The absorption of long-chain fatty acids from the small intestine of the sheep

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1. Three sheep, each of which was fitted with a rumen cannula and with two pairs of re-entrant cannulas in different parts of the small intestine, were used in this study. They were fed on dried grass cubes or hay plus linseed meal and oats: an aqueous solution of polyethylene glycol (PEG) was infused continuously into the rumen.

2. Total lipids were extracted from samples of the chyme entering and leaving the different lengths of the small intestine embraced by the respective cannulas. The lipids were fractionated into unesterified fatty acids, neutral lipids and phospholipids and the contribution of each fraction to the total fatty acids was determined. The samples were also analysed for their PEG content, thus affording an index of the extent to which water had been absorbed from each particular length of intestine.

3. From the above findings and a knowledge of the flow-rate of the digesta, the uptake of unesterified fatty acids and the degree of dissimilation or uptake, or of both, of esterified fatty acids was calculated.

4. The results indicated that, by the time the digesta reached the ileum (i.e. the distal half of the small intestine), the uptake of fatty acids was almost complete, as was also the hydrolytic release of esterified fatty acids.

5. Though there were no gross differences in the overall composition of the unesterified and esterified fatty acids in different parts of the small intestine, it appeared that C_{18} mono-unsaturated acid, the principal unsaturated unesterified acid, was absorbed somewhat more efficiently than were the major saturated acids (palmitic acid and stearic acid).

Following the studies described in the foregoing paper (Lennox, Lough & Garton, 1968) on the nature and origin of the lipids in the jejunum of the sheep, it was deemed desirable to make a quantitative assessment of the changes which take place in the amounts of unesterified and esterified fatty acids in the digesta during its passage through the small intestine. As a result of bacterial activity in the rumen of the sheep, dietary lipids are subjected to extensive hydrolysis and unsaturated fatty acids can be hydrogenated either partially or completely. Thus, in the lipids entering the small intestine of the sheep, unesterified fatty acids predominate and these usually consist mainly of stearic acid and positional and geometrical isomers of C_{18} unsaturated acids (see Garton (1967) for review of lipid metabolism in the rumen).

To determine the extent to which fatty acids are absorbed from different parts of the intestine, analyses were made of digesta samples obtained from three sheep, each of which was fitted with two pairs of re-entrant intestinal cannulas in different positions from the upper jejunum to the terminal ileum. A very brief account of some of the results obtained was included in a contribution to a recent symposium (Lough & Garton, 1968).

EXPERIMENTAL

Animals and their treatment

Three Scottish Blackface ewes, subsequently referred to as sheep 8, 9 and 10, were used in this study. At least 3 months before the experiments were undertaken each animal was fitted with a rumen cannula and with two pairs of re-entrant cannulas in the small intestine as described by Scott (1965). In this form of cannulation (see Fig. 1) the intestine is transected in two places and the ends are closed and cannulated; the cannulas (Ash, 1962) which comprise a pair are united by a short length of polythene tubing which can be removed to allow samples of digesta to be collected from the proximal cannula. The positions of the pairs of cannulas (subsequently referred to as cannulas 1 and 2, and cannulas 3 and 4), in terms of their approximate distance (in m) distal to the point of entry of the common bile duct, were as follows: sheep 8, 0·5 and

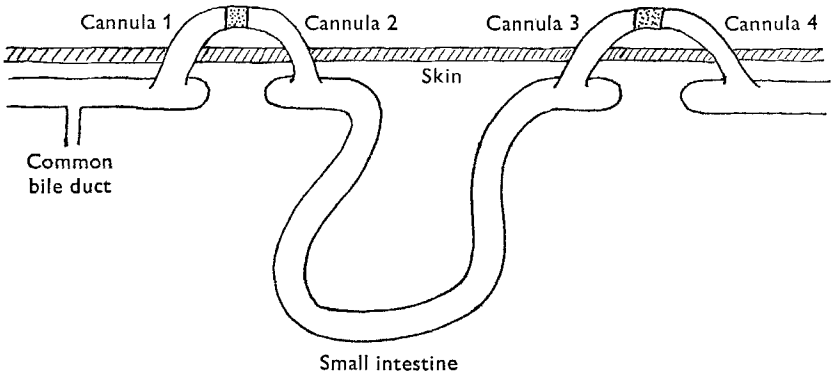

Fig. 1. Diagrammatic representation of re-entrant cannulation of the small intestine of the sheep (after Scott, 1965).

