated with rumen bacteria suspended in phosphate buffer (Wright, 1960). In the rumen, less than 0.1 gram of lipid is contained within the bacterial cells in each litre of fluid (Garton and Oxford, 1955), and if hydrogenation does occur within the cells, the rate of turnover of the bacterial lipid must be very high. Under these circumstances, it may be expected that substantial amounts of the lipids would be metabolised by the micro-organisms. Although many species of aerobic bacteria can oxidise fatty acids (Silliker and Rittenberg, 1951), Hobson and Mann (1961) found that when some species of rumen bacteria were incubated with emulsified linseed oil under anaerobic conditions, there was no evidence that oxidation of the fatty acids was taking place, although hydrolysis of the triglycerides did occur, and the glycerol which was liberated was fermented.
The role of lipases and bile in the digestion of fat.

The glycerides which are present in the diet are partially or completely hydrolysed by lipases, and the products of hydrolysis are converted into a more stable physical form by the constituents of the bile before they are absorbed from the small intestine.

Lipase activity has been detected in the secretions of small glands in the pharynx and base of the tongue of young calves (Ramsey, Young and Wise, 1960; Young, Ramsey and Wise, 1960), but this lipase activity disappears at or about weaning time (Nelson, unpublished; cited by Garton, 1960 a) after the microbial population has become established. The micro-organisms in the rumen may also hydrolyse triglycerides and phospholipids (Garton, Hobson and Lough, 1958; Garton, Lough and Vioque, 1959; Dawson, 1959). When vegetable oils were incubated anaerobically for 24 hours with sheep rumen contents, 40-75 percent of the glyceride fatty acids were liberated, and the extent of hydrolysis appeared to be related to the degree of unsaturation of the component fatty acids (Garton, Hobson and Lough, 1959; Garton, Lough and Vioque, 1959). Garton, Hobson and Lough (1958) found that the lipids in the ruminal fluid in sheep fed on hay and vegetable concentrates contained from 50-60 percent of free long-chain fatty acids. The experiments described in this thesis have shown however that in sheep and lambs deprived of pancreatic juice the concentration of lipids in the intestinal lymph falls to very low levels, indicating that little dietary fat is being absorbed. If hydrolysis of triglycerides precedes fat absorption and this is the main function of pancreatic
lipase, it would seem that lipolysis occurring proximal to the small intestine is unimportant in influencing the absorption of long-chain triglycerides in both sheep and lambs.

Pancreatic juice from sheep and lambs appears to have an activity towards glycerides of long-chain fatty acids similar to pancreatic lipase from the rat (Borgström, 1952 e; 1954 b), pig (Borgström, 1957 a; Wills, 1961) and human (Borgström, 1956; 1957 a). Purified lipase preparations have been produced from extracts of swine pancreas, and swine and rat pancreatic juice (Sarda, Marchis-Mouren, Constantin and Desnuelle, 1957; Wills, 1958; 1961; Marchis-Mouren, Sarda and Desnuelle, 1959; 1960) and have been shown to hydrolyse only triglycerides in emulsion form; an "esterase" present in low concentration in pancreatic juice is active against solutions of short-chain fatty acid esters (Desnuelle, 1961). The dietary fat enters the duodenum as an emulsion stabilised by phospholipid (Borgström, 1962), and it is likely that the lipase is active against this fat only when absorbed to the oil/water interface of the emulsion particles (Schönheyder and Volqvartz, 1943; Sarda and Desnuelle, 1958; Desnuelle, 1961).

The optimum pH for the hydrolysis of triglycerides in in vitro experiments has been found to vary with the substrate and buffer system used (Weinstein and Wynne, 1935-36). Schönheyder and Volqvartz (1945) using pig pancreas extract found that the optimum increased from pH 7 for triacetin to pH 8.8 for tristearin, but the presence of calcium ions did not change the pH optimum. Variations have also been noted
with lipases from different animals (Borgstrom, 1956).

Borgstrom (1957 a) found that the hydrolysis of triolein by pancreatic juice from rats proceeded most rapidly at pH 8.7, whilst for pancreatic juice from pigs and humans the optimum pH was 9.0-9.1 and 9.1-9.2. The results obtained when sheep pancreatic juice was incubated with an emulsion of cottonseed oil, which contains linoleic, oleic and palmitic acids as major components (Hilditch, 1956), suggest that the pH at which most rapid hydrolysis occurs is about 7.8. This is slightly lower than the optimum pH for lipolysis by pancreatic juice from monogastric animals.

Bile salts will also cause variations in the optimum pH for lipolysis. Borgstrom (1954 a) found that the optimum pH for lipolysis by rat pancreatic juice decreased from 8 to 6 with increasing concentration of taurocholic acid up to 0.2 percent. A second pH optimum appeared in the region of pH 9. The rate of lipolysis at pH 8 was reduced in the presence of a high concentration of bile salts. Thus, although in vitro the optimum pH for the hydrolysis of triglycerides by sheep pancreatic lipase was about 7.8, it is likely to be rather lower than this in the presence of bile salts in the lumen of the small intestine. In the absence of bile salts, the rate of lipolysis is limited by a non-temperature dependent reaction (Sarda and Desmuelle, unpublished; cited by Desmuelle, 1961) which may be the removal of the liberated fatty acids from the oil-
water interface of the glyceride particles (Desnuelle, 1961; cf. Hofmann, 1961; Hofmann and Borgstom, 1962). When this process is stimulated by bile salts, a temperature dependent reaction becomes rate limiting; this reaction is probably the hydrolysis of monoglycerides (Borgstrom, 1962).

Calcium ions also stimulate the hydrolysis of fats by pancreatic lipase in monogastric animals as well as in sheep (Schnheyder and Volqvartz, 1945; Borgstrom, 1954 b). It is likely that the calcium ions act by removing the liberated fatty acids from the oil-water interface of the glyceride particles as insoluble calcium soaps. The rate of resynthesis of glyceride ester bonds is decreased (Borgstrom, 1954 b), and the reaction proceeds more rapidly in the direction of net hydrolysis. In addition, calcium ions have been shown in in vitro experiments to increase the stability of pancreatic lipase (Wills, 1960) which rapidly loses activity in the lumen of the gut (Pelot and Grossman, 1962). Each day up to 2-5 grams of calcium is excreted into the gut of sheep (Hill, 1962), and this may help to maintain the activity of the lipase in the intestine.

