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The C_{18} fatty acids of ox plasma lipids

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SUMMARY

The fatty acid composition of the cholesterol esters, triglycerides, phospholipids, and free fatty acids from the pooled plasma of four grass-fed, maiden heifers was determined by gas-liquid chromatography. Special attention is drawn to the composition of the C_{18} fatty acids, and particularly to the presence of C_{18} tri-unsaturated fatty acid as a major component (22.9% w/w) of the acids esterified to cholesterol since workers in other laboratories have not detected this acid in ox plasma cholesterol esters or have found it in only very small amount.

Kelsey and Longenecker (1) and Lough and Garton (2) have described the fatty acid composition of plasma lipids from the blood of pasture-fed, lactating cows, and Garton *et al.* (3) examined the lipids in pooled samples of ox blood that contained contributions from lactating animals. In all three studies, C_{18} tri-unsaturated acid was found to be a significant component of the acids esterified to cholesterol; it formed only a very small proportion of the fatty acids of the phospholipids (3) and was not detected in the triglycerides or free fatty acids (1, 2).

On the other hand, Dole *et al.* (4) found that C_{18} tri-unsaturated acid comprised only 2% of the fatty acids of "cow" plasma neutral lipids (i.e., cholesterol esters plus triglycerides) and detected none in phospholipids or free fatty acids. Hanahan *et al.* (5) apparently did not detect this species of acid in the cholesterol esters, triglycerides, or lecithins of ox plasma (of unstated origin), although they stated that the fatty acid analyses were in general agreement with our previous observations (2).

It was considered possible, though perhaps unlikely, that the amounts of C_{18} tri-unsaturated acid previously found (1, 2, 3) in ox plasma lipids might be associated with the fact that all or part of the blood used was obtained from lactating animals. A further investigation was therefore made of the lipids extracted from the plasma of maiden heifers (*i.e.*, nonpregnant, nonlactating animals).

Unsaturated C_{18} fatty acids occurring in ruminant tissues probably contain positional and geometrical isomers of oleic, linoleic, and linolenic acids due to microbial hydrogenation in the rumen of feed lipids containing these acids (6). Hence, in this paper, the terms C_{18} mono-, di-, and tri-unsaturated acid are used.

EXPERIMENTAL METHODS

Plasma Samples. Approximately 2 liters of jugular venous blood was obtained, at slaughter, from each of four grass-fed, maiden heifers; the blood was citrated (40 ml saturated sodium citrate solution per liter of blood) and centrifuged. After allowing for the citrate solution present, a volume corresponding to 500 ml of each plasma sample was taken; these were pooled, giving a final volume representing 2 liters of plasma.

Extraction of Lipids. The plasma was poured slowly into 4 liters of absolute ethanol. The mixture was filtered and the filtrate reduced to 200 ml by distillation under reduced pressure at about 35°. The protein precipitate, containing most of the lipid, was thoroughly mixed with 4 liters of chloroform and 2 liters of methanol and the mixture was heated to boiling. It was then filtered and the extraction repeated three times. The concentrated aqueous ethanol from the initial precipitation was added to the pooled filtrates and the whole was reduced to about 250 ml by distillation at about 35° under reduced pressure. This solution was then taken to dryness in a stream of N_2 on a warm water bath to yield the crude lipids (7.59 g).

Fractionation and Analysis of Lipids. The crude lipids were chromatographed on a column of silicic acid prepared as previously described (7). The eluting solvents used successively were: petroleum ether (b.p. 40°–60°) to remove hydrocarbons; petroleum ether containing 1% (v/v) diethyl ether to remove cholesterol esters; diethyl ether alone to remove triglycerides, free fatty acids, and cholesterol; and chloroform-methanol, 1:1 (v/v) to remove phospholipids collectively together with nonlipid contaminants. The free fatty acids were extracted from the fraction eluted with diethyl ether by washing with 0.5% (w/v) aqueous KOH, and the remainder of the fraction was hydrolyzed by refluxing with

excess 0.5 N ethanolic KOH to yield the glyceride acids and the free cholesterol; glycerol was determined by periodate oxidation. The cholesterol esters were similarly hydrolyzed. The fatty acids of each fraction were converted to methyl esters by refluxing with excess methanol containing 1% (w/w) H_2SO_4. The fraction containing phospholipids yielded fatty acid methyl esters directly by methanolysis under reflux with 6 N methanolic HCl for 6 hours.