2·5; sheep 9, 2·5 and 8·5; sheep 10, 0·5 and 15·5. It was thus possible to obtain samples representative of the digesta entering and leaving the upper jejunum (sheep 8), the rest of the jejunum (sheep 9) and almost the entire length of the small intestine (sheep 10).

During the experiments and for several days previously each sheep was housed in a metabolism cage (Duthie, 1959) and given daily a diet of 800 g grass cubes from a continuous feed-delivery device (Murray, Reid & Sutherland, 1962) or a daily diet of 800 g hay, in two feeds at 08.30 and 16.30 h, plus 225 g of a mixture of linseed meal and crushed oats at 16.00 h daily. Throughout the experiment and for at least 5 preceeding days each animal received, via the rumen cannula, a continuous drip infusion of about 2·2 l. per day of an aqueous solution (0·5 %, w/v) of the non-absorbable solute, polyethylene glycol (PEG).

Collection of digesta

Digesta was collected for a period of 6–8 h from cannulas 1 and 3 and the flow-rate (ml/h) from cannula 1 was recorded. Following the removal of a 5 ml sample from each 50 ml, the pH was measured (glass electrode); the digesta was then heated to 37° and

returned to the intestine via the adjacent cannula. Each 5 ml sample of digesta was heated to 90° for 15 min and then frozen at −20° until it could be analysed. Two such collections of digesta were made on different occasions from each of the three sheep.

Analytical procedures

Determination of PEG. From each 5 ml portion of digesta a sample (1 ml) was removed and analysed for its content of PEG by the method of Hydén (1955) as modified by Smith (1958), except that the sample was not subjected to preliminary centrifugation.

Extraction and fractionation of lipids. The 4 ml portions of digesta remaining after removal of the samples for PEG determination were pooled and treated for the extraction and separation of unesterified fatty acids, neutral lipids and total phospholipids as previously described (Lennox *et al.* 1968) for the analysis of intestinal digesta from cannulated sheep. From each lipid fraction, methyl esters of the component fatty acids were prepared (Duncan & Garton, 1962) and weighed and their composition was determined by gas–liquid chromatography (Duncan & Garton, 1963); in Table 4, and as appropriate in the text, the acids are designated by the shorthand nomenclature of Dole, James, Webb, Rizack & Sturman (1959), which indicates the number of carbon atoms/molecule, followed by the number of double bonds. The *trans* fatty acid content (expressed as elaidic acid, i.e. 18:1) of the unesterified fatty acids from the digesta of sheep 10 was determined by submitting the methyl esters to infrared absorption analysis (Garton & Duncan, 1964).

Calculation of results

Given the flow-rate of digesta from cannula 1 and making allowance for the 10% retained for analysis, the mean amounts (mg/h) of unesterified and esterified fatty acids which entered the length of intestine at cannula 2 could readily be calculated. Values for the mean amounts of fatty acids in the digesta leaving the length of intestine at cannula 3 were calculated from the expression

$$\frac{(\text{mg fatty acid/ml}) \times (\text{vol. digesta entering cannula 2/h})}{\text{PEG concentration factor}},$$

the PEG concentration factor being the ratio of the values for the PEG content/unit volume of digesta from cannulas 3 and 1. Though Coombe & Kay (1965) showed that PEG was retained for a very slightly longer period in the small intestine of the sheep than was particulate matter (stained straw), for the purpose of these experiments the PEG concentration factor was taken as an index of the degree to which both liquid and solid phases were concentrated during their passage along the intestine.

RESULTS AND DISCUSSION

The mean flow-rates of digesta from cannula 1 of each animal are given in Table 1, together with the pH of the digesta samples and the values for the corresponding PEG concentration factors, and in Table 2 are shown the results of the two experiments on

each sheep with respect to the amounts of unesterified and esterified fatty acids entering and leaving the different portions of the small intestine. The proportions of fatty acids which remained in combination as neutral lipid and phospholipid following passage of the digesta through the different parts of the intestine are shown in Table 3.