The mode of action of pancreatic lipase on triglycerides has been shown to be a three-stage reaction (Borgstrom, 1954 b; cf. Bergstrom and Borgstrom, 1956) and the hydrolysis proceeds largely via the pathway:

\[
\text{Triglyceride} \rightarrow 1,2-\text{Diglyceride} \rightarrow 2-\text{Monoglyceride} \rightarrow \text{Glycerol}
\]

Although pancreatic lipase will hydrolyse tri-, di and monoglycerides, the reaction rate decreases in this order (Marchis-Mouren, Sarda and
Thus partial glycerides accumulate during lipolysis in vivo (Desnuelle and Constantin, 1952; Mattson, Benedict, Martin and Beck, 1952; Borgstrom, 1952 e) and in vitro (Frazer and Sammons, 1945; Desnuelle, Naudet and Rouzier, 1948; Desnuelle, Naudet and Constantin, 1950; Borgstrom, 1952 e; 1954 b; Mattson and Beck, 1955). During lipolysis by sheep pancreatic juice, negligible amounts of free glycerol were liberated during the early stages of the incubation, suggesting a very slow rate of hydrolysis of monoglycerides which apparently accumulated in the incubation medium. This decrease in the rate of hydrolysis as the number of fatty acid chains attached to primary alcohol linkages decreases, may be partly explained by the strong specificity shown by the lipase for these primary linkages (Mattson and Beck, 1956; Desnuelle, 1961).

The rate of hydrolysis of artificial triglycerides is not related to the chain length (12-18 carbon atoms) or degree of unsaturation of the fatty acids (Savary and Desnuelle, 1956). In most vegetable and animal fats however, the fatty acids esterified to the primary alcohol groups are more saturated than those esterified to the 2-position (Savary, Flanzy and Desnuelle, 1957; Mattson and Lutton, 1958) and it may be expected that during the initial stages of the hydrolysis of these fats in the lumen of the intestine, more saturated fatty acids will be liberated. As the hydrolysis proceeds, the iodine number of the fatty acids which are liberated will tend to increase.

The results of experiments in which sheep and lambs were deprived of pancreatic juice suggest that partial or complete hydrolysis of the dietary fat by pancreatic lipase is a
necessary prerequisite to fat absorption in these animals. It may be suggested however, that the decrease in fat absorption following diversion of pancreatic juice from the duodenum of the sheep and lambs may be due in part to an increase in the acidity of the intestinal contents. In animals with simple stomachs, the pancreatic juice bicarbonate helps to maintain a stable pH in the intestinal lumen. An decrease in the pH of the duodenal contents, which occurs after a meal, promotes the release of secretin, which in turn stimulates the flow of bicarbonate-rich pancreatic juice which helps to neutralise the excess acid in the duodenum (Gregory, 1962). It is possible that the increase in acidity of the intestinal contents following deprivation of pancreatic juice in monogastric animals may decrease the absorption of fatty acids present in the intestine: the incorporation of fatty acids into soluble bile salt micelles in vitro is almost negligible at very low pH values (Hofmann, 1961). In sheep however, there is no evidence that significant changes in intestinal pH occur in animals deprived of pancreatic juice. Magee (1961) found that in normal fed sheep, pancreatic juice flowed at an average of 12.4 ml./hr. and contained 10-35 mequiv./litre of bicarbonate: about 0.25 mequiv. of bicarbonate were secreted each hour in the pancreatic juice. In the experiments of Masson and Phillipson (1952), the mean total acidity of the duodenal contents (collected from an area proximal to the entrance of the common bile duct) of sheep was 54 mequiv./litre, and 400-500 ml. of material passed from the abomasum into the duodenum each hour. From these results it can be calculated that about 25 mequiv. of titratable acidity
pass into the duodenum each hour, and it is not likely that the removal of 0.25 mequiv/hr. of bicarbonate in pancreatic juice would greatly affect the intestinal pH. In fact, Masson and Phillipson (1952) found that the mean pH of material entering the duodenum in sheep was 3.0 (the mean value for abomasal pH found in our experiments was 3.1) whereas in the above experiments the pH of samples of duodenal contents collected from the duodenum distal to the entrance of the common bile duct was 4.2 (Heath and Morris, 1963). Magee (1961) found that the pH of the duodenal contents is generally below 4.5. Samples of intestinal contents collected from the mid-jejunum had a much higher pH, but it is not known whether the acid which leaves the abomasum is absorbed in the proximal small intestine, or whether the intestinal secretions of sheep have a high buffering capacity.

In dogs, the administration of fat into the duodenum is followed by an increase in the flow and enzyme concentration of the pancreatic juice (Wang and Grossman, 1951). In response to a high fat meal, increased amounts of lipase are secreted to effect the digestion of the fat as it passes through the proximal small intestine. In sheep however, the dietary fat is released continuously from the rumen into the abomasum and duodenum, and the administration of fat into the duodenum of these animals was without effect on the rate of secretion and enzyme content of the pancreatic juice (Magee, 1961).

In both ruminant and nonruminant animals therefore, dietary triglycerides are hydrolysed in the small intestine. In man, about 40
percent of the dietary triglycerides are completely hydrolysed, while most of the remaining 60 percent are partially hydrolysed, prior to their absorption (Borgstrom, Tryding and Westo, 1957). In the nonruminant animals, most if not all of the hydrolysis of triglycerides is catalysed by pancreatic lipase in the small intestine. In ruminant animals however, two sites of hydrolysis have been proposed, the rumen and the small intestine, but studies on the dynamic aspects of glyceride hydrolysis and resynthesis in these regions of the gut are lacking.

In sheep deprived of bile, the concentration of lipids in the intestinal lymph decreased rapidly and after 6-10 hours the absorption of dietary fat had ceased. In lambs, some dietary fat was absorbed into the lymph in the absence of bile, but the absorption of fat was greatly impaired. This contrasts with the situation in monogastric animals: in humans deprived of bile, the absorption of fat from the gut is reduced by only about 40 percent (Borgstrom, 1962), and Bernhard, Ritzel and Hug (1952) found in dogs with bile fistulae that 50-80 percent of a meal of deuterium labelled fat was absorbed. It is possible that some of the effect of bile deprivation on fat absorption may be due to intestinal stasis and a decreased rate of movement of material through the gut. There is however, no evidence that bile deprivation has this effect, and in fact Morgan and Simmonds (1962) have shown that in rats deprived of bile the rate of stomach emptying is increased. Although no $^{14}$C tripalmitin was absorbed in sheep deprived of bile, all the radioactive fat was recovered in the faeces within 2-3 days. The sheep and lambs deprived of bile appeared
clinically normal with good ruminal and intestinal movements; they ate well and passed normal amounts of faeces. Another possible explanation of the depression in fat absorption in these experiments may be that the metabolism of the mucosal cells of the intestine was altered. In vitro, conjugated bile salts have been shown to stimulate the esterification of fatty acids which have been absorbed by segments of rat intestine (Dawson and Isselbacher, 1960 b). When segments of jejunum from sheep and lambs were incubated with $^{14}$C palmitate in soluble form as an albumin complex however, there was no evidence that bile deprivation affected the uptake or esterification of the fatty acids, nor was there any evidence that bile was necessary for this process. For these reasons, the effects of bile deprivation in sheep and lambs were thought to be due to a change in the normal processes which occur in the intestinal lumen during fat absorption.

