An Investigation of Serum Lipoproteins and of their Contribution to Milk Fat in the Dairy Cow

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1. Tritium-labelled olive-oil triglycerides were introduced into the rumens of lactating cows and the specific activities of the lipids of milk and plasma and of serum lipoproteins were measured. 2. On treatment of serum with dextran sulphate it was found that the lipid of the precipitated β -lipoproteins consistently had a specific activity-time curve with a maximum comparable in value with, and occurring earlier than, that of the milk fat. 3. On fractionation of the lipids of these lipoproteins it was found that only the triglycerides and diglycerides had specific activity-time curves with maxima greater than that of milk fat, and on radioactivity data alone they are the only blood constituents studied that meet the requirements for being the precursor of milk fat. 4. From a consideration of abundances and the mean specific activities over the period of the experiment it is shown that the contribution of the diglycerides to the radioactivity in the milk fat must have been negligible and that only the triglycerides could have been responsible for all the radioactivity found in it. 5. Although no other fraction could alone have been responsible for all the radioactivity in the milk fat, at least one, the phospholipids, could have made some contribution. 6. It is calculated that 35-48% by weight of milk fat was derived from the β -lipoprotein triglycerides, according to whether the phospholipids made an improbably large contribution or none at all.

It was previously reported from this Laboratory (Glascock, Duncombe & Reinius, 1956) that when tritium-labelled stearic acid, either free or in the form of triglycerides, was fed to a lactating cow, up to 59% of the radioactivity was transferred to the milk fat. It was already known that milk fat is synthesized in the udder from small molecules such as acetate (Popják, French & Folley, 1951), but this work showed that it is also derived from lipid precursors, as, in fact, had been concluded many years earlier by Graham, Jones & Kay (1936). The specific activity of the mixed plasma lipids reached a maximum about 40 hr. after dosing, whereas that of the milk fat did so at 23 hr.; and the maximum specific activity of the milk fat was 5-10 times as great as that of the plasma lipids, which could not therefore be wholly or mainly its precursor (Glascock, McWeeny & Smith, 1958).

It was concluded that freshly absorbed fat circulates in the form of a compound that is selectively removed by the udder for conversion into milk fat. In these experiments it must have had a maximum specific activity greater than that of the

milk fat and therefore more than 10 times as great as that of the mixed plasma lipids, in which its abundance must have been correspondingly low. The present work was designed to identify this fatty compound. Preliminary accounts of various parts of this work have already been published (Welch, Noble, Wright, Bishop & Glascock, 1963; Glascock, Welch, Bishop & Davies, 1965).

EXPERIMENTAL

Chemicals

Solvents were all reagent grades. Ether was dried over sodium and redistilled. Hexane, benzene, chloroform, ethanol and methanol were distilled once. Toluene, for scintillation counting, was distilled three times. Diphenyl-oxazole was supplied by Thorn Electronics Ltd. Hyamine hydroxide was prepared from Hyamine 10X, supplied by Charles Lennig and Co. (Gt. Britain) Ltd., London, W.C. 1, by the method of Eisenberg (1958). Silicic acid for column chromatography was Mallinckrodt 100-mesh grade and supplied by Kodak Ltd., Kirkby, Liverpool. Merck Silica Gel G for thin-layer chromatography was supplied by Camlab (Glass) Ltd., Cambridge. Florisil (magnesium trisilicate) was 60-100 mesh and supplied by L. Light and Co. Ltd., Colnbrook, Bucks. Cellulose powder was Whatman standard grade. Purified phosphatide was a gift from the

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Upjohn Company, Kalamazoo, Mich., U.S.A., which is gratefully acknowledged. Pitocin at 10 international oxytocin units/ml. was supplied by Parke, Davis and Co. Ltd., Hounslow, Middlesex. Dextran sulphate was clinical grade, supplied by Glaxo Laboratories Ltd., Greenford, Middlesex.

Dextran sulphate reagent. This consisted of a filtered mixture of 1 vol. of a 10% (w/v) solution of dextran sulphate and 5 vol. of M-CaCl₂ solution.