In sheep 8, in which the two pairs of cannulas embraced about 2·0 m of the upper jejunum just distal to the bile duct, only very limited hydrolytic release of esterified fatty acids took place (Table 2) and the absorption which occurred was almost entirely at the expense of the unesterified fatty acids which were present initially in the incoming digesta. The 6·0 m length of intestine, to which access was afforded by the cannulas in sheep 9, represents that portion of the jejunum immediately distal to the lower pair of cannulas in sheep 8. In this part of the intestine there was not only a significant uptake of unesterified fatty acids, but also a very marked hydrolysis or

Table 1. *Diets, rate of flow of digesta from cannula* 1 *of each sheep, pH of digesta samples and polyethylene glycol concentration factors*

Sheep no.	Experiment no.	Diet	Mean flow-rate from cannula 1 (ml/h)	PEG concentration factor (mean value)	pH of digesta	
					Entering cannula 2	Leaving cannula 3
8	1	Hay, linseed and oats	358	1·04	2·8–4·6	3·8–5·0
	2	Grass cubes	223	1·24	3·2–3·9	3·9–4·2
9	1	Grass cubes	157	1·41	4·3–4·8	7·6–7·9
	2	Grass cubes	117	1·32	4·6–5·0	7·2–7·6
10	1	Grass cubes	238	2·56	2·5–4·0	7·6–7·9
	2	Hay, linseed and oats	533	2·47	2·6–4·2	7·5–7·6

uptake, or both, of fatty acids in ester combination as neutral lipids and phospholipids (see Table 3). With respect to the phospholipids (mostly biliary phosphatidylcholine and lysophosphatidylcholine) which enter this part of the jejunum, it seems likely, as discussed in the preceding paper (Lennox *et al.* 1968), that lysophosphatidylcholine is progressively formed from phosphatidylcholine by the action of pancreatic phospholipase and that it may facilitate the micellar solubilization of unesterified fatty acids and be concomitantly assimilated by the mucosal cells. The values in Table 1 of about 79% for the degree of absorption of fatty acids from virtually the entire length of the small intestine (sheep 10) are probably slightly low since the fatty acids apparently remaining esterified (notably as phospholipids) probably included structural lipids of ileal bacteria.

Taken together, the results from the experiments on the three sheep indicate that, by the time the digesta reached the ileum (i.e. the distal half of the small intestine), the assimilation of unesterified fatty acids and the dissimilation or uptake, or both, of ester-bound fatty acids were almost complete.

The compositions of the unesterified and ester-bound fatty acids in the digesta obtained in the two experiments with each sheep were similar, although sheep 8 and 10 received diets of grass cubes in one experiment and hay, linseed and oats in the other.

For clarity of presentation the results from one experiment with each animal are shown in Table 4 and the values are restricted to those for palmitic acid and the C_{18} fatty acids, since these comprised the greater part of the total acids in each class of lipid, notably in the unesterified fatty acids.

Table 2. *Amounts of unesterified and esterified fatty acids entering and leaving different lengths of the small intestine of sheep*

Experiment no.*	Nature of fatty acids	Amount (mg/h) in digesta: Entering cannula 2	Amount (mg/h) in digesta: Leaving cannula 3	Amount absorbed: mg/h	Amount absorbed: %
	Sheep 8 (2·0 m length of upper jejunum)				
1	Unesterified	599	505		
	Esterified				
	In neutral lipids	49	43		
	In phospholipids	135	118		
	Total	783	666	117	15·0
2	Unesterified	329	229		
	Esterified				
	In neutral lipids	60	45		
	In phospholipids	96	85		
	Total	485	359	126	26·0
	Sheep 9 (6·0 m length of lower jejunum)				
1	Unesterified	369	205		
	Esterified				
	In neutral lipids	58	29		
	In phospholipids	77	6		
	Total	504	240	264	52·4
2	Unesterified	217	96		
	Esterified				
	In neutral lipids	38	11		
	In phospholipids	35	6		
	Total	290	113	177	61·0
	Sheep 10 (15 m length of intestine, upper jejunum to lower ileum)				
1	Unesterified	379	79		
	Esterified				
	In neutral lipids	73	15		
	In phospholipids	79	18		
	Total	531	112	419	78·9
2	Unesterified	490	81		
	Esterified				
	In neutral lipids	48	22		
	In phospholipids	125	35		
	Total	663	138	525	79·2

* Details of the diet in each experiment are given in Table 1.