It has long been thought that bile salts aid the absorption of fat by converting the products of hydrolysis into a physical form suitable for absorption. Frazer, Schulman and Stewart (1944) found that bile salts formed a stable emulsion with free fatty acids and monoglycerides in the pH range 4-8.5, and they suggested that fat is absorbed from the intestine as an emulsion of this type with a particle size of less than 0.5 µ. Borgstrom (1962) claimed that studies on the ultrastructure of the intestinal mucosa, and on the physical state of the lipids during digestion did not favour Frazer's hypothesis. As the fatty acids and monoglycerides released during lipolysis can be incorporated into soluble micelles of conjugated bile salts or lyso-
phosphatidyl choline, Borgstrom (1962) and Hofmann and Borgstrom (1962) suggested that the dietary fat may be absorbed in micellar form. The output of bile salts in the bile of sheep and lambs appears, as in other animals, to be dependent on the resorption of bile salts from the intestine (Josephson, 1941; Harrison and Hill, 1960; Wheeler and Ramos, 1960).

In the bile of humans, it appears that the concentration of phospholipids may approach 1 gram percent (Blomstrand, 1960; Blomstrand and Ekdahl, 1960). Phosphatidyl choline comprises the major part of the bile phospholipids in humans, but small amounts of lysophosphatidyl choline are also present (Phillips, 1960). Earlier work, however, suggested that the concentration of phospholipids in human bile was much lower than the concentrations found in the bile from sheep and lambs (Sobotka, 1937; Deuel, 1955). In the bile from sheep, taurine conjugates of di- and tri-hydroxy bile salts are present (Deuel, 1955), together with high concentrations of phosphatidyl choline and lysophosphatidyl choline. It is likely that the phosphatidyl choline which enters the small intestine in the bile, is hydrolysed to lysophosphatidyl choline and glycerophosphoryl choline by pancreatic phospholipases (Shapiro, 1952; 1953). In man, most of the phospholipids in the lumen of the small intestine are present as lysophosphatidyl choline (Borgstrom, 1957 b).

The region of the intestine which is most important for the absorption of these products of glyceride hydrolysis is not known, but the marked differences in the rate of uptake of \(^{14}\)C palmitate between
segments of proximal jejunum and distal ileum suggest that the proximal segment of the intestine may be more active in fat absorption. This view is consistent with the conclusions of Borgstrom, Dalqvist, Lundh and Sjovall (1957) following studies in man, but in rats, fat absorption appears to occur more rapidly in the distal parts of the small intestine (Bennett and Simmonds, 1962).

**The intestinal lymph and fat absorption.**

As in animals with simple stomachs, a large part of the fat which was absorbed from the intestines of sheep and lambs was esterified in the intestinal mucosa and appeared as triglyceride in the intestinal lymph. When lambs received a drink of milk or an injection of fat into the abomasum, the concentration of fat in the lymph increased to a maximum at about 5-8 hours then declined; the response was essentially similar to that which occurs in cats, rats and rabbits during fat absorption (Borgstrom and Laurell, 1953; Morris, 1954; Simmonds, 1955b). In sheep, the long period (up to four days) over which radioactive fat injected into the rumen is absorbed, suggests that the absorption of dietary fat into the lymph is a continuous process.

When large amounts of fat were injected into the abomasum of sheep, the fat content of the lymph decreased at first and then increased to show characteristically two peaks of fat concentration. It is possible that the decrease in the absorption of dietary fat which follows the injection of fat into the abomasum of sheep, may be due to a decrease in the rate of gastric emptying with a consequent decrease in the amount of fat present at the absorbing sites in the intestine.
The immediate nature of the response however, suggests that some factor which influences the absorption of fat from the lumen of the intestine may be responsible. When fat is injected into the abomasum of sheep, the rate of bile flow and the concentration of total solids in the bile fluctuates in a manner similar to the changes which occur in the fat concentration in the lymph during this period. The mechanisms which activate the gall-bladder under these circumstances are not known. Although a substance capable of causing gall-bladder contraction in small animals has been extracted from the intestine of sheep (Ivy, Kloster, Lueth and Drewyer, 1929; Kloster, Ivy and Lueth, 1929), the changes in bile flow in sheep bear little resemblance to the "cholecystokinin effect" in monogastric animals (Ivy, 1929; Greengard, 1948; Jorpes and Mutt, 1961). This effect is characterised in humans by a contraction of the gall-bladder during the first 30 minutes after the ingestion of a meal containing fat (Borgstrom, Dahlqvist, Lundh and Sjovall, 1957). It is possible that the fluctuations in fat absorption in sheep may merely reflect the availability of bile constituents in the small intestine; when the output of bile salts and phospholipids in the bile decreases, less of the fat in the intestine can be converted into a form suitable for absorption, and the content of fat in the lymph decreases.

Although at least 90 percent of the fat absorbed into the lymph of the sheep and lambs was present as triglycerides, the concentration of nonesterified fatty acids and phospholipids in the lymph increased during fat absorption. Similarly, when sheep and lambs
were given a feed of $^{14}$C tripalmitin, a small percentage of the radioactivity in the lymph was present in the nonesterified fatty acid and phospholipid fractions. It is likely that the exogenous nonesterified fatty acids in the lymph resulted from incomplete esterification of the fatty acids absorbed from the intestine. The increase in phospholipid concentration in the lymph probably reflects an increased rate of phospholipid synthesis in the intestinal wall. L-α-glycerophosphate and phosphatidic acid play an important part in the esterification of absorbed fatty acids in the intestinal mucosa of monogastric animals (Kennedy, 1957; Hubscher and Clark, 1961), and glycerophosphatides may be formed during this process. The results of experiments in which segments of small intestine from sheep and lambs were incubated with labelled palmitic acid suggest that similar reactions may occur in ruminant animals.