The fatty acid methyl esters were analyzed by gas-liquid chromatography before and after hydrogenation using a Pye Argon Chromatograph (Cambridge, Great Britain). Columns were packed with acid-washed Embacel® (Kieselguhr, 60–100 mesh; May and Baker, Dagenham, Great Britain) impregnated with polymerized ethylene glycol succinate (prepared as for the adipate [8]) in the proportions of 11:2 by weight of solid support to liquid phase. The operating temperature was 173° and the argon flow was 50 cc per minute. The ionization detector (radium-D source) of the type described by Lovelock *et al.* (9) was used and calculation of the proportions in each mixture was made directly from the relative peak areas on the chromatograms, determined by the "triangulation" procedure. The chromatograms of the hydrogenated esters were used to confirm the chain length of the unsaturated components.

RESULTS

In Table 1 the results of the fractionation of the plasma lipids on silicic acid are given, together with other analytical results. It is evident that each fraction, except that eluted with chloroform-methanol, was free from nonlipid contaminants. Methanolysis of the fraction eluted with chloroform-methanol yielded fatty acid methyl esters in amount corresponding to 1,500 mg phospholipids, calculated as lecithin, which is the major phospholipid component present (5, 10). It is also apparent from Table 1 that the esterified fatty acids present in the fraction eluted with diethyl ether were present initially essentially as triglyceride. From the results given in Table 1, the proportions of each class of lipid present originally in the plasma were calculated (Table 2).

Of the total fatty acids in the plasma (142.5 mg per 100 ml), 50.7% were esterified to cholesterol, 9.1% were present as glycerides, 35.4% were in phospholipid combination, and 4.8% in the free state. The analyses of the component acids of each fraction are recorded in Table 3.

The retention times of the C_{18} mono-, di-, and tri-unsaturated acids relative to that of methyl stearate were, respectively, 1.17, 1.48, and 2.00. These values are in conformity with those repeatedly found in this laboratory for methyl oleate, methyl linoleate, and methyl linolenate as components of mixed esters prepared from linseed oil. Further, hydrogenation of the methyl esters prepared from the fatty acids of the cholesterol esters yielded the predicted increment to the proportion of methyl stearate.

DISCUSSION

As Table 3 indicates, C_{18} fatty acids comprised a considerable proportion of the total fatty acids of each group of plasma lipids; the amounts of the individual C_{18} components, expressed as percentages of the total C_{18} fatty acids of each group, are recorded in Table 4.

Previous observations (3) on the over-all composition of ox plasma lipids were confirmed, and it is apparent that the presence of C_{18} tri-unsaturated fatty acid in the plasma cholesterol esters is not peculiar to lactating cows. It does, however, appear to be characteristic of the cholesterol esters that they contain C_{18} tri-unsaturated fatty acid as a major component, accounting for 27.8% of the total C_{18} acids of this fraction (11.5% of the plasma total acids) in the present study; in the other lipid fractions it represented but a minor component acid.

