The effects of intravenous infusions of triglycerides on the secretion of milk fat in the cow

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1. Artificial emulsions of nine synthetic triglycerides ranging from tripropionin to triolein were given as continuous infusions into the jugular vein of lactating cows for periods of 2 days. The effects of these infusions on the composition of blood lipids and on the secretion of the component fatty acids in milk were examined.

2. Tricaproin, tricaprylin, tripelargonin, tricaprin, trilaurin, trimyristin and triolein, in contrast to tripropionin and tributyrin, increased the yields in milk of the acid contained in the triglyceride. The increased yield of acid was positively correlated with chain length of the infused triglyceride, and with triglycerides above tricaprin the transfer of fatty acid to milk was sufficiently large to give consistently increased yields of total milk fat.

3. The infusions increased the concentrations in blood plasma of cholesterol, phospholipid and in some experiments also of triglyceride. Concentrations of these lipids returned to normal by 2 days after the infusion. The fatty acid compositions of the plasma triglycerides were not affected by the infusion of triglycerides up to tripelargonin but with triglycerides longer than this the plasma triglycerides were altered in composition towards that of the infused triglyceride.

4. Short- and intermediate-chain fatty acids of the infused triglycerides were elongated by the successive addition of two carbon units to give increased yields in milk of acids ranging up to C_{15} and C_{16} when they contained acids with an even and odd number of carbon atoms respectively. Possible interference with the conversion of stearic into oleic acid in the mammary gland owing to the infused emulsions is also discussed.

Arterio-venous difference studies in conscious lactating goats (Barry, Bartley, Linzell & Robinson, 1963), perfusion of isolated goat mammary glands (Lascelles, Hardwick, Linzell & Mepham, 1964; Linzell, Annison, Fazakerley & Leng, 1967) and feeding radioactive triglycerides to lactating cows (Glascock, Welch, Bishop, Davies, Wright & Noble, 1966) have clearly demonstrated the importance of the triglycerides of the chylomicra and low-density lipoproteins as precursors of milk fat. The contribution of these plasma lipids to the formation of milk fat will depend on the nutrition and body fat reserves of the cow and may account for as little as 35 % or as much as 75 % by weight of the fatty acids in milk (Riis, 1964; Barry, 1966; Glascock *et al.* 1966). The remaining fatty acids in milk are synthesized within the mammary gland from acetate and β-hydroxybutyrate (Barry, 1964).

In the rumen, dietary glycerides are hydrolysed and any liberated unsaturated acids modified by hydrogenation and isomerization (Garton, 1965, 1967), so that the fatty acids reaching the small intestine differ in composition from those contained in the food. When the diet contains fatty acids which are not metabolized in the rumen the fatty acid composition of blood plasma triglycerides is altered towards the composition of fatty acids in the diet (Storry, Rook & Hall, 1967) but the concentration of total plasma triglycerides is not influenced by the amount of fat in the diet (Leat & Gillman, 1964; Tove, 1965; Hartmann, Harris & Lascelles, 1966) because of the rapid clearing of absorbed triglycerides from the plasma. The intravenous

infusion of emulsified cottonseed oil into lactating cows (Tove & Mochrie, 1963; Storry & Rook, 1964), however, produces changes in the concentration and composition of blood plasma triglycerides and in the yield and composition of milk fat which indicate that artificial emulsions can be utilized in the formation of milk fat. In the present experiments the effects of intravenous infusions of a wide range of synthetic triglycerides on the composition of blood lipids and on the secretion of milk fat are studied.

EXPERIMENTAL

Animals and their management

Lactating Friesian cows were housed in metabolism stalls and fed on a diet consisting of 7–8 kg hay/day and a concentrate mixture balanced for milk production given at the rate 1·8 kg/gal of milk produced. The yield of milk was recorded, and from samples of milk taken at each milking daily composite samples were prepared for milk fat analysis. At 12·00 h on each day of the experiment a sample of blood was taken through a cannula maintained in the jugular vein on the side of the neck opposite to that used for infusion. The emulsions were infused for a period of 2 days between initial and final control periods of 2 and 6 days respectively.

Preparation and infusion of emulsions

Mixed phosphatides were prepared from the yolks of fresh eggs (Singleton, Gray, Brown & White, 1965) and stored in ethyl alcohol containing 0·02% butylated hydroxytoluene under an atmosphere of N_2 at $-20°$ until required for an emulsion. The yield of crude phosphatides was about 8% of the weight of fresh yolks and no further purification of the mixed phosphatides was carried out. Thin-layer chromatography of the phosphatides which had been extracted and stored in this way showed that they contained phosphatidyl choline, phosphatidyl ethanolamine, traces of free cholesterol and no lysolecethin.

'Technical' grade tripropionin and trimyristin and 'Practical' grade tributyrin, tricaproin, tricaprylin, tripelargonin, tricaprin, trilaurin and triolein (Fluka, A. G., Switzerland) were used without further purification.

Emulsions were prepared in batches of 7 l. by an adaptation of the method of Zeringue, Brown & Singleton (1964). Six litres 2·5% glycerol solution and 1200 g triglyceride were heated separately to 60–70°. An ethanolic solution of egg phosphatides (100 ml containing 72 g phosphatides for all emulsions except trilaurin and trimyristin which contained 108 and 144 g respectively) was added to the hot triglyceride, and the triglyceride–phosphatide mixture was then blended with the glycerol solution for 2 min in a Kenwood Rotoblend. The pH of the resulting emulsion was adjusted to 6·8 by the addition of 10 N-NaOH. Sometimes it was necessary to add a few drops of NaOH before blending in order to raise the pH sufficiently to produce this initial emulsion. The resulting crude emulsion was passed continuously through a Rannie Homogenizer (Hiron and Rempler Ltd, Wembley, Middlesex) at an applied pressure of 3500–4000 lb/in² for 45 min and at a temperature of about 70°. During homogenization the emulsion circulated through a stainless

steel mixing bath which was continuously flushed with N_2 to prevent oxidation. The pH of the emulsion was finally adjusted to 6·8 and homogenization continued for a further period of 20 min. The pH remained stable during this second period of homogenization. After preparation all emulsions were examined under the microscope and the emulsion droplets measured by means of a calibrated eye-piece. The majority of fat particles in the emulsions were 0·5 μm or less in diameter with a few particles reaching 1·0–1·5 μm.