Tritium-labelled triglycerides. [³H]Stearic acid was prepared as described by Glascock & Reinius (1956) by the catalytic addition on a micro-scale of tritium, in the chemical form of hydrogen gas, to elaidic acid. [³H]Stearic acid thus prepared was usually pure white with m.p. 71° and a specific activity of about 200 mc/mg. The tritium is known to be unevenly distributed on the carbon chain and not specifically in the 9,10-positions (Glascock & Reinius, 1956).

A suitable total activity was transesterified with 0.5 g. of olive oil by the method of Bergström, Borgström & Rottenberg (1952). The product was purified by chromatography on silicic acid as described below for blood lipids.

Oil emulsion. The oil emulsion for oral administration was prepared as described by Meyer, Fansher, Schurr & Webster (1957); the purified labelled triglyceride was dissolved in 5 g. of olive oil together with 2.5 g. of monostearin and 100 mg. of purified phosphatide. The mixture was vigorously stirred in 100 ml. of boiling water for 10 min. by means of an MSE homogenizer, yielding a stable emulsion.

Experimental animals

Three separate experiments (numbered 62/1, 62/2 and 63/1) were carried out on Friesian cows of the Institute herd. Each animal was starved for 24 hr. before dosing and had a polythene cannula inserted in the right jugular vein to enable blood samples to be taken without disturbing her. During the experiment, which lasted up to 4 days, she received 4.5 kg. of hay and 2.7 kg. of concentrates twice daily (at 6.00 a.m. and 4.00 p.m.) with free access to water. After the injection of 2 ml. of Pitocin through the cannula, she was milked out completely just before dosing and when milk samples were taken. In Expts. 62/1 and 62/2 the labelled triglyceride was given by stomach tube in 100 ml. of emulsion followed by 100 ml. of unlabelled emulsion, making a total dose of 15 g. of fat. In Expt. 63/1 these quantities were halved and the emulsion was given by

intraruminal injection through a previously inserted polythene cannula. The points on the graphs show the times at which blood samples were taken and the midpoints of the periods between milkings. Other experimental details are summarized in Table 1.

Treatment of samples

Milk. A 50 ml. portion was withdrawn from each sample and freeze-dried.

Separation of plasma and red cells. The samples of citrate-treated blood were centrifuged for 30 min. at 1400g. Plasma was separated, its volume was measured and it was freeze-dried. The red cells were suspended in 45 ml. of iso-osmotic solution and were centrifuged for 10 min. at 1400g, the supernatant was discarded, the procedure was repeated twice more and the cells were freeze-dried.

Standardization of lipoprotein precipitation. Before each experiment, the optimum amount of dextran sulphate reagent required to precipitate the β -lipoproteins was determined by titrating the serum with dextran sulphate reagent to maximum turbidity (Oncley, Walton & Cornwell, 1957).

Separation of lipoproteins. The method was based on that of Burstein & Samaille (1957). The samples of blood were allowed to stand for 12 hr. at 4° and they were then centrifuged for 30 min. at 1400g. Serum was aspirated from the cells, its volume was measured, the calculated amount of dextran sulphate reagent was added and the mixture left in the refrigerator at 4° for 12 hr. It was then allowed to warm to room temperature and centrifuged at 2800g for 1 hr. at 20°. The supernatant, which contained the α -lipoproteins and the unesterified fatty acids, was freeze-dried. The precipitated lipoproteins were redissolved in a solution of NaCl (5%, w/v) equivalent in volume to one-twentieth of the original volume of serum, and the lipoproteins were reprecipitated by the addition to that solution of 10 times its own volume of CaCl₂ solution (0.1M). The mixture was stored at 4° for 2½ hr. and the lipoproteins were separated by centrifugation at 20° for 30 min. at 2800g. The supernatant solution was discarded.