TABLE 1. FRACTIONATION AND ANALYSIS OF HEIFER PLASMA CRUDE LIPIDS

Silicic Acid Chromatography			
Eluting Solvent	Weight of Fraction (mg)	Nature of Fraction	Analysis of Fractions
Petroleum ether	23	Hydrocarbons	
1% Diethyl ether in petroleum ether	3,323	Cholesterol esters	Hydrolysis gave 56.6% cholesterol and 43.4% fatty acids
Diethyl ether	990	Triglycerides, free fatty acids, and free cholesterol	Contained 136 mg free fatty acids, 566 mg free cholesterol, 266 mg glyceride-bound fatty acids, and 25 mg glycerol
Chloroform-methanol (1:1)	2,857	Phospholipids plus non-lipid material	Methanolysis gave 1,040 mg fatty acid methyl esters

TABLE 2. LIPIDS IN HEIFER PLASMA

Nature of Lipid	Amount in Plasma (mg/100 ml)
Hydrocarbons	1
Cholesterol esters	166
Free cholesterol	28
Free fatty acids	7
Triglycerides	14
Phospholipids (as lecithin)	75

TABLE 3. PERCENTAGE COMPOSITION (BY WEIGHT) OF FATTY ACIDS OF HEIFER PLASMA LIPIDS

Fatty Acid*	Source of Fatty Acids			
	Cholesterol Esters	Glycerides	Free Fatty Acids	Phospholipids
12:0	—	—	0.6	—
14:0	0.7	1.1	1.8	trace
15:0	2.3	1.8	1.0	0.3
16:0	5.5	24.0	18.5	16.2
16:1	2.8	4.8	3.9	1.1
16:2	0.3	0.7	0.3	trace
17:0	0.5	6.8	2.0	4.4
18:0	1.5	30.0	24.1	26.8
18:1	5.6	24.3	39.3	16.3
18:2	52.4	4.6	5.2	14.1
18:3	22.9	1.9	3.3	2.4
20:0	0.9	—	—	trace
20:?†	4.6	—	—	9.7
22:? ↓ 24:?‡	—	—	—	8.7

* Shorthand nomenclature of Dole *et al.* (4), indicating chain length and number of double bonds.

† Including 20:4 and another (unidentified) unsaturated component.

‡ Comprising saturated and unsaturated acids of chain length 22, 23, and 24 carbon atoms; unsaturated components could not be unequivocally identified owing to the unavailability of suitable reference acids.

TABLE 4. PERCENTAGE COMPOSITION (BY WEIGHT) OF C_{18} FATTY ACIDS OF HEIFER PLASMA LIPIDS

Nature of Acid*	Source of C_{18} Fatty Acids			
	Cholesterol Esters	Glycerides	Free Fatty Acids	Phospholipids
18:0	1.8	49.3	33.5	45.0
18:1	6.8	40.0	54.7	27.3
18:2	63.6	7.6	7.2	23.7
18:3	27.8	3.1	4.6	4.0

* Nomenclature explained in footnote to Table 3.

The fatty acid composition of serum cholesterol esters of several species (man, dog, rat, rabbit, guinea pig, pig, hen, and goose) was determined by Swell *et al.* (11); noteworthy in all cases was the very small proportion of C_{18} tri-unsaturated fatty acid found, the amount ranging from traces (dog) to 1.1% (rabbit). As previously mentioned, similar findings with respect to ox plasma cholesterol esters were made by Dole *et al.* (4) and Hanahan *et al.* (5). By contrast, the present analyses and those previously reported (2), in which C_{18} tri-unsaturated fatty acid was identified by a combination of reversed-phase chromatography and alkali-isomerization, have demonstrated unequivocally that this acid can be a major component of the acids esterified to cholesterol in ox plasma.

The presence of C_{18} tri-unsaturated fatty acid in the plasma lipids of pasture-fed cattle would appear to be related to the relatively high content of linolenic acid in their feed lipids (12, 13). It is known, however, that much of the linolenic acid of these lipids is subjected to microbial hydrogenation in the rumen (see review by Garton [14]), although small amounts of unchanged acid apparently reach the site of intestinal absorption and are subsequently preferentially esterified to cholesterol. In other studies,[1] C_{18} tri-unsaturated fatty acid has been found as a minor component of the total fatty acids in the duodenum and small intestine of sheep given a diet that included dried grass.

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[1] G. A. Garton, unpublished experiments.