In monogastric animals in the post-absorptive state, the lipids in the lymph are derived from the plasma as constituents of the capillary filtrate (Morris, 1954; Courtice and Morris, 1955). Under these conditions, the concentration of lipids in the intestinal lymph does not exceed the plasma levels. In adult sheep however, no true post-absorptive state occurs, and the plasma concentrations of non-esterified fatty acids, phospholipids and cholesterol are always less than the levels in the lymph which contains considerable amounts of exogenous or newly synthesised lipid. When bile is diverted from the intestines however, no exogenous lipid enters the lymph and the level of lipids in the lymph falls below the plasma concentration. Under
these conditions the lipids of the intestinal lymph are probably derived from the plasma.

The fatty acids which appear in the intestinal lymph are not distributed at random between the different lipid fractions. In lymph from rats, the phospholipids contain a high concentration of stearic and linoleic acids but there is discrimination against the incorporation of oleic acid into phospholipids. Oleic acid is preferentially esterified to cholesterol (Whyte, 1963). In the phospholipids from ox plasma, Garton, Duncan and Lough (1961) and Duncan and Garton (1962) found that the proportion of linoleic acid is much higher, and that of oleic acid much lower than the levels in the glycerides. These workers also found a very high level of linoleic and linolenic acids in the cholesterol esters from ox plasma (Lough and Garton, 1957; Garton, Duncan and Lough, 1961; Duncan and Garton, 1962) and suggested that linolenic acid was preferentially esterified to cholesterol in the gut wall (Duncan and Garton, 1962). There was no evidence to suggest that this occurs in sheep. In the intestinal lymph of sheep fed on lucerne chaff, the fatty acid composition of the cholesterol esters did not differ from that of the glycerides, and did not change during the absorption of polyunsaturated fatty acids from the gut; minimal changes did occur in the cholesterol ester composition during the absorption of maize oil by lambs. The cholesterol esters in the lymph collected from the liver and hind limbs of sheep however, contained appreciably higher levels of linoleic acid than the cholesterol esters from the intestinal lymph (Adams and Morris, 1963). In sheep it was apparent that during the esterification
of the absorbed fatty acids, the 1,2 diglycerides containing linoleic acid tended to be phosphorylated to form phospholipids, but the cis and trans-isomers of \( C_{18} \) monoenoic acids were preferentially esterified to form triglycerides. During the absorption of maize oil however, progressively more of the linoleic acid was carried in the glyceride fraction, which suggests that the absolute amount of this acid incorporated into phospholipids in the intestinal mucosa is restricted by the rate of synthesis of the phospholipids. It is likely that a similar condition holds for rats. When Morris and Simpson-Morgan (1963) fed to rats \(^{14}C \) labelled linoleic acid dissolved in commercial oleic acid (containing about 1 percent of linoleic acid), 18 percent of the labelled fatty acids in the lymph were present in the phospholipids. When the labelled linoleic acid was dissolved in an artificial oil containing 34 percent of linoleic acid however, less than 5 percent of the radioactivity in the lymph was present in the phospholipid fraction (Simpson-Morgan and Morris, 1962).

The rate of flow of lymph from the intestines increased during the absorption of fat in sheep and lambs. In lambs given a drink of milk, the increase in lymph flow was accompanied by a decrease in the protein concentration in the lymph and this was probably due to the absorption of water from the intestine (Korner, Morris and Courtice, 1954; Simmonds, 1954). However in adult sheep, as in monogastric animals (Borgstrom and Laurell, 1953; Simmonds, 1954; 1955 b), the increases in lymph flow which occurred during fat absorption were not accompanied by consistent changes in the protein concentration in the
Borgstrom and Laurell (1953) and Simmonds (1955 b) found in rats that the lymph flow reached a peak about two hours before the maximum concentration of fat in the lymph, and Simmonds (1955 b) suggested that the lymphagogue effect was related to some event preceding the expulsion of fat into the intestinal tissue fluid. When Simmonds (1957) studied the effect of various pharmacological preparations on lymph flow during fat absorption, he concluded that the changes in lymph flow were not due to alterations in intestinal motility. It is likely that the increase in lymph flow during fat absorption is mediated by some humoral effect which influences blood flow and lymph formation in the intestinal mucosa (cf. Borgstrom and Laurell, 1953; Simmonds, 1955 b). It is interesting to note in this connection that some commercial preparations of secretin have been found to stimulate lymph flow from the intestines (Razin, Feldman and Dreiling, 1961).

It is likely that in both sheep and lambs, the dietary fat which enters the circulation as chylomicron triglyceride may make a significant contribution to the total energy requirements of the animals. The oxidation of continuously infused chylomicrons has been studied in rats by Morris and Simpson-Morgan (1963). When chylomicrons containing $^{14}C$ labelled fatty acids were infused into 220 gram rats at rates up to 100 mg. of total esterified fatty acid per hour, about 40 percent of the label infused each hour was oxidised to $^{14}CO_2$ during the third hour of the infusion. At the highest infusion rate (100 mg. fat/hour), about one-quarter of the rats' total energy was derived from the
oxidation of the infused chylomicrons. In the experiments on lambs (5-7 Kg.) described in this thesis, up to 30-50 µEq./minute (500-800 mg./hr.) of dietary fat was transported to the blood by the chylomicrons of the intestinal lymph, and it may be expected that an appreciable proportion of this chylomicron triglyceride would be oxidised directly.

In fasting adult sheep, the total energy utilized has been estimated to be about 1,100 Kcal./day (Annison and Lewis, 1959). Blaxter (1961) calculated that in a 50 Kg. sheep fed on a ration sufficient to maintain body weight, the material absorbed from the gut each day has a heat of combustion of about 1,350 Kcal. The sheep used in the experiments described in Chapter 3 weighed about 30 Kg. and absorbed up to 8 grams (70-80 Kcal.) of dietary fat into the lymph each day. Thus of the total energy-yielding substances absorbed from the gut of sheep, up to one-tenth may be provided by chylomicron triglycerides.

The quantitative importance of the lymphatic system in the transport of absorbed fat.