Electrophoresis. This was carried out on Whatman 3MM paper for 5 hr. at a potential difference of 240 v in veronal buffer, pH 8.6 and *I* 0.05. Protein bands were stained with bromophenol blue and lipoprotein bands with Sudan Black B as recommended by Swahn (1953). On electrophoresis, whole serum gave four well-defined protein bands, which are identified as albumin, α -globulin, β -globulin and

Table 1. *Experimental animals*

Expt. no.	Date	Weight of animal (kg.)	Day of lactation	Milk yield (kg./day)	Total radioactivity given (mc)	Volumes of blood samples
62/1	February 1962	473	124	12	45.5	75 ml.* 125 ml.
62/2	October 1962	525	59	9	36	225 ml.
63/1	May 1963	605	241	13.6	46	225 ml. (jugular vein) 225 ml. (mammary vein)

* Taken into citrate for plasma separation.

γ -globulin (which remained at the origin or moved slightly behind it). The supernatant, after separation of the lipoprotein-dextran sulphate precipitate, gave a lipoprotein band lying between the α -globulin and the albumin, and in conformity with the nomenclature used by workers on human serum (e.g. Smith, 1960) we provisionally call this fraction α -lipoprotein. The redissolved lipoprotein-dextran sulphate precipitate gave a band migrating close to the β -globulin and another remaining at the origin with diffuse staining in between. For convenience we call the whole of the material precipitated by dextran sulphate β -lipoprotein.

Extraction of lipids. Freeze-dried plasma, α -lipoproteins, milk and red cells were shaken for 3 hr. with a volume of chloroform-methanol (2:1, v/v) equal to that of the original fresh sample. A mixture of chloroform-methanol and red cells gave a rubbery mass which could be avoided by the addition of one-third of a volume of ethanol to the solvent mixture. The mixture was filtered on a Buchner funnel, and the residue was washed and then shaken for a further 24 hr. with the same volume of solvent. The suspension was again filtered and the procedure repeated. Finally, the residue was submitted to Soxhlet extraction with the same solvent mixture for 3 hr. The extracts were combined, concentrated to 20 ml. under reduced pressure and freed from protein by passing through a column of cellulose powder, as described by Böttcher, Woodford, Boelsma-Van Houte & Van Gent (1959).

The extracts of plasma and milk were concentrated to small volume by distillation under reduced pressure, made up to 100 ml. with chloroform-methanol (2:1, v/v) and stored at -10° . The extract of α -lipoproteins was concentrated under reduced pressure almost to dryness and further purified by redissolving in 100 ml. of chloroform-methanol (2:1, v/v) and partitioning with one-quarter of its volume of water as described by Folch, Lees & Sloane-Stanley (1957). The lower phase was concentrated to small volume under reduced pressure, 50 ml. of benzene was added and the mixture was distilled almost to dryness to remove water. This procedure was repeated and the residue was finally dissolved in hexane, made up to 100 ml. and stored at -10° .

β -Lipoproteins. To the precipitated β -lipoproteins was added 25 ml. of chloroform-methanol (2:1, v/v), and the mixture was filtered through a Soxhlet thimble (7.5 cm. $\times$ 2.5 cm.). The small residue was extracted in a Soxhlet apparatus for 3 hr. with the same solvent. The extract was concentrated nearly to dryness, a few millilitres of chloroform-methanol (2:1, v/v) were added and the solution was combined with the first extract and made up to approx. 80 ml. It was then partitioned with one-quarter of its volume of water and the procedure continued as described for the α -lipoproteins.

Fractionation of lipids

The technique used for fractionation on columns of silicic acid was basically that of Barron & Hanahan (1958). This permitted the separation of all main classes of lipids except the free fatty acids, which were separated on a Florisil column as described by Carroll (1961). A maximum of 50 mg. of mixed lipids was applied to a column prepared from 3 g. of activated silicic acid and eluted by a stepwise procedure in which the volumes of various solvents required were determined by reference to a radiochromatogram.