The emulsions were held in a glass reservoir and continuously infused into the jugular vein by means of a diaphragm-operated micropump (type S with multiple capacity selector; Distillers' Company Ltd, Epsom, Surrey). Before starting the infusion the reservoir, pump and infusion lines were assembled and sterilized by pumping absolute ethanol through the system. The ethanol in the reservoir was then replaced with the freshly prepared emulsion and the system flushed through to remove any alcohol. After connecting the infusion line to the cannula in the jugular vein, the emulsion was given at an initial rate of 50 ml/h and the rate gradually increased over a period of 2–3 h to about 150 ml/h. The emulsions were infused at ambient temperatures with the exception of trimyristin which was kept in a water bath at 50°, the reservoir being continuously flushed with N_2 to reduce oxidation. The infusion line was also heated by means of a warm water jacket. Generally the emulsions were well accepted by the animals but in one cow receiving tributyrin and in both cows receiving trimyristin there was an initial rise in body temperature and in these experiments the infusion rate was reduced to about 85 ml/h.

Methods of analysis

Blood lipids. The lipids in blood plasma were extracted in 2:1 (v/v) chloroform–methanol and the major components separated on columns of silicic acid in experiments TR 2–TR 12 (Storry & Rook, 1965) and by thin-layer silicic acid chromatography in experiments TR 13–TR 32 (Storry & Tuckley, 1967). In the thin-layer method a known quantity of total lipid was applied to the plate in the following way. The extracted plasma lipids were dissolved in chloroform to give a concentration of approximately 100 mg/ml, and 80 μl of this lipid solution were applied directly to the plate. From the same syringe a further 80 μl of this lipid solution were transferred to a pre-weighed tube (made from Quickfit SRB 7 and CBB 7 sockets and cones) and dried to constant weight. The areas of the developed plate containing the individual lipid fractions were scraped into centrifuge tubes and the cholesterol esters, free cholesterol and triglycerides eluted by shaking for 2 min with 10 ml 2:1 (v/v) chloroform–methanol and the phospholipids by shaking with 10 ml 10% (v/v) methanolic HCl. The individual lipid components contained in the eluates obtained with either the column or thin-layer methods of chromatography were calculated from the chemically determined cholesterol (Brown, 1959) phosphorus (Chen, Toribara & Warner, 1956) or glycerol (Moore, 1962). When used in conjunction with thin-layer chromatography, the sensitivity of the glycerol determination was increased by using 0·2 ml 0·025 M-sodium metaperiodate, 0·2 ml 0·25 M-sodium arsenite and 5 ml 0·12% chromotropic acid reagent instead of the concentrations

originally proposed by Moore (1962). With freshly prepared lipid standards of cholesterol stearate, free cholesterol, tripalmitin and lecithin recoveries with the thin-layer method were respectively $97·6 \pm 0·87$, $92·6 \pm 1·37$, $99·8 \pm 1·24$ and $103·0 \pm 0·91 \%$. Also, analysis of plasma lipid samples by the column and thin-layer methods of chromatography gave good agreement.

Fatty acids in plasma triglycerides and milk. Methyl esters of the fatty acids of plasma triglycerides and milk fat were prepared by transesterification with sodium methoxide in sealed tubes. The composition of the methyl esters of milk fat in all the experiments and of the methyl esters of the plasma triglycerides in the experiments ranging from tripropionin to tricaprin was determined by gas–liquid chromatography with a Perkin–Elmer model F 11 gas chromatograph (Storry *et al.* 1967). In the experiments in which emulsions of trilaurin, trimyristin and triolein were infused, the methyl esters of plasma triglycerides were determined with a Pye argon chromatograph (W. G. Pye & Co. Ltd, Cambridge) and columns of 20% polyethylene glycol adipate on 100–120 mesh celite at 180°.

<div align="center">RESULTS</div>

<div align="center">*Effect of triglyceride infusions on milk secretion*</div>

Yield of milk and milk fat. The fatty acid compositions of the triglyceride emulsions are given in Table 1, and the effects of the infusions on the yield of milk and milk fat in Table 2. The effects of the infusions on milk and fat yields were assessed by comparing the mean value for the period of infusion with the combined mean values for the two control periods but omitting the 1st day of the post-infusion period to avoid any carry-over effect.

<div align="center">Table 1. *Fatty acid* composition (g/100 g) of emulsions*</div>

Emulsion	3:0	4:0	6:0	7:0	8:0	9:0	10:0	12:0	14:0	16:0	17:0	18:0	18:1	18:2	18:3
Tripropionin	99·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tributyrin	—	99·0	tr	—	tr	—	tr	tr	—	tr	—	tr	—	—	—
Tricaproin	—	1·1	96·6	—	—	—	0·8	tr	—	0·8	0·4	—	0·3	—	—
Tricaprylin	—	1·4	6·6	—	74·7	—	11·6	5·6	—	—	—	—	—	—	—
Tripelargonin	—	—	—	0·4	2·1	95·6	1·7	tr	—	0·2	—	—	—	—	—
Tricaprin	—	—	—	—	tr	—	99·9	—	—	—	—	—	—	—	—
Trilaurin	—	—	—	—	—	—	1·0	95·7	3·0	0·4	—	—	—	—	—
Trimyristin	—	—	—	—	—	—	—	15·2	80·3	4·6	—	—	—	—	—
Triolein†	—	—	—	—	—	—	0·1	0·7	4·4	8·2	0·8	2·6	64·7	7·0	0·6

tr, trace.
* Number of carbon atoms and number of double bonds (Farquhar, Insull, Rosen, Stoffel & Ahrens, 1959).
† In addition to the fatty acids given there were small concentrations of $C14:1$, $C15:0$ and $C16:1$ acids amounting to about 10 %.

Both cows infused with tributyrin and one cow of each pair infused with tricaprylin and tricaprin showed small, but nevertheless significant, falls in the yield of milk. Changes in the milk yield of all other animals were not significant. With the exception of one animal infused with tricaproin and one infused with tripelargonin, which showed a significant increase and decrease respectively, the yield of milk fat was not

significantly altered by the infusion of triglycerides ranging from tripropionin to tricaprin although the general pattern of response was a decrease in yield. The response in milk fat yield to triglycerides longer than tricaprin was a consistent increase which in some of the experiments reached statistical significance.