Although the lymphatic system plays an important part in the transport of absorbed fat, an appreciable amount of the radioactive fat absorbed from the gut of sheep did not appear in the lymph collected from intestinal or thoracic duct fistulae. Similar results were obtained when labelled palmitic acid was fed to rats (Bloom, Chaikoff, Reinhardt, Entenman and Dauben, 1950; Bloom, Chaikoff, Reinhardt and Dauben, 1951; Borgstrom, 1952 d), and Bloom, Chaikoff, Reinhardt, Entenman and Dauben (1950) suggested two pathways by which this fat
may enter the blood. On the one hand, they suggested that fat may be absorbed directly into the tributaries of the portal vein. The portal vein had been implicated in the transport of absorbed long-chain fatty acids by Frazer (1940; 1946). Although the portal vein transports most of the short-chain fatty acids from the gut (Bloom, Chaikoff and Reinhardt, 1951; Kiyasu, Bloom and Chaikoff, 1952), there is no evidence that fatty acids with 16 or more carbon atoms are transported by this route. On the other hand, Bloom and his colleagues suggested that all of the fat which is absorbed from the gut may appear in the intestinal lymphatics, but that some of this may enter the blood through lymphatic-venous anastomoses. Anastomoses of this type have been demonstrated in rats (Job, 1918; Threefoot, Kent and Hatchett, 1963), dogs (Freeman, 1942), cattle (Baum, 1911) and some monkeys (Silvester, 1912), but Engeset (1959) using rats and Carlsten and Olin (1952) using cats failed to find any evidence of anastomoses on X-Ray examination of the mercury filled lymphatics. The observations of Threefoot, Kent and Hatchett (1963) suggest that although lymphatic-venous anastomoses are normally present in rats, during some experimental procedures they may be non-functional, but may re-open following an injection of hexamethonium. When anaesthetised sheep were examined immediately after a thoracic duct fistula was established, it appeared that if connections do exist between the lymphatics from the intestine and the abdominal veins, they are not common. It is possible however, that in these sheep some anastomoses may have been present but were not patent during the experiments.
In most of the experiments involving chronic lymphatic fistulae, especially those of the thoracic duct, the flow of lymph was occasionally obstructed due to the formation of clots in the cannulae. An attempt was made in some experiments to minimise clot formation by coating the inner surfaces of the cannulae with silicone (Siliclad; Clay Adams). Although it was generally possible to remove the clots by infusing a solution of trypsin (British Drug Houses) or fibrinolysin (Elase; Parke Davis) into the cannulae, it was thought that new pathways for the drainage of lymph may have developed during the period of lymphatic obstruction. Under these circumstances it was not possible to ascertain whether the lymph draining from an area was being collected quantitatively.

When the flow of lymph through the thoracic duct was obstructed experimentally, it was found that the lymph was in fact draining to the blood through other channels. Immediately after the thoracic duct flow was obstructed in sheep, as in cats (Lee, 1922) and dogs (Blalock, Robinson, Cunningham and Gray, 1937), the lymphatics distal to the obstruction became distended and the valves incompetent, and lymph extravasated through the walls. Although leakage of large molecules through the walls of the larger lymphatics is negligible in normal animals (Mayerson, Patterson, McKee, LeBrie and Mayerson, 1962), after ligation of the lymphatics, protein may escape into the surrounding tissues (Courtice and Steinbeck, 1951). In the experiments with sheep, it is likely that the lymph which escaped through the walls of the
dilated lymphatics was subsequently absorbed into the lymphatics of the pleura and mediastinum and returned to the circulation through the right lymph duct (Yoffey and Courtice, 1956).

It may be expected that the extravasation of lymph through the walls of the occluded lymphatics, and its subsequent return to the circulation, is a relatively slow process. In sheep during the first few days after the thoracic duct was ligated, the passage of labelled protein from the mesenteric lymphatics to the blood was very slow. After 5-8 days however, the labelled protein was transferred from the mesenteric lymphatics to the blood much more quickly, and the evidence suggests that this transfer was occurring within the substance of lymph nodes. It has been shown in dogs that when the efferent lymphatic from a single lymph node is occluded, plasma protein will pass from lymph nodes directly into the blood (Pressman, Simon, Hand and Miller; 1962), and Zhdanov (1952) considers that when lymph drainage is obstructed, the increased pressure in the sinusoids of the lymph nodes may be followed by transfer of lymph to the blood within the lymph nodes. Macroscopic and microscopic changes occur in the lymph nodes of the abdomen after ligation of the thoracic duct: the nodes become large and oedematous with a decreased amount of lymphoid tissue (Lee, 1922; Blalock, Robinson, Cunningham and Gray, 1937). It is possible that lymph may enter the blood through stomata in the post-capillary veins within the nodes (Schulze, 1925). These stomata may serve as communications between blood vessels and lymph pathways in the cortical substance of the lymph nodes (Rusznyak, Foldi and Szabo, 1960). It is also possible that some transfer may take
place between lymphatics and small veins in the wall of lymph nodes (Engeset, 1959). When Engeset injected mercury into the lumbar lymph nodes of rats after the thoracic duct was ligated, he noticed that in two of the rats, mercury entered small veins in the walls of the renal lymph nodes. However the possibility that this transfer may have occurred within the substance of the nodes was not investigated.

Tjernberg (1962) noted that when iodinated benzoic acid derivatives were injected into the lymphatics of rabbits, they diffused rapidly through the walls of the lymphatics, and he considered that the contrast media had caused an increase in the permeability of the vessel walls. If these contrast media had increased the permeability of the lymphatics in the sheep used in the experiments described in Chapter 6, it is possible that they may also have acted on the blood vessels within lymph nodes, allowing the passage of lymph to the bloodstream. The rate of extravasation of lymph through the walls of the main lymphatics in sheep, even after ligation of the thoracic duct, was however, much slower than in rabbits (Tjernberg, 1962).

Indirect pathways of lymph drainage to the bloodstream may be replaced later by more direct connections between the thoracic duct and the hemiazygos vein. Anastomoses of this type have been demonstrated in cats (Lee, 1922; Carlsten and Olin, 1952), rats (Threefoot, Kent and Hatchett, 1963) and dogs (Blalock, Robinson, Cunningham and Gray, 1937) during the first two months after ligation of the thoracic duct. Freeman (1942) killed dogs at periods up to 225 days after the thoracic duct was tied at the base of the neck and in each dog he found lymphatic-
venous anastomoses. Yoffey and Courtice (1956) consider that these were probably enlargements of pathways normally present. New lymphatics may also develop to bypass the obstructed thoracic duct and drain directly into the large veins (Lee, 1922; Threefoot, Kent and Hatchett, 1963).

It is clear that the lymphatic system responds promptly and efficiently to the increase in pressure which follows the development of an obstruction. Means are provided whereby the excess tissue fluid with the plasma protein and absorbed fat are returned to the circulation so maintaining the volume and composition of the tissue fluid constant. This plasticity of the lymphatic system presents a problem when it is required to collect lymph from an area of the body over a long period. Although it is likely that initially all of the lymph from the intestines of sheep is collected from a freely flowing cannula in the thoracic duct, it may be that if there are temporary obstructions to flow through the cannula, this may stimulate the development of alternate routes of drainage and quantitative collection of the lymph would not occur. It was thought however, that this possibility did not provide the complete explanation of the failure to recover all of the absorbed fat in the intestinal lymph of sheep. In these animals therefore, the portal vein must be considered as a possible route for the transport of a proportion of the fat which is absorbed from the gut.