This was previously prepared by eluting one sample and measuring the total radioactivity in 5 ml. fractions; elution with each solvent was continued until the radioactivity diminished to a negligible level. The volumes required did not greatly differ from experiment to experiment.

Thin-layer chromatography. Thin-layer chromatography of spots containing not more than 200 μ g. of mixed lipids was done on layers of Merck Silica Gel G, 250 μ thick. The chromatograms were developed for about 30 min. in ether-hexane-acetic acid (10:90:1, by vol., for separation of sterol esters, sterols, triglycerides and fatty acids, or 50:50:1, by vol., for separation of monoglycerides, diglycerides and triglycerides; phospholipids remain at the origin in both systems). Spots were stained with dodecamolybdo phosphoric acid [10% (w/v) in 95% (v/v) ethanol].

Measurement of radioactivity. Specific radioactivity was determined by the liquid-scintillation counting of weighed samples in an Ecko single-channel scintillation counter. The scintillation system was diphenyloxazole (3 g./l.) in a 9:1 (v/v) mixture of toluene and ethanol. In Expts. 62/1 and 62/2 samples of total lipids were assayed in duplicate but only single samples of the fractions were assayed. In Expt. 63/1 most samples were assayed in duplicate.

Titration and counting of diglyceride fatty acids. Thin-layer chromatography showed that the diglycerides obtained in Expt. 63/1 were contaminated with sterols. In this experiment only therefore, the diglycerides were saponified and the fatty acids were liberated, extracted, titrated and counted.

Monitoring of samples. In Expts. 62/2 and 63/1 the radioactivity of samples of blood and milk was measured as soon as possible after taking them, to ensure that sampling continued until specific activities passed their maximum. Serum was counted directly by adding 0.1 ml. to a mixture of 5 ml. of phosphor solution and 1 ml. of Hyamine hydroxide. Milk was diluted with an equal volume of water and 0.2 ml. added to the same mixture.

RESULTS

Experiment 62/1. Fig. 1(a) shows the specific activity-time curves of the total mixed lipids of milk, plasma, α - and β -lipoproteins and blood cells. The collection of samples ceased before the specific activity of the milk fat reached a maximum and in this respect the experiment was anomalous. In all other experiments the maximum had been reached at about 23 hr., but in this one the specific activity of the milk fat was still rising 36 hr. after dosing. Until it reached its maximum at 27 hr. the specific activity of the β -lipoprotein lipids was about the same as that of the milk fat. The specific activity of the α -lipoprotein lipids, however, was even lower than that of the total plasma lipids. This was to be expected since the β -lipoproteins, whose lipids had a high specific activity, had been removed. The specific activity of the lipids of the red blood cells was lower than that of any of the other samples collected, showing that they make little or no contribution to the radioactivity in the milk fat.

The specific activity of none of the fractions of plasma lipids reached values even approaching the

Fig. 1. Expt. 62/1: specific activity of milk and blood lipids of a cow after administration of [³H]stearic acid as olive-oil triglycerides. (a) Mixed lipids of milk (O), plasma (●), serum β-lipoproteins (□), serum α-lipoproteins (▲) and red cells (Δ); (b) milk mixed lipids (O) and serum β-lipoprotein triglycerides (●).

Fig. 2. Expt. 62/2: specific activity of milk and blood lipids of a cow after administration of [³H]stearic acid as olive-oil triglycerides. (a) Milk mixed lipids (O) and serum β-lipoprotein mixed lipids (▲); (b) milk mixed lipids (O) and serum β-lipoprotein triglycerides (Δ).

highest observed in the samples of milk fat. The finding in a previous experiment (Glascocks & Wright, 1962) that the specific activity of an unidentified fraction reached a maximum earlier and greater than that of the milk fat was therefore not confirmed.