Table 2. *Effect of intravenously infused triglyceride emulsions on the yields of milk and milk fat in the cow*

Expt no.	Cow	Triglyceride infused	Milk yield (kg/day)		Milk-fat yield (g/day)	
			Control	Infusion	Control	Infusion
TR 31	Brilliant 9	Tripropionin	19.4 ± 0.25	19.1 ± 0.75	746 ± 31.9	676 ± 7.0
TR 32	Brilliant 8	Tripropionin	17.0 ± 0.22	17.1 ± 0.75	739 ± 38.4	772 ± 31.0
TR 5	Sylph	Tributyrin	11.5 ± 0.12	$9.9 \pm 0.02^{**}$	478 ± 13.6	473 ± 49.0
TR 8	Bride 19	Tributyrin	12.1 ± 0.41	$9.1 \pm 2.10^{**}$	497 ± 8.5	364 ± 147.0
TR 17	Bugle 8	Tricaproin	14.2 ± 0.31	14.2 ± 0.10	576 ± 12.3	560 ± 48.0
TR 18	Gallant 8	Tricaproin	17.2 ± 0.35	17.6 ± 0.15	708 ± 15.4	$787 \pm 6.0^{**}$
TR 3	Sylph	Tricaprylin	11.2 ± 0.14	$10.4 \pm 0.18^{**}$	516 ± 13.2	495 ± 15.5
TR 6	Bride 19	Tricaprylin	13.8 ± 0.15	13.7 ± 0.58	513 ± 12.9	526 ± 8.0
TR 13	Perdita	Tripelargonin	13.5 ± 0.17	13.0 ± 0.23	592 ± 8.5	$513 \pm 4.5^{***}$
TR 14	Grace 11	Tripelargonin	15.3 ± 0.30	14.6 ± 0.00	682 ± 12.4	644 ± 17.0
TR 4	Gallant 8	Tricaprin	9.9 ± 0.30	9.8 ± 0.10	386 ± 10.6	376 ± 4.5
TR 7	Perdita	Tricaprin	11.4 ± 0.17	$10.5 \pm 0.03^{***}$	519 ± 15.0	501 ± 13.5
TR 15	Gallant 8	Trilaurin	21.4 ± 0.23	21.6 ± 0.60	768 ± 52.0	$912 \pm 32.0^{*}$
TR 16	Bugle 8	Trilaurin	15.7 ± 0.08	15.7 ± 0.10	594 ± 38.9	653 ± 7.6
TR 28	Sylph	Trimyristin	12.0 ± 0.30	12.5 ± 0.80	379 ± 55.1	426 ± 52.5
TR 29	Brilliant 9	Trimyristin	24.6 ± 0.30	24.7 ± 0.78	938 ± 67.9	1128 ± 165.0
TR 10	Perdita	Triolein	15.8 ± 0.21	17.1 ± 0.68	640 ± 19.3	$738 \pm 31.0^{*}$
TR 11	Grace 11	Triolein	18.8 ± 0.33	18.9 ± 0.49	751 ± 10.8	$850 \pm 17.5^{**}$

$* \; P < 0.05$; $** P < 0.01$; $*** P < 0.001$, where P is the probability that the observed difference from the control value could have arisen by chance.

Yields of the major fatty acids of milk fat. The changes in pattern of secretion of the major fatty acids were similar for both animals infused with a given triglyceride. For simplicity, the results from the two experiments with each triglyceride have therefore been averaged and they are presented in Figs. 1–6.

Infusion of the short-chain triglycerides, tripropionin and tributyrin, did not lead to increased yields of propionic and butyric acids respectively in milk (Figs. 1*b* and 2*a*), whereas triglycerides containing acids with more than four carbon atoms gave increased yields of the corresponding acids in milk (Figs. 2*b*–6). The yields of fatty acids in milk, increased with increase in chain length of the infused glyceride; this is shown in Table 3 after correcting to a common dose of 500 g pure triglyceride/day.

In addition to increased yields in milk of fatty acids contained in the infused glycerides, there were changes in the yields of other acids associated with the infusions. First, there were increased yields of odd carbon numbered acids, up to C_{15}, with the infusions of tripropionin and tripelargonin (Figs. 1*b* and 4*b*) and increased yields of even carbon numbered acids, up to C_{16}, with the infusions of tricaproin (Fig. 2*b*), which suggested that these shorter-chain triglycerides were being elongated by the successive addition of two carbon units. Similar chain elongation appeared to occur

also with the infusion of tricaprylin (Fig. 3*a*) but no doubt, in these experiments, part of the increased yields of C_{10} and C_{12} acids, also could be attributed directly to the C_{10} and C_{12} 'contaminants' in this particular emulsion. Secondly, with the infusion of all triglycerides except triolein, there were reduced yields in milk of oleic acid,
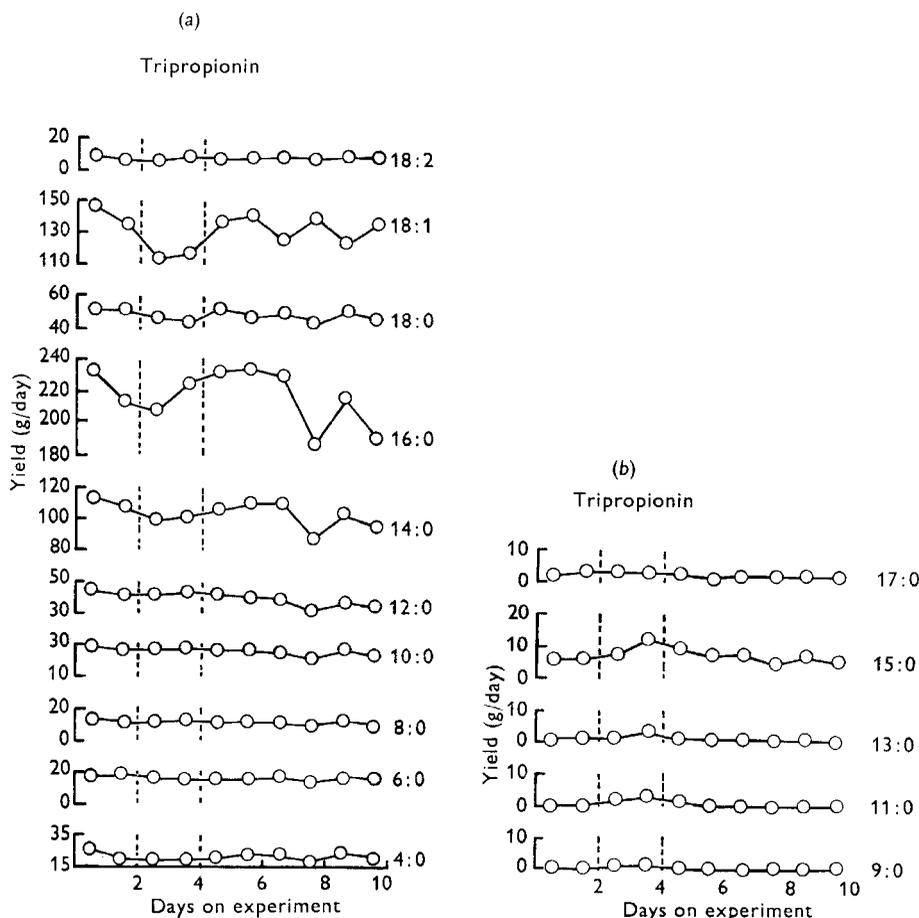

Fig. 1. Effects of intravenous tripropionin infusions on the yields in cow's milk of fatty acids containing (*a*) an even number and (*b*) an odd number of carbon atoms.

sometimes in association with a less marked decrease in stearic acid, but the yield of linoleic acid was not affected.