**GENERAL SUMMARY.**

During the growth of a lamb, changes occur in the pattern of digestion and absorption of the dietary fat. In the young lamb, the milk which is suckled bypasses the undeveloped rumen and the fat enters
the duodenum unchanged. As the lamb grows and begins to eat grass, the dietary fat is modified by events which occur during its passage through the rumen. Although some hydrolysis of triglycerides probably does occur in the rumen, it is likely that this does not greatly influence the absorption of fat from the intestine. Negligible amounts of the linoleic and linolenic acids from the diet appear in the intestinal lymph. It was concluded that the highly unsaturated C\textsubscript{18} fatty acids in the dietary lipids of sheep are hydrogenated in the rumen with the formation of stearic acid and cis and trans-isomers of C\textsubscript{18} monoenoic acids. The fatty acids of low iodine number which are characteristic of the depot fats of sheep are not derived entirely from endogenous synthesis, but partly from the dietary fatty acids which have been hydrogenated by the symbiotic micro-organisms in the rumen before being absorbed into the intestinal lymph.

In the small intestine of both sheep and lambs, the fat is hydrolysed by pancreatic lipase and the resultant products of hydrolysis are dependent on bile for their absorption. It is probable that up to 80 percent or more of the dietary fat is absorbed, and that this absorption occurs mainly in the proximal small intestine. When large amounts of fat are present in the small intestine of sheep, the rate of absorption of fat is probably regulated by the output of bile salts and phospholipids in the bile, and this in turn is controlled by the presence of fat in the abomasum or duodenum. Most of the absorbed fatty acids are esterified in the mucosal cells, probably through a phospholipid intermediate, and appear in the intestinal lymph as triglyceride;
small amounts may also be present in the phospholipids and non-
esterified fatty acids.

Linoleic acid is preferentially esterified to phospholipids, and oleic acid to glycerides in the intestinal mucosa. During the absorption of large amounts of linoleic acid however, the percentage of the linoleic acid in the lymph which is transported by phospholipids appears to be limited by the rate of synthesis of phospholipids in the gut wall. The proportion of linoleic acid in the glyceride fraction consequently increases with the increase in concentration of linoleic acid in the lymph. Although most of the absorbed fatty acids are transported in the intestinal lymph, it is possible that a small amount of the absorbed fat may enter the blood through lymphatic-venous anastomoses in the thorax or abdomen. The possibility that some of the absorbed fatty acids may enter the bloodstream directly, must be considered. During the absorption of fat into the intestinal lymph, the rate of flow of lymph increases in both sheep and lambs. Due to the continuous nature of fat absorption in sheep, it is likely that the intestinal lymph flow is continuously stimulated to a degree which varies with the rate of fat absorption.

The most conspicuous changes which occur in the processes of fat absorption during the growth of the lamb are doubtless referable to the development of a microbial population in the rumen capable of hydro-
genating unsaturated fatty acids, thus modifying the fatty acid composition of the lipids in the tissues. The sheep provides an example of a host animal whose long-chain fatty acid metabolism is regulated to an extent
by the presence of symbiotic organisms in its gut. The metabolic transformations effected by the micro-organisms on the dietary lipids are accepted by the host animal and the modified fatty acids used as such.
The work reported in this thesis was carried out in the Department of Experimental Pathology, John Curtin School of Medical Research, Australian National University, during the tenure of an Australian National University Scholarship. I am indebted to Professor F.C. Courtice for the opportunity to undertake this work and for his encouragement during its prosecution.

My best thanks are due to my supervisor, Dr. B. Morris for suggesting the topic, for his guidance during the course of the work and for his constructive criticism during the preparation of the manuscript.

I also wish to thank my friends in the School for their advice. Particular thanks are due to Mr. E.P. Adams who has provided the answers to many chemical problems, and to Mrs. H. Kobau for her conscientious technical assistance.

The plates used in Figures 3 and 5 were provided by Drs. A.K. Lascelles and B. Morris.

Finally, I thank my wife and Miss J. O'Connor for their assistance in the preparation of the manuscript.
BIBLIOGRAPHY.


Asellius G. (1627) "De Lactibus sive Lacteis venis"; Medicina; J. Baptam Bidellius; cited by Drinker (1942).


Baker, J.R. (1942) Quart. J. micr. Sci. 84, 73.


Bennett, S. and Simmonds, W.J. (1962) Quart. J. exp. Physiol. 47, 32.


Borgstrom, B. (1952 b) Acta physiol. scand. 25, 111.
Borgstrom, B. (1952 c) Acta physiol. scand. 25, 140.
Borgstrom, B. (1957 b) Acta chem. scand. 11, 749.
Carpenter, W.B. (1842) "Principles of Human Physiology", cited by
Olmsted (1939).
Cornwell, D.G., Backderf, R., Wilson, C.L. and Brown, J.B. (1953)
Arch. Biochem. 46, 364.
Sci. 29, 451.
Cremer, E. and Muller, R. (1950) Mikrochmeie 36-37, 553.
Cruikshank, W. (1786) "Anatomy of the Absorbing Vessels of the Human
Body", cited by Gulliver (1846).
de Graaf, R. (1664) "De natura est usu succi pancreatici", Leiden.
Cited by Gastiglioni (1947).
Acta 2, 561.
Acta 5, 561.
Publishers Inc.
Drinker, C.K. (1942) "The Lymphatic System" Lane Medical Lectures; Stanford University; Stanford University Press.
Eimer, Th. (1869) Virchows Arch. 48, 119.
Elkes, J.J., Frazer, A.C. and Stewart, H.C. (1939) J. Physiol. 95, 68.
Elkes, J.J. and Frazer, A.C. (1943) J. Physiol. 102, 24 P.
Frazer, A.C. (1943 a) J. Physiol. 102, 306.
Frazer, A.C. (1943 b) J. Physiol. 102, 329.
Harrison, F.A. and Hill, K.J. (1960) J. Physiol. 154, 61 P.


Hewson, W. (1769 b) Phil. Trans. 59, 204.


Hill, K.J. (1961) in "Digestive Physiology and Nutrition of the Ruminant"; edited by D. Lewis; London; Butterworths; p. 48.


Hunter, W. (1784) "Two introductory lectures to his last course of
Anatomical Lectures at His Theatre in Windmill St.; Cited by Drinker (1942).