The specific activity-time curves of milk fat and β-lipoprotein triglycerides are shown in Fig. 1(b). These triglycerides were the only fraction whose maximum specific activity was greater than that of the milk fat at 36 hr., when the shape of the curve suggests that it was nearing its maximum. The maximum specific activity of the β-lipoprotein triglycerides occurred at 27 hr., when it was 2½ times as great as that of the milk fat at 36 hr. The fall in specific activity observed in the β-lipoprotein mixed lipids between 20 and 22 hr. is also shown by the triglycerides, and it is therefore possible that the curve should be drawn through both these points instead of as shown in Fig. 1(b).

Thin-layer chromatography in a later experiment (63/1) showed that the diglycerides, as eluted from the silicic acid column, were often heavily contaminated with sterols. It is therefore possible in this experiment that the diglycerides had specific activities as high as those of the triglycerides.

Experiment 62/2. The specific activities of milk fat and of β-lipoprotein mixed lipids had about the same value at their maxima, as shown in Fig. 2(a). The maximum specific activity of the β-lipoprotein lipids occurred earlier than that of the milk fat, which was at the usual time of 24 hr. In this experiment, as in Expt. 62/1, the maximum specific activities of all fractions of β-lipoprotein lipids except the triglycerides were less than that of the milk fat. The maximum specific activity of the triglycerides was 2½ times as great as that of the milk fat, as shown in Fig. 2(b). It is possible in this experiment, as in Expt. 62/1, that the diglycerides, as separated, were contaminated with sterols and

Fig. 3. Expt. 63/1: specific activity of milk and blood lipids of a cow after administration of $[^3\text{H}]$ stearic acid as olive-oil triglycerides. (a) Milk mixed lipids (O) and serum β -lipoprotein mixed lipids (Δ); (b) milk mixed lipids (O), serum β -lipoprotein triglycerides (Δ) and diglycerides ($\blacktriangle$).

had a specific activity about the same as that of the triglycerides.

Experiment 63/1. Although the results of this experiment were substantially in agreement with those of Expts. 62/1 and 62/2 there were some differences in detail, as shown in Fig. 3. The maximum in the specific activity-time curve of the milk fat is less sharp than in the earlier experiments and is almost flat over the period 14–26 hr. The maximum specific activity of the mixed lipids of the β -lipoproteins was considerably lower than that of the milk fat and the curve showed three maxima, of which the first, at 12 hr. is less well-defined by the experimental points than the other two at 22 and 30 hr. It might be thought that these maxima were artifacts arising from experimental error in the determination of specific activities, particularly since their existence depends so much on the validity of the minima occurring at 18 and

26 hr. If these two points are regarded as aberrant then a curve with a single maximum at about 16 hr. can be drawn without any of the other experimental points lying very far off it. Such a curve was in fact published in a preliminary account of this work (Glascocock *et al.* 1965). However, the two minima are the result of duplicate analyses in very good agreement and there appears to be no justification for discarding them. Further, the specific activity-time curves of the diglycerides and triglycerides, which were the fractions of greatest specific activity contained in these mixed lipids, also showed three strongly marked maxima (Fig. 3b). It thus appears that the time-course of the specific activity of the mixed lipids of the β -lipoproteins is best represented by the curve given in Fig. 3(a).

In Fig. 3(b) are shown the specific activity-time curves of the β -lipoprotein diglycerides and triglycerides and of the milk fat. In previous experiments only the triglycerides had a maximum specific activity greater than that of the milk fat. As noted above, however, it was found that the diglycerides in this experiment were contaminated with sterols. Assay of the fatty acids extracted from the hydrolysed diglyceride fraction showed that it had a specific activity-time curve similar to that of the triglycerides.

Both of these fractions show maxima that may also have existed in earlier experiments, as shown by the points that have been plotted but through which it was not thought justifiable to draw the curve. The maxima are also much more pronounced than they could have been in those experiments, which may be because the curves more closely reflect the time-course of specific activities as a result of improved techniques. It is also possible that a different method of administration of the labelled oil produced a different pattern of absorption.

DISCUSSION

The origin of the maxima in the specific activity-time curves of some of the lipids of the serum β -lipoproteins is at present a matter for conjecture. Absorption from one or more of the compartments of the stomach would result in the lipids' being carried to the liver. This could delay their appearance in peripheral blood whereas absorption from the intestine into the lymphatic system would not. The simultaneous operation of these processes might well produce the complex specific activity-time curves actually observed in the peripheral blood lipids.