The increased yields of fatty acids in milk, due to the infusions of the various triglycerides, returned to normal values by the 2nd day after the infusion but the decreased yields of oleic acid often took 3 or 4 days to recover.

Effects of infusions on blood composition

Plasma lipids. All the infusions increased the concentration of total lipid in the plasma and the mean values for the two experiments with each triglyceride are

presented in Fig. 7. There was no consistent pattern in the change in concentration with the various triglycerides except that the increase in concentration tended to be greater with trilaurin, trimyristin and triolein than with the shorter-chain triglycerides.
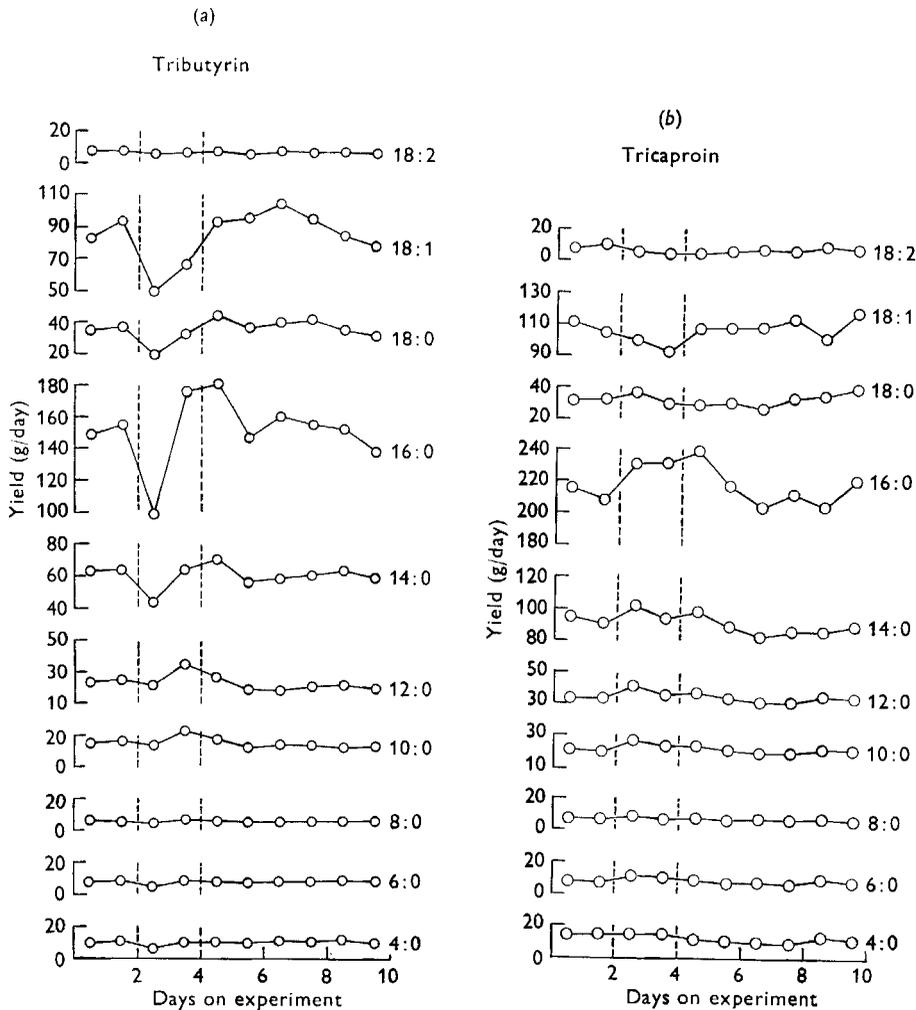

Fig. 2. Effects of (a) tributyrin and (b) tricaproin intravenous infusions on the yields of the major fatty acids in cow's milk.

The effects of the infusions on the individual components of plasma lipid are given in Table 3. The concentrations of cholesterol esters were not consistently affected whereas with the exceptions of experiment TR 13 and experiments TR 3 and TR 14 the concentrations of free cholesterol and phospholipid respectively were increased. The effects of the infusions on the concentrations of plasma triglycerides were variable with little effect for triglycerides below tricaprylin. Triglycerides above tricaprylin tended to give on average a bigger increase in the concentration of plasma triglycerides during their infusion.

Fatty acid composition of plasma triglycerides (*Tables* 4 *and* 5). With triglycerides below tricaprin there was no increase in the content of the corresponding fatty acid in the plasma triglycerides during the infusions. Changes in the yields of the respective
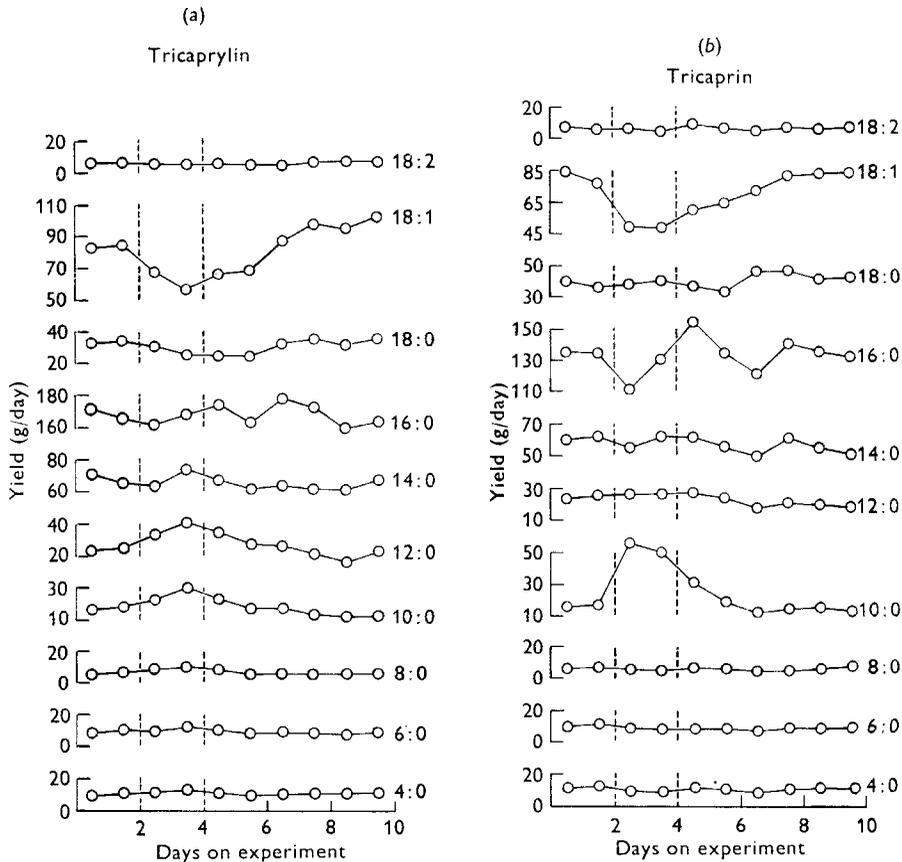

Fig. 3. Effects of (*a*) tricaprylin and (*b*) tricaprin intravenous infusions on the yields of the major fatty acids in cow's milk.

fatty acids in milk with these short-chain triglycerides were, at the most, small and the absence of these short-chain acids in the plasma triglycerides suggests that they were very rapidly metabolized on infusion. With triglycerides above tricaprin the fatty acid composition of the plasma triglycerides was altered towards that of the infused emulsion.