Josephson, B. (1941) Physiol. Rev. 21, 463.
Lieberkuhn (1745) cited by Drinker (1942).
Magendie, F. (1817) Precis v. 2, p. 366; Cited by Olmsted (1944).
Moore, B. and Rockwood, D.P. (1897) J. Physiol. 21, 58.
Müller, (1838) "Physiologia" tr. Dr. Baly, i, 266. Cited by Gulliver (1846).
Munk, I. and Rosenstein, A. (1891) Virchows Arch. 123, 484.
Olmsted, J.M.D. (1944) "François Magendie"; New York; Schumans.
Pecquet, J. (1651) "Experimenta nova anatomica, quibus incognitum chyli receptaculum et ab eo per thoracem in ramos usque subclavios vasa lactea deteguntur". Cited by Drinker (1942), Rusznyak, Foldi and Szabo (1960).
Rachford, B.K. (1891) J. Physiol. 12, 72.
Ross, G. (1844) Lancet, 1, 625.
Rudbeck, O. (1653) "Nova Excercitatio Anatomica". Translated by Nielsen (1942).
Simmonds, W.J. (1957) Quart. J. exp. Physiol. 42, 205.
Sobotka, H. (1937) "Physiological Chemistry of the Bile"; London; Balliere, Tindall and Cox.
Stern, I. and Shapiro, B. (1953) J. clin. Path. 6, 158.


Wills, E.D. (1958) Biochem. J. 69, 17 P.


THE FATTY ACID COMPOSITION OF VARIOUS LIPID FRACTIONS ISOLATED DURING THE EXPERIMENTS DESCRIBED IN CHAPTER 5.

In Appendix Tables I, II and III, the proportions of the various fatty acids are shown as the percentages of the weight of the total fatty acids or of the \( C_{18} \) fatty acids. The fatty acids are designated by the number of carbon atoms, followed by the number of double bonds in the molecule: thus \( 18:0 \) represents stearic acid, \( 18:3 \) represents linolenic acid etc. Different samples of maize oil were used in the experiments on sheep and in those on lambs; the maize oil used in the sheep experiments is designated as "maize oil\(^1\)" and that used in the lamb experiments is designated as "maize oil\(^2\)."

Lamb 31 was suckling its mother (sheep milk\(^1\)) while lambs 32 and 33 were fed with cow's milk (cow's milk\(^1\)) from a bottle. During the analysis of the fatty acids from bile phospholipids, one or two peaks which had a retention time slightly longer than methyl stearate, could not be identified. These are listed as "p.c. of unident. acids" in Appendix Table III.

Abbreviations used in these Tables include:

- G - glyceride
- PL - phospholipid
- CE - cholesterol ester
- TL - total lipid
- T - traces were present
- NM - not measured
- DW - of dry weight
- O - 50 ml. of maize oil given by oesophageal tube
- R - 10 ml. of maize oil given into the rumen
- AB - 25 ml. of maize oil given into the abomasum
Appendix Table I. The fatty acid composition of lipids from sheep lymph, and from lucerne chaff and maize oil.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. frac-ment</td>
<td>µEq./unsat. trans C\textsubscript{18} -ion</td>
<td>ml. acids</td>
<td>acids</td>
<td>acids</td>
<td>18:0</td>
<td>18:1</td>
</tr>
<tr>
<td>36 G4CE</td>
<td>-</td>
<td>3.8</td>
<td>42</td>
<td>NM</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>36 PL</td>
<td>-</td>
<td>1.6</td>
<td>47</td>
<td>NM</td>
<td>64</td>
<td>40</td>
</tr>
<tr>
<td>40 G4CE</td>
<td>-</td>
<td>11.3</td>
<td>60</td>
<td>NM</td>
<td>69</td>
<td>20</td>
</tr>
<tr>
<td>40 PL</td>
<td>-</td>
<td>3.4</td>
<td>50</td>
<td>NM</td>
<td>68</td>
<td>28</td>
</tr>
<tr>
<td>48 G4CE</td>
<td>-</td>
<td>14.2</td>
<td>42</td>
<td>10.2</td>
<td>54</td>
<td>44</td>
</tr>
<tr>
<td>48 AB</td>
<td>-</td>
<td>22.7</td>
<td>71</td>
<td>T</td>
<td>74</td>
<td>12</td>
</tr>
<tr>
<td>48 PL</td>
<td>-</td>
<td>3.2</td>
<td>52</td>
<td>-</td>
<td>73</td>
<td>29</td>
</tr>
<tr>
<td>48 AB</td>
<td>-</td>
<td>3.5</td>
<td>54</td>
<td>-</td>
<td>70</td>
<td>29</td>
</tr>
<tr>
<td>49 G4CE</td>
<td>-</td>
<td>5.6</td>
<td>52</td>
<td>-</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>49 AB</td>
<td>-</td>
<td>22.1</td>
<td>65</td>
<td>-</td>
<td>68</td>
<td>13</td>
</tr>
<tr>
<td>49 PL</td>
<td>-</td>
<td>2.1</td>
<td>49</td>
<td>-</td>
<td>72</td>
<td>32</td>
</tr>
<tr>
<td>49 AB</td>
<td>-</td>
<td>3.0</td>
<td>55</td>
<td>-</td>
<td>71</td>
<td>27</td>
</tr>
<tr>
<td>48/49 CE</td>
<td>-</td>
<td>-</td>
<td>65</td>
<td>NM</td>
<td>61</td>
<td>22</td>
</tr>
<tr>
<td>48/49 CE AB</td>
<td>-</td>
<td>-</td>
<td>69</td>
<td>NM</td>
<td>73</td>
<td>16</td>
</tr>
<tr>
<td>54 G4CE</td>
<td>-</td>
<td>59.4</td>
<td>45</td>
<td>9.1</td>
<td>63</td>
<td>48</td>
</tr>
<tr>
<td>54 G4CE O</td>
<td>-</td>
<td>57.5</td>
<td>47</td>
<td>25</td>
<td>73</td>
<td>43</td>
</tr>
<tr>
<td>54 PL</td>
<td>-</td>
<td>10.6</td>
<td>51</td>
<td>-</td>
<td>77</td>
<td>34</td>
</tr>
<tr>
<td>54 PL O</td>
<td>-</td>
<td>10.9</td>
<td>53</td>
<td>15</td>
<td>79</td>
<td>32</td>
</tr>
<tr>
<td>63 G</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>NM</td>
<td>53</td>
<td>50</td>
</tr>
<tr>
<td>63 PL</td>
<td>-</td>
<td>-</td>
<td>45</td>
<td>NM</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>63 CE</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>NM</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>64 G</td>
<td>-</td>
<td>-</td>
<td>39</td>
<td>NM</td>
<td>60</td>
<td>54</td>
</tr>
<tr>
<td>64 PL</td>
<td>-</td>
<td>-</td>
<td>53</td>
<td>NM</td>
<td>69</td>
<td>32</td>
</tr>
<tr>
<td>64 CE</td>
<td>-</td>
<td>-</td>
<td>49</td>
<td>NM</td>
<td>63</td>
<td>44</td>
</tr>
</tbody>
</table>