In a precursor-product relationship the precursor reaches a maximum specific activity both greater and earlier than that of its product (Zilversmit, Entenman & Fishler, 1943). If it is the sole precursor and if the observations continue until

the bulk of the tracer has been swept through the system, the mean specific activities of precursor and product tend to equality. If there is another and unlabelled precursor the mean specific activity of the product is less than that of the labelled precursor in proportion to the degree of dilution of isotope produced by the unlabelled precursor.

A precursor-product relationship can be inferred from the curves for the β -lipoprotein triglycerides and milk fat of Expt. 62/2 (Fig. 2b), and also from Expt. 62/1 (Fig. 1b) as similar curves would almost certainly have been obtained had it been allowed to continue long enough. This inference is not invalidated by the brief minimum in the curve at 14 hr. in Expt. 63/1 (Fig. 3b) since this would merely have had the effect of flattening the maximum of the product curve, as was, in fact, observed in the curve of the milk fat. Similarly the third smaller maximum would be expected to produce a change in the slope of the new descending product curve, and this again was observed.

Expt. 63/1 was the only one in which observations were continued long enough for most of the tracer to be swept through the system and hence which permits an estimate of the contribution of precursor to product from a comparison of specific activities. Such a calculation involves the assumption that the precursor was responsible for all the radioactivity in the product.

Graphical integration between the limits of 0 and 72 hr. of the curves shown in Fig. 3(b) gave a mean specific activity for the milk fat of 2.25×10^4 disintegrations/min./mg., which is in good agreement with the value of 2.27×10^4 disintegrations/min./mg. observed. Similarly the mean specific activities of the diglycerides and triglycerides were found to be 3.57×10^4 and 4.86×10^4 disintegrations/min./mg. These values, together with those of the other β -lipoprotein lipids, are shown in Table 2.

The diglycerides and triglycerides are the only

fractions whose maximum and mean specific activities were both greater than that of the milk fat and the only ones therefore that could have been responsible, on the evidence of specific activities alone, for all the radioactivity in the milk fat. The diglycerides can be excluded as a sole precursor, or even as an appreciable co-precursor, however, because of their low abundance, which (Table 2) was only about 1mg./l. of whole blood. The probable upper limit for the rate of blood flow through the udder is 17l./min. [Glascock & Wright (1962) suggest a range 7–17l./min.]. Complete removal of these diglycerides during passage through the udder could therefore provide a rate of secretion into milk of not more than 17mg./min. for fat and 6.1×10^5 disintegrations/min./min. for radioactivity. These are only 6 and 10% respectively of the observed mean rates of secretion into milk, which were 275mg./min. for fat and 6.25×10^6 disintegrations/min./min. for radioactivity. Further, provisional results indicate that in fact very little, if any, of the diglycerides was actually removed during passage through the udder.

The triglycerides are the only other possible single source of radioactivity in the milk fat and a comparison of mean specific activities gives a value of 46.3% as the fraction by weight of total milk fat derived from them. Extrapolation shows that this value would not have been more than 48% even if the experiment had been continued until all specific activities were virtually zero. The mean rate of secretion of milk fat was 275mg./min., and if 48% of this were derived from the triglycerides they must have been removed from the blood at a rate of 133mg./min. With only a 50% arterio-venous difference (say 10mg./l.) such a rate of loss would require a rate of blood flow through the udder of 13l./min., which is well within the estimated range. The contribution of the β -lipoprotein

Table 2. Concentrations and mean specific activities of radioactive lipid components of the jugular serum β -lipoproteins in the lactating cow (Expt. 63/1)

Concentrations expressed as mg./100mg. of total lipids are given as means $\pm$ S.E.M. with the numbers of determinations in parentheses. Mean specific activities were obtained by graphical integration between the limits 0 and 72 hr. of specific activity-time curves.