DISCUSSION

Acids containing up to ten carbon atoms are absorbed from the digestive tract directly into the portal vein as free acids whereas long-chain acids are absorbed into the lymph as a lipoprotein complex (chylomicra) which enters the jugular vein via the thoracic lymph duct (Senior, 1964). The infusion of triglycerides containing fatty acids of less than twelve carbon atoms directly into the jugular vein and the

use of egg phosphatides as an emulsifying agent thus cannot be regarded as entirely physiological. Nevertheless, the fact that the different triglycerides produced specific effects on the secretion of individual fatty acids in milk fat, which fall into line with established metabolic pathways, indicates that the emulsions were utilized, at least in part, in a way similar to that for naturally occurring chylomicra. In this respect it is of interest that in the dog and man there is no difference in the pattern of clearance from blood plasma for naturally occurring chylomicra and artificial emulsions of
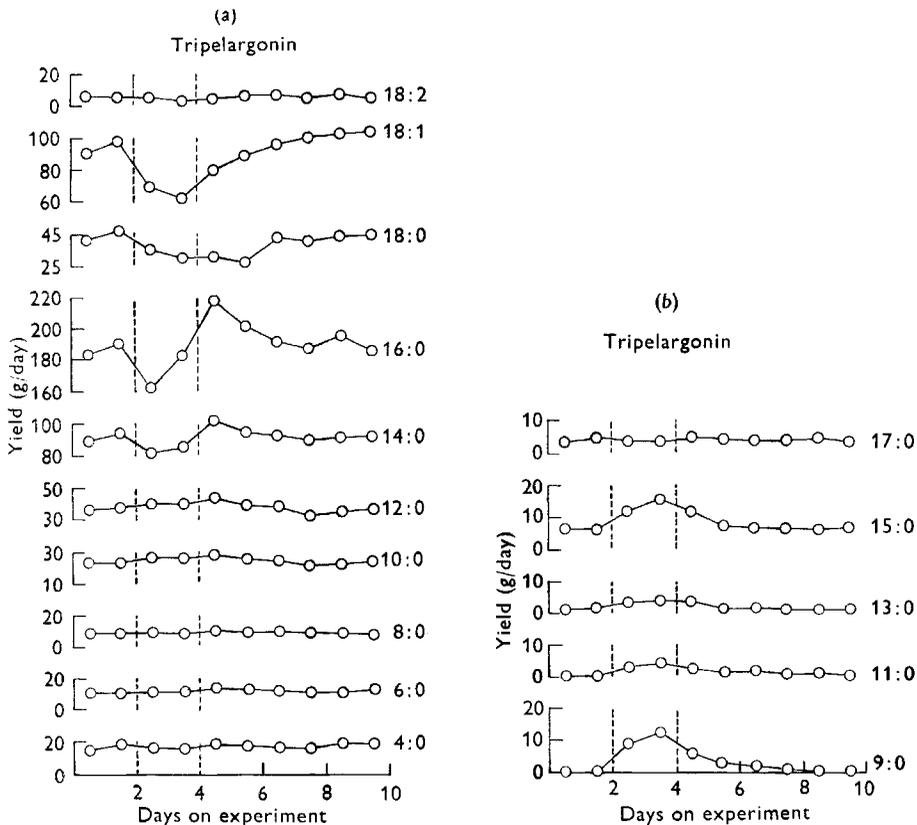

Fig. 4. Effects of intravenous tripelargonin infusions on the yields in cow's milk of fatty acids containing (a) an even number and (b) an odd number of carbon atoms.

soya-bean oil with the same basic composition as that of the emulsions used in the present experiments (Hallberg, 1965). Furthermore, the rat mammary gland does not in its mode of uptake and utilization differentiate between naturally occurring chylomicra and artificial triglyceride emulsions of composition similar to those used in the present experiments (Schoefl & French, 1968).

The size of the particles in the emulsions used in the present experiments was very similar to that of chylomicra, and generally there were no clinical signs of stress similar to those reported by other workers (Glascock, McWeeny & Smith, 1957; Leat & Gillman, 1964). In one cow receiving tributyrin and in both cows

receiving trimyristin, however, the infusions were initially accompanied by a rise in body temperature and increased respiration rate which subsided when the infusions were continued at a lower rate. These toxic reactions could not be attributed to any particular factor, but it is of interest that low molecular triglycerides have produced
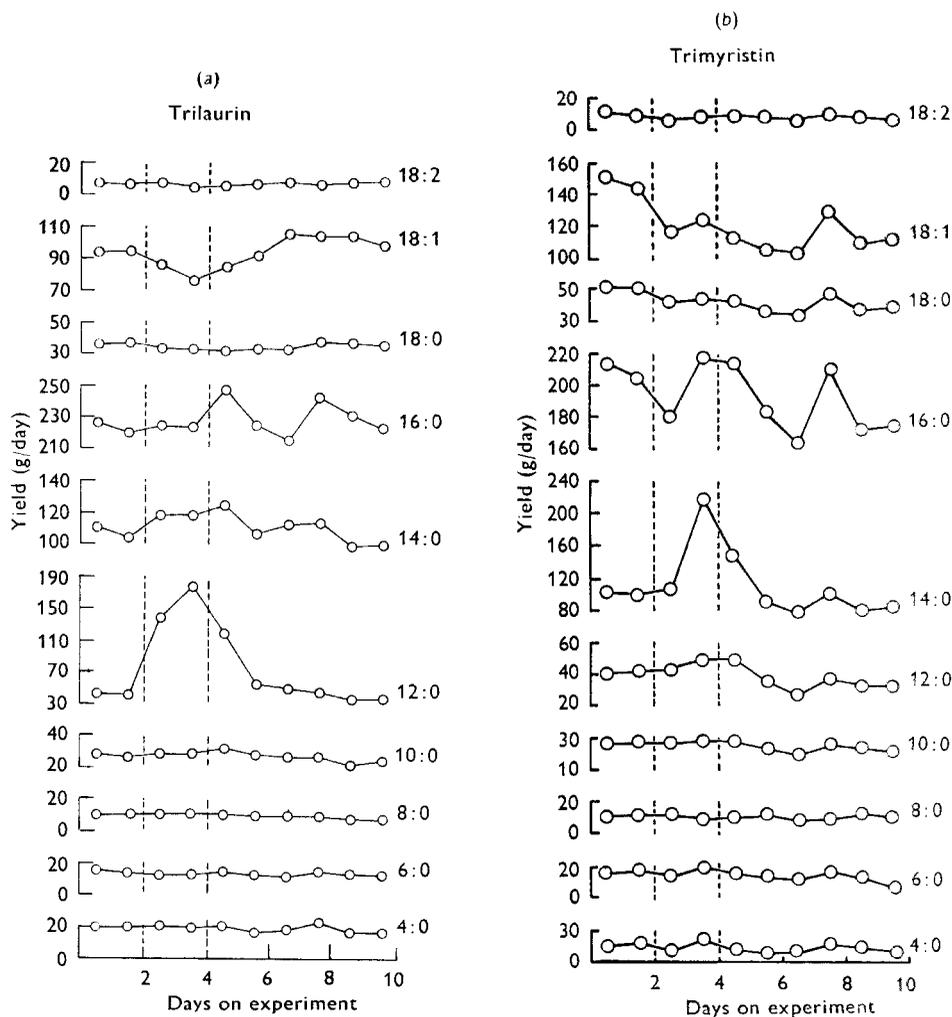