Lucerne chaff: 3.0% DW 

Maize oil: 1

| DW | 72 | - | 61 | 5.1 | 5.5 | 30 | 60 | 1.7 | 0.4 | 24.7 | 8.6 | 1.2 | 3.1 | 3.4 | 18.0 | 35.3 |
|    | 84 | - | 86 | 2.2 | 41 | 56 | 0.7 | - | - | - | - | - | - | - | - | 3.52 | 4.8 | 0.6 |
Appendix Table II. The fatty acid composition of the lipids from lamb lymph and from milk and maize oil.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Lipid T</td>
<td>freat-ment</td>
<td>unsat.</td>
<td>trans</td>
<td>G18 acids</td>
<td>16:0</td>
</tr>
<tr>
<td>31</td>
<td>G</td>
<td>-</td>
<td>40</td>
<td>4.8</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>G</td>
<td>-</td>
<td>50</td>
<td>NM</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>G</td>
<td>R</td>
<td>54</td>
<td>NM</td>
<td>50</td>
<td>19</td>
</tr>
<tr>
<td>32</td>
<td>PL</td>
<td>-</td>
<td>58</td>
<td>NM</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>32</td>
<td>PL</td>
<td>R</td>
<td>59</td>
<td>NM</td>
<td>75</td>
<td>32</td>
</tr>
<tr>
<td>32</td>
<td>CE</td>
<td>-</td>
<td>70</td>
<td>NM</td>
<td>62</td>
<td>10</td>
</tr>
<tr>
<td>32</td>
<td>CE</td>
<td>R</td>
<td>69</td>
<td>NM</td>
<td>62</td>
<td>11</td>
</tr>
<tr>
<td>32</td>
<td>TL</td>
<td>-</td>
<td>50</td>
<td>NM</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>32</td>
<td>TL</td>
<td>R</td>
<td>56</td>
<td>NM</td>
<td>56</td>
<td>22</td>
</tr>
<tr>
<td>33</td>
<td>G</td>
<td>-</td>
<td>46</td>
<td>11</td>
<td>41</td>
<td>24</td>
</tr>
<tr>
<td>33</td>
<td>G</td>
<td>R</td>
<td>57</td>
<td>5.9</td>
<td>54</td>
<td>17</td>
</tr>
<tr>
<td>33</td>
<td>PL</td>
<td>-</td>
<td>57</td>
<td>T</td>
<td>73</td>
<td>32</td>
</tr>
<tr>
<td>33</td>
<td>PL</td>
<td>R</td>
<td>56</td>
<td>T</td>
<td>73</td>
<td>32</td>
</tr>
<tr>
<td>33</td>
<td>CE</td>
<td>-</td>
<td>67</td>
<td>-</td>
<td>66</td>
<td>14</td>
</tr>
<tr>
<td>33</td>
<td>CE</td>
<td>R</td>
<td>54</td>
<td>-</td>
<td>55</td>
<td>21</td>
</tr>
<tr>
<td>33</td>
<td>TL</td>
<td>-</td>
<td>48</td>
<td>8.8</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>33</td>
<td>TL</td>
<td>R</td>
<td>57</td>
<td>4.3</td>
<td>57</td>
<td>21</td>
</tr>
</tbody>
</table>

Sheep Milk\(^1\) 37 5.9 43 27 57 11 5.1 5.7 3.7 10.2 1.4 2.3 27.8 3.6 1.5 11.6 24.5 4.6 2.2
Cows' Milk\(^1\) 44 11 47 30 59 15 2.1 2.6 2.5 9.0 3.3 2.5 23.7 6.9 2.0 14.2 27.9 3.6 1.0
Maize oil\(^2\) 89 - 92 3.4 31 65 0.5 - - - - - - 8.0 - - 3.1 28.5 59.8 0.4
Appendix Table III.  The fatty acid composition of the phospholipids from bile.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. mg./ml.</td>
<td>酸 unsat.酸</td>
<td>酸 unident.酸</td>
<td>酸 trans酸</td>
<td>酸 C18酸</td>
<td>酸 18:0酸 18:1酸 18:2酸 18:3酸</td>
</tr>
<tr>
<td>Sheep</td>
<td>47</td>
<td>15.3 54</td>
<td>-</td>
<td>55</td>
<td>25 62 10 3</td>
<td>0.8 0.9 29.3 6.0 4.6 13.6 34.5 5.5 1.7</td>
</tr>
<tr>
<td>Sheep</td>
<td>61</td>
<td>14.3 56</td>
<td>2.8</td>
<td>58</td>
<td>23 49 18 10</td>
<td>0.7 0.8 28.4 5.6 2.2 13.2 28.7 10.2 6.1</td>
</tr>
<tr>
<td>Sheep</td>
<td>62</td>
<td>10.9 56</td>
<td>12.6</td>
<td>62</td>
<td>23 57 8 12</td>
<td>T T 20.9 2.8 1.5 14.2 35.6 5.2 7.2</td>
</tr>
<tr>
<td>Lambs</td>
<td>27/28</td>
<td>17.3 61</td>
<td>6.2</td>
<td>61</td>
<td>14 39 35 12</td>
<td>0.7 0.9 29.8 9.2 2.3 6.8 19.4 17.2 6.0</td>
</tr>
<tr>
<td>Lamb</td>
<td>30</td>
<td>28.8 51</td>
<td>-</td>
<td>53</td>
<td>19 37 27 17</td>
<td>2.7 2.9 32.0 4.5 3.4 10.2 19.7 14.4 9.0</td>
</tr>
<tr>
<td>Steer</td>
<td>1</td>
<td>11.5 45</td>
<td>4.6</td>
<td>48</td>
<td>31 42 17 10</td>
<td>0.6 0.9 36.7 6.0 2.2 14.7 20.2 8.0 4.7</td>
</tr>
<tr>
<td>Steer</td>
<td>2</td>
<td>13.4 53</td>
<td>6.0</td>
<td>53</td>
<td>25 51 15 9</td>
<td>0.9 1.1 33.2 6.4 2.0 11.8 24.5 7.4 4.4</td>
</tr>
</tbody>
</table>