Component	Concentration (mg./100mg. of total lipids)	Concentration (mg./l. of serum)	$10^{-4} \times$ Mean specific activity (disintegrations/ min./mg.)
Unidentified fraction	1.14 ± 0.07 (30)	4.43	0.48
Free fatty acids	2.52 ± 0.21 (36)	9.80	0.53
Monoglycerides	1.17 ± 0.06 (32)	4.55	0.30
Diglycerides	0.50 ± 0.07 (15)	1.94	3.57
Triglycerides	10.07 ± 0.61 (43)	39.15	4.86
Phospholipids	24.44 ± 0.86 (33)	95.02	0.80
Sterol esters	41.63 ± 0.88 (31)	161.86	0.10

triglycerides calculated from specific-activity data is therefore consistent with other known facts. The value would, however, be too high if other labelled lipids contributed to the radioactivity in the milk fat, and of the present results it cannot be said with certainty that they did not do so at all. The contribution of the lipids of low abundance (monoglycerides, unesterified fatty acids and the unidentified fraction) can be shown to be negligible, however, on the same grounds as that of the diglycerides: at any reasonable rate of blood flow through the udder even a 100% arteriovenous difference would have provided an insignificant fraction of the radioactivity actually found in the milk fat; and, further, preliminary results indicate that the arteriovenous differences for all these fractions was much less than 100%.

Of the two β -lipoprotein lipids of high abundance the sterol esters can have made no appreciable contribution to the radioactivity in the milk fat because their mean specific activity was so low compared with that of the triglycerides. The mean specific activity of the phospholipids, however, was one-sixth that of the triglycerides and some of the radioactivity in the milk fat might therefore have been derived from them. It is calculated that if the phospholipids were the only other precursor the radioactivity in the milk fat could not be accounted for by a contribution of less than 35% by weight of triglycerides. In this extreme and unlikely case, therefore, the real contribution of the triglycerides would be three-quarters of that calculated. It therefore seems safe to say that although a value of 48% is a maximum the true value for the proportion of milk fat derived from the β -lipoprotein triglycerides is unlikely to be much less.

Evans, Patton & McCarthy (1961) have shown that the lipoproteins migrating with approximately the same electrophoretic mobility as the β -globulins are those of low density ($d < 1.063$); and Smith (1960) states that the lipid-containing material remaining at the origin on electrophoresis of human serum consists of lipoproteins of very low density in the form of particles greater in diameter than 1000 Å and of chylomicrons (diameter 5000 Å). It thus appears that the material precipitated with dextran sulphate in our work consists of lipoproteins of low density and, insofar as they exist in the cow, of chylomicrons. The results reported here are therefore in qualitative agreement with those of Barry, Bartley, Linzell & Robinson (1963), who found a substantial arteriovenous difference in the triglyceride fatty acids of chylomicrons and low-density lipoproteins ($d < 1.019$) across the udder of the lactating goat. These workers also found that the arteriovenous differences in stearic acid and palmitic acid combined in these triglycerides were proportionately equal, indicating that there is no

selective use or rejection of stearic acid relative to the other long-chain fatty acids. The assumption in our work that the [^3H]stearic acid effectively labels all the long-chain fatty acids of the β -lipoprotein triglycerides and is not selectively used by the udder for secretion into milk is therefore probably justified.

That circulating triglyceride can give rise to milk fat was demonstrated in this Laboratory as long ago as 1957 (Glascock *et al.* 1958) by the collection of radioactive milk fat after the injection of tritium-labelled olive-oil triglycerides into a lactating cow. This has been elegantly confirmed by Lascelles, Hardwick, Linzell & Mephram (1964), who injected chylomicrons, labelled *in vitro* with [^3H]stearic acid, into a lactating goat and showed that there was an efficient transfer of radioactivity into milk fat. The present work, which shows that only a small proportion of the total circulating triglycerides is used for milk-fat synthesis, is therefore in agreement with those results also.

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