Fig. 5. Effects of (a) trilaurin and (b) trimyristin intravenous infusions on the yields of the major fatty acids in cow's milk.

unphysiological responses in cats and mice (Wretlind, 1957, 1964). The reaction to trimyristin in the present experiments may have been related to the use of a less pure triglyceride in that emulsion.

With the exception of tricaprylin, trimyristin and triolein, the triglyceride emulsions contained over 95 % of the designated fatty acid. Although the responses in yield of total milk fat to the infusions of triglycerides below trilaurin were variable the responses in the yields of individual fatty acids, though small, were more specific.

Tripropionin and tributyrin did not increase the yield in milk of propionic or butyric acid respectively whereas triglycerides containing fatty acids of six or more carbon atoms did lead to increased yields in milk of the fatty acid contained in the
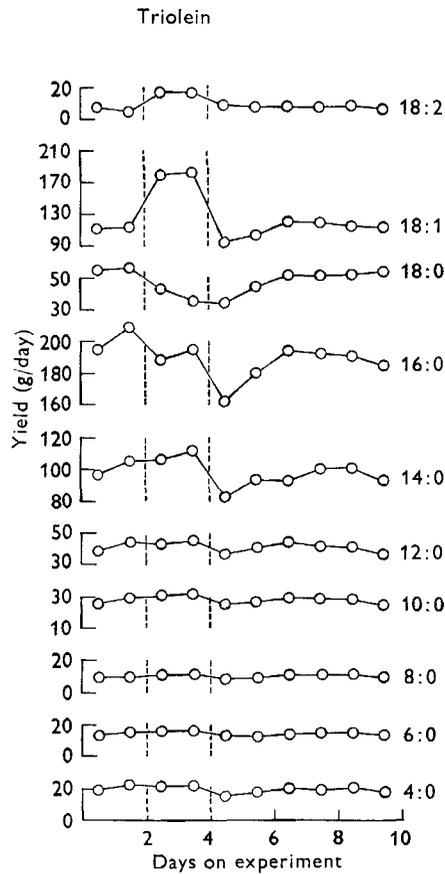

Fig. 6. Effects of intravenous triolein infusions on the yields of the major fatty acids in cow's milk.

Table 3. *Effects of chain length of infused triglyceride on the yields of the corresponding acids in cow's milk. Values are corrected to a common dose of 500 g triglyceride/day*

Chain length of glyceride fatty acid	Mean change in fatty acid yield during infusion (g/day)
3:0	0
4:0	−3
6:0	+4
8:0	+5
9:0	+10
10:0	+37
12:0	+91
14:0	+102
18:1	+102

triglyceride. The yield of fatty acid in milk increased with increase in chain length of the infused triglyceride.

Normally fatty acids with less than twelve carbon atoms are not significant components of plasma triglycerides in the cow (Evans, Patton & McCarthy, 1961;
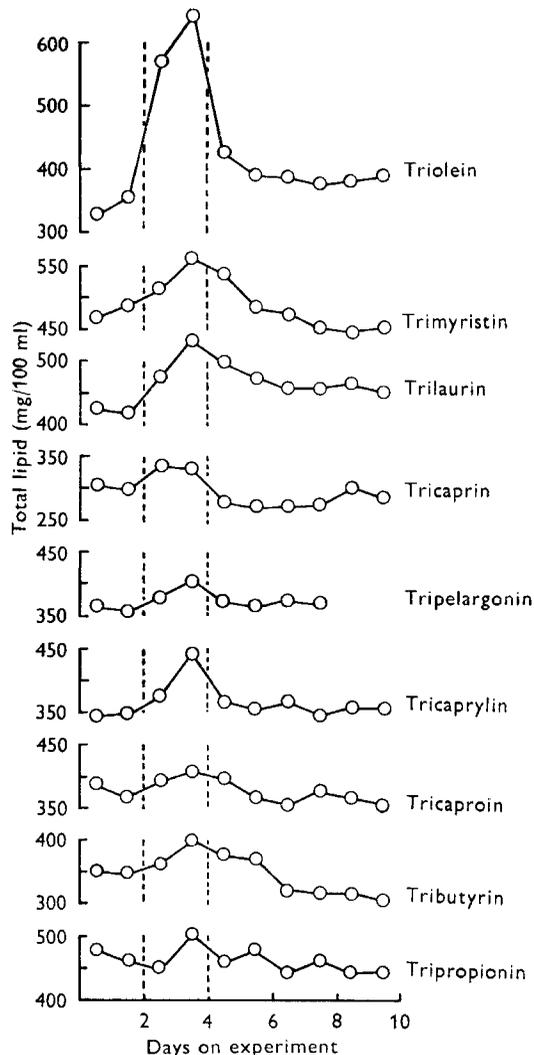

Fig. 7. Effects of intravenous infusions of different triglycerides on the concentrations of total lipid in blood plasma of cows.

McCarthy, Chandler, Griel & Porter, 1968), probably because of extensive metabolism of these shorter-chain acids in the liver as they are absorbed from the digestive tract via the portal system. In rats, for example, the liver is an active site for either oxidation or elongation of short- and intermediate-chain acids (Schieg & Klatskin, 1968) and deposition of caprylic and pelargonic acids in adipose tissue of rats following

the feeding of tricaprylin and tripelargonin only occurs when metabolism in the liver is circumvented by means of postcaval shunts (Zurier, Campbell, Hashim & Van Itallie, 1967). In the present experiments the emulsions were infused directly into the jugular vein so that some of the short- and intermediate-chain triglycerides would escape metabolism by the liver; this is supported by the fact that triglycerides from tricaproin upwards produced increased yields of the corresponding acids in milk fat.