Though there were no gross differences in overall composition of either the unesterified or the esterified fatty acids in different parts of the small intestine, the uptake of unesterified fatty acids was apparently not completely unselective. A comparison of the composition of the unesterified fatty acids entering and leaving the cannulas of sheep 9 and 10 shows that, though palmitic acid (16:0) and stearic acid (18:0) were

quite efficiently absorbed, there was a somewhat preferential uptake of C_{18} mono-unsaturated acid. Analyses of duodenal and ileal contents of two slaughtered sheep by Ward, Scott & Dawson (1964) indicated that C_{18} mono-unsaturated acid of the *trans* configuration, which is produced in the rumen during bacterial hydrogenation of dietary C_{18} unsaturated acids (Shorland, Weenink, Johns & McDonald, 1957; see also Garton, 1967), was absorbed almost completely from the small intestine. Though in the experiment with sheep 10 no such markedly selective uptake of *trans* acid was apparent, this acid did comprise a smaller proportion of the 18:1 leaving the terminal ileum than it did of the 18:1 entering the upper jejunum (see footnotes to Table 4), showing that the *trans* acid had been absorbed to a relatively greater extent than had the accompanying 18:1 of the *cis*-configuration; it is unlikely (cf. Ward *et al.* 1964) that any of the *trans* 18:1 in the ileal contents arose by bacterial hydrogenation in this part of the small intestine.

Table 3. *Proportions of fatty acids which remained in ester combination following passage of digesta through different lengths of the small intestine of sheep*

(For lengths of intestine, see Table 2)

Sheep no.	Experiment no.*	Proportion (%) remaining esterified as: Neutral lipid	Phospholipid
8	1	88	87
	2	75	89
9	1	50	8
	2	29	17
10	1	21	23
	2	46	28

* Details of the diet in each experiment are given in Table 1.

The pH values of the digesta samples (Table 1) supplement those recorded in the preceding paper (Lennox *et al.* 1968) in showing that conditions in the upper jejunum are notably acidic (pH about 2–3) and that a pH of 6–7 does not obtain until the chyme reaches the lower jejunum. This is in marked contrast to the rapid neutralization of gastric hydrochloric acid in the human upper small intestine (see Borgström, Dahlqvist, Lundh & Sjövall, 1957) and is probably associated with the comparatively low content of bicarbonate (15–30 m-equiv./l.) in sheep pancreatic juice (Taylor, 1962) compared with 120–130 m-equiv./l. in human pancreatic juice (Oser, 1965). The high degree of acidity in the upper jejunum of the sheep is presumably responsible for the precipitation of conjugated bile acids, of which about 40% are present in association with the particulate matter of the intestinal chyme (Lennox *et al.* 1968). Though the solubility of long-chain fatty acids in bile acid micelles is greatly enhanced when some ionization of the fatty acids has taken place (i.e. when the pH of the digesta is 6·5 or higher (see Hofmann, 1966)), it is evident from the results of the experiments with sheep 8 that, under conditions when very low pH values prevail, fatty acid uptake can take

Table 4. *Proportions of palmitic acid* (16:0) *and* C_{18} *fatty acids in the lipids of the digesta entering and leaving different lengths of the small intestine of three sheep*

(Values as % by weight of the total fatty acids in each class of lipid)

Sheep no.*	Source of digesta	Unesterified fatty acids					Fatty acids of neutral lipids					Fatty acids of phospholipids				
		16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
8 (Expt 2)	Upper jejunum															
	Entering cannula 2	9·9	60·5	19·7	1·2	1·4	16·7	29·9	14·0	3·6	10·6	36·8	13·8	18·7	2·7	2·4
	Leaving cannula 3	10·5	52·3	20·3	3·0	3·0	13·8	29·6	14·3	2·5	6·1	43·6	18·6	17·9	2·0	3·2
9 (Expt 2)	Mid to lower jejunum															
	Entering cannula 2	10·0	50·6	28·2	2·6	2·5	15·0	58·4	15·6	0·6	0·1	56·6	25·5	6·5	0·5	0·5
	Leaving cannula 3	13·0	57·6	18·0	1·2	3·6	16·8	36·7	14·0	2·8	10·4	45·8	33·5	5·1	0·3	0·3
10 (Expt 1)	Upper jejunum to lower ileum															
	Entering cannula 2	7·6	59·5	24·0†	1·7	3·2	17·8	37·1	22·4	3·6	9·1	35·8	14·0	20·0	3·8	5·4
	Leaving cannula 3	10·4	64·8	18·7‡	1·4	0·8	19·8	41·9	11·0	3·9	8·7	38·3	27·6	14·7	0·6	0·9

* Details of the diet in each experiment are given in Table 1.
† Includes 14·6% 18:1 *trans*, i.e. 60·8% of total 18:1.
‡ Includes 7·3% 18:1 *trans*, i.e. 39·0% of total 18:1.

place, albeit to a limited extent. This finding is consistent with the observations of Heath & Morris (1963) that the transfer of fatty acids from chyme to intestinal lymph was not completely abolished in sheep deprived of pancreatic juice, though it was in the absence of bile.

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