The effect of triglyceride chain length on the secretion of fatty acids in milk

Table 4. *Effect of intravenously infused triglyceride emulsions on the composition of blood plasma lipids in the cow*

Expt no.	Cow	Triglyceride infused	Cholesterol ester* (mg/100 ml)		Free cholesterol (mg/100 ml)		Phospholipid (mg/100 ml)		Triglyceride† (mg/100 ml)	
			Control	Infusion	Control	Infusion	Control	Infusion	Control	Infusion
TR 31	Brilliant 9	Tripropionin	237	231	33	39	172	177	9·4	8·4
TR 32	Brilliant 8	Tripropionin	217	205	30	37	155	181	10·0	9·6
TR 5	Sylph	Tributyrin	159	180	26	36	125	155	8·2	9·5
TR 8	Bride 19	Tributyrin	169	187	28	39	138	163	8·1	6·3
TR 17	Bugle 8	Tricaproin	160	186	25	30	137	146	9·2	11·6
TR 18	Gallant 8	Tricaproin	206	212	31	38	161	184	10·9	12·4
TR 3	Sylph	Tricaprylin	161	146	26	27	113	111	7·8	8·5
TR 6	Bride 19	Tricaprylin	206	206	31	38	157	203	11·0	16·2
TR 13	Perdita	Tripelargonin	189	202	25	23	126	133	7·5	12·5
TR 14	Grace 11	Tripelargonin	215	196	31	33	154	153	8·1	14·0
TR 4	Gallant	Tricaprin	135	118	22	27	109	129	8·3	11·9
TR 7	Perdita	Tricaprin	150	155	24	28	108	142	7·9	17·3
TR 15	Bugle 8	Trilaurin	285	285	37	44	189	231	6·8	10·0
TR 16	Gallant 8	Trilaurin	188	174	25	27	143	154	8·0	11·8
TR 28	Sylph 11	Trimyristin	233	233	35	39	167	199	10·0	19·3
TR 29	Brilliant 9	Trimyristin	251	245	35	43	187	237	12·1	10·8
TR 10	Perdita	Triolein	183	196	26	32	130	201	8·0	20·0
TR 11	Grace 11	Triolein	225	215	28	38	163	249	3·9	188·2

* Expressed as cholesterol oleate.
† Expressed as triolein.

Table 5. *Effect of intravenously infused triglyceride emulsions on the composition (g/100 g) of the major fatty acids in blood plasma triglycerides in the cow*

Fatty acid	Tripropionin		Tributyrin		Tricaproin		Tricaprylin		Tripelargonin	
	Control	Infusion	Control	Infusion	Control	Infusion	Control	Infusion	Control	Infusion
3:0	—	—	—	—	—	—	—	—	—	—
4:0	—	—	tr	tr	—	—	tr	tr	tr	tr
6:0	—	—	tr	tr	—	—	—	tr	—	—
8:0	0·3	0·6	tr	tr	—	—	tr	tr	tr	tr
9:0	0·1	0·5	—	—	—	—	—	—	—	—
10:0	0·1	0·2	0·2	0·2	—	—	tr	tr	—	tr
12:0	1·0	1·2	1·7	1·7	1·5	1·4	1·6	1·1	0·9	1·0
14:0	4·7	5·5	6·1	6·1	5·5	6·5	6·1	7·0	4·8	5·6
16:0	19·9	19·3	26·3	27·5	25·4	24·4	30·1	27·9	22·8	21·7
18:0	29·9	23·3	29·8	26·9	25·6	25·6	30·3	27·5	28·3	26·1
18:1	15·6	17·1	15·7	16·6	15·2	15·4	14·5	14·7	18·8	17·9
18:2	3·0	3·4	1·7	1·5	3·4	1·3	1·5	1·4	2·9	2·8

tr, trace.

observed in the present experiments may be due to one or more of several factors. The simplest explanation is that the short- and intermediate-chain triglycerides were extensively metabolized by extramammary tissues resulting in less of these shorter-chain glycerides reaching the mammary gland for utilization in milk-fat synthesis. In rats, for example, the rate of catabolism and the proportion of acid oxidized to CO_2 increase with decrease in carbon chain length from palmitic to butyric acids (Kirschner & Harris, 1961; Bollinger & Reiser, 1965). In the present experiments the fact that infusion of triglycerides below tricaprin did not increase the content of the respective acid in the plasma triglycerides suggests that these short-chain triglycerides are also rapidly metabolized in the cow. However, other possibilities such as low activity of mammary gland lipoprotein lipase for triglycerides below trilaurin, or a lower rate of incorporation into milk-fat triglycerides of acids released from

Table 6. *Effect of intravenously infused triglyceride emulsions on the composition (g/100 g) of the major fatty acids in blood plasma triglycerides in the cow*

Fatty acid	Tricaprin		Trilaurin		Trimyristin		Triolein	
	Control	Infusion	Control	Infusion	Control	Infusion	Control	Infusion
10:0	0·5	17·0	tr	tr	tr	tr	—	—
12:0	1·4	1·5	1·5	5·1	2·3	5·1	1·2	1·0
14:0	5·5	5·9	6·4	13·6	8·8	38·7	4·8	4·1
16:0	25·4	21·1	27·7	25·2	26·1	18·9	29·0	26·0
18:0	30·8	21·7	32·2	22·3	35·4	17·3	33·4	35·9
18:1	15·9	13·4	13·9	12·1	11·9	7·5	19·1	22·1
18:2	1·9	0·7	2·8	2·5	2·7	1·3	1·1	1·1

tr, trace.

these shorter-chain triglycerides, cannot be excluded. The effect of triglyceride chain length on the activity of mammary gland lipoprotein lipase is not known, but Patton & McCarthy (1963) have postulated the existence in alveoli of one pool of long-chain fatty acids at the base of the cell which is derived from the plasma triglycerides and another pool of intermediate- and short-chain acids in the upper reaches of the cell which arises by synthesis *de novo* from acetate. Also tracer studies in goats (Annison, Linzell, Fazakerley & Nichols, 1967; West, Annison & Linzell, 1967) have shown that fatty acids synthesized within the secretory cells are not in equilibrium with the long-chain acids of the triglycerides and free fatty acids in blood supplying the mammary gland; Wood (1966) has, furthermore, suggested that the pathway for incorporation of short-chain acids into milk triglycerides may differ from that for long-chain acids. In the light of these findings of other workers it is thus conceivable that in the event of short- and intermediate-chain triglycerides reaching the mammary gland their uptake and secretion in milk fat may be limited.

In addition to increased yields of fatty acids in milk corresponding to those contained in the infused triglycerides, there were increased yields of other acids which indicated that the fatty acids of the short-chain triglycerides were being elongated by the successive additions of two carbon units. This phenomenon was best illustrated in the experiments with tripropionin and tripelargonin, where the yields of C_9 to

C_{15} and C_{11} to C_{15} acids respectively were increased in spite of the fact that the emulsions did not contain these acids. The infusion of tricaproin similarly increased the yields of C_8 to C_{16} acids, showing that this chain elongation was not limited to acids with an odd number of carbon atoms. Chain elongation of propionate has been observed by other workers in tracer studies with the perfused bovine mammary gland (James, Peeters & Lauryssens, 1956).

In all the experiments except those with triolein, there was a notable depression in the yield of oleic acid during the period of infusion. This decreased yield of oleic acid cannot be attributed simply to competition for glycerol by fatty acids liberated from the infused triglycerides since simultaneous decreases in the yield of other C_{18} acids, which are derived from the same pool of fatty acids, did not always occur. Milk fat contains three or four times as much oleic as stearic acid and, since uptake by the mammary gland of oleic acid from the low-density lipoproteins is much less than that of stearic acid (Barry *et al.* 1963), the dehydrogenation of stearic to oleic acid which occurs in bovine (Lauryssens, Verbeke & Peeters, 1961) and goat (West *et al.* 1967; Annison *et al.* 1967) mammary gland must be a quantitatively important pathway for the secretion of oleic acid in milk fat. As this dehydrogenation of stearic acid occurs at a site in the mammary gland which is in equilibrium with the long-chain free fatty acids of blood plasma it seems possible that the presence of infused triglyceride emulsions in some way inhibited this pathway.

REFERENCES

Annison, E. F., Linzell, J. L., Fazakerley, S. & Nichols, B. W. (1967). *Biochem. J.* **102**, 637.
Barry, J. M. (1964). *Biol. Rev.* **39**, 194.
Barry, J. M. (1966). *Outl. Agric.* **5**, 129.
Barry, J. M., Bartley, W., Linzell, J. L. & Robinson, D. S. (1963). *Biochem. J.* **89**, 6.
Bollinger, J. N. & Reiser, R. (1965). *J. Am. Oil Chem. Soc.* **42**, 1130.
Brown, W. D. (1959). *Aust. J. exp. Biol. med. Sci.* **37**, 523.
Chen, P. S., Toribara, T. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.
Evans, L., Patton, S. & McCarthy, R. D. (1961). *J. Dairy Sci.* **44**, 475.
Farquhar, J. W., Insull, W. Jr, Rosen, P., Stoffel, W. & Ahrens, E. H. Jr (1959). *Nutr. Rev* **17**, Suppl.
Garton, G. A. (1965). In *Physiology of Digestion in the Ruminant*, p. 390. [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors.] Washington: Butterworths.
Garton, G. A. (1967). *Wld Rev. Nutr. Diet.* **7**, 225.
Glascock, R. F., McWeeny, D. J. & Smith, R. W. (1957). *Proc. int. Conf. Radioisotopes scient. Res.* **1**. *Paris* Vol. 3, p. 146.
Glascock, R. F., Welch, V. A., Bishop, C., Davies, T., Wright, E. W. & Noble, R. C. (1966). *Biochem. J.* **98**, 149.
Hallberg, D. (1965). *Acta physiol. scand.* **65**, Suppl. no. 254.
Hartmann, P. E., Harris, J. G. & Lascelles, A. K. (1966). *Aust. J. biol. Sci.* **19**, 635.
James, A. T., Peeters, G. & Lauryssens, M. (1956). *Biochem. J.* **64**, 726.
Kirschner, S. L. & Harris, R. S. (1961). *J. Nutr.* **73**, 397.
Lascelles, A. K., Hardwick, D. C., Linzell, J. L. & Mepham, T. B. (1964). *Biochem. J.* **92**, 36.
Lauryssens, M., Verbeke, R. & Peeters, G. (1961). *J. Lipid Res.* **2**, 383.
Leat, W. M. F. & Gillman, T. (1964). In *Metabolism and Physiological Significance of Lipids*, p. 257. [R. M. C. Dawson and D. N. Rhodes, editors.] London: John Wiley & Sons Ltd.
Linzell, J. L., Annison, E. F., Fazakerley, S. & Leng, R. A. (1967). *Biochem. J.* **104**, 34.

McCarthy, R. D., Chandler, P. T., Griel, L. C. & Porter, G. A. (1968). *J. Dairy Sci.* **51**, 392.

Moore, J. H. (1962). *J. Dairy Res.* **29**, 141.

Patton, S. & McCarthy, R. D. (1963). *J. Dairy Sci.* **46**, 916.

Riis, P. H. (1964). Investigations on lipid metabolism in cattle. PhD Thesis, Royal Veterinary and Agricultural College, Copenhagen, Denmark.

Schieg, R. & Klatskin, G. (1968). *J. Am. Oil Chem. Soc.* **45**, 31.

Schoefl, G. I. & French, J. E. (1968). *Proc. Roy. Soc.* B **169**, 153.

Senior, J. R. (1964). *J. Lipid Res.* **5**, 495.

Singleton, W. S., Gray, M. S., Brown, M. L. & White, J. L. (1965). *J. Am. Oil Chem. Soc.* **42**, 53.

Storry, J. E. & Rook, J. A. F. (1964). *Biochem. J.* **91**, 27C.

Storry, J. E. & Rook, J. A. F. (1965). *Biochem. J.* **96**, 210.

Storry, J. E., Rook, J. A. F. & Hall, A. J. (1967). *Br. J. Nutr.* **21**, 425.

Storry, J. E. & Tuckley, B. (1967). *Lipids* **2**, 501.

Tove, S. B. (1965). In *Physiology of Digestion* in the *Ruminant*, p. 399. [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors.] Washington: Butterworths.

Tove, S. B. & Mochrie, R. D. (1963). *J. Dairy Sci.* **46**, 686.

West, C. E., Annison, E. F. & Linzell, J. L. (1967). *Biochem. J.* **102**, 23P.

Wood, G. E. (1966). *Diss. Abstr.* **27**B, 394.

Wretlind, A. (1957). *Acta physiol. scand.* **40**, 59.

Wretlind, A. (1964). *Acta chir. scand.* Suppl. no. 325, p. 31.

Zeringue, H. J., Brown, M. L. & Singleton, W. S. (1964). *J. Am. Oil Chem. Soc.* **41**, 688.

Zurier, R. B., Campbell, R. G., Hashim, S. A. & Van Itallie, T. B. (1967). *Am. J. Physiol.* **212**, 291.