The Effect of Diet on the Phospholipid Composition of the Red Blood Cells of Man

J. G. HILL, A. KUKSIS and J. M. R. BEVERIDGE, Department of Biochemistry, Queen's University, Kingston, Ontario

Abstract

Short term (16 day) controlled fat (formula type diet) feeding to 10 healthy adult males led to no detectable change in the total amt or the relative proportions of the individual phospholipids of the red blood cells, although limited changes did occur in the fatty acids of certain of the phospholipids.

The total phospholipid content of the red blood cells was 315 ± 10 mg/100 ml (average of 20 samples). Lecithin accounted for 34% of the total, with sphingomyelin, phosphatidyl ethanolamine and phosphatidyl serine representing 25, 25 and 16%, respectively. Approx 36% of the phosphatidyl ethanolamine, 4% of the phosphatidyl serine and 6% of the lecithin was present in the plasmalogen form.

Each phospholipid class was found to have a distinctive fatty acid spectrum. The M ratio of saturated to unsaturated fatty acids in all three phosphoglycerides was nearly 1:1. Behenic, lignoceric and nervonic acids made up almost half of the sphingomyelin fatty acids, and the M ratio of saturated to unsaturated fatty acids in this lipid was 3:1.

When compared with red cells from subjects consuming a diet with a high butter fat content, red cells from subjects on a diet rich in corn oil were found to contain higher levels of linoleic acid in the lecithin and phosphatidyl serine fractions, and lower levels of oleic acid in the lecithin fraction. No changes were observed in the fatty acids of the phosphatidyl ethanolamine and sphingomyelin fractions. It is probable that these alterations represent the result of highly specific exchanges with plasma fatty acids, and it is suggested that three levels of specificity are involved: class of phospholipid, type of fatty acid, and specific fatty acid.

Introduction

ANY ATTEMPT TO INCREASE our understanding of the relationship between membrane function and structure, at the molecular level, will require suitable data on the chemical composition of the particular membrane involved. Ideally, such data will provide a measure of the normal composition of the membrane, as well as some indication of the variation which might be encountered in response to a physiological stress, e.g., a change in diet. For a variety of reasons the red cell membrane has been widely used in this type of investigation, and in a previous paper we recorded some observations with respect to the type and stability of its lipid components. Serial analyses on the red cells of subjects consuming diets of radically different fat content revealed no change in the total amt of any of the cell lipids, although alterations in the phospholipid fatty acid spectra did occur (1). Such constancy in the total amt of lipid is probably essential to any concept of the cell membrane requiring the lipid molecules to play an important structural role. On the other hand, changes in the relative amt of the various fatty acids may or may not be compatible with such a concept, depending on their type and extent. For this reason, it was of interest to determine more presisely the effect of dietary fats on red cell phospholipids.

Although a number of reports (2–6) have contribute valuable information on the relationship between diet and red cell lipids, data from rigidly controlled dietary experiments, providing details about the various phospholipid classes, are still lacking. This investigation was undertaken, therefore, to obtain data of this nature.

Experimental

Blood Samples. Ten male university students volunteered for the experiment. After a control blood sample (unrestricted diet, A) had been taken for each student, five were placed on a diet providing 40% of calories in the form of butter fat (diet BF), and five on a diet in which corn oil provided 40% of calories (diet CO). Both diets BF and CO were of the homogenized formula type (1). After 16 days a second blood sample was taken from each student. Each blood sample was 20 ml in volume, and was taken after an overnight fast. Heparin was used to prevent coagulation.

Lipid Samples. Red cell lipids were prepared as previously described (1). After removing an aliquot for the determination of phosphorus (7), the lipid was subjected to a three-stage fractionation procedure on silicic acid (8,9) as outlined in Figure 1. The progress of elution was followed by measurement of phosphorus in individual fractions and thin layer chromatography (TLC). Overall phosphorus recovery averaged 91%.

Reference Materials. Fresh reference phospholipids were obtained from suitable natural sources as required. Sphingomyelin was recovered from cow red cells by the method of Hanahan et al. (10). Lecithin

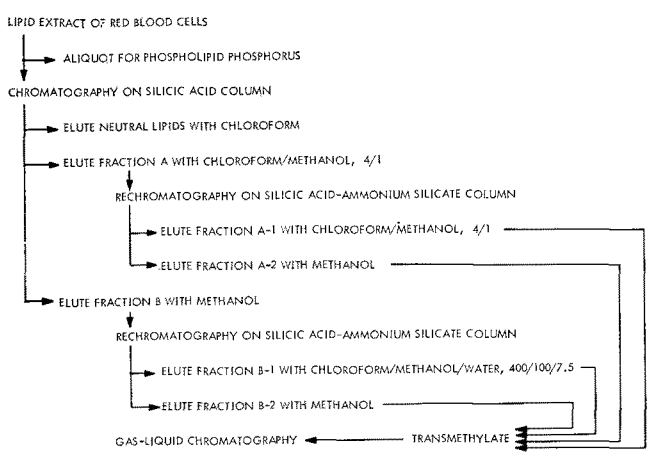

FIG. 1. A summary of the procedure for the fractionation of red blood cell lipids.

was isolated from dog bile as described by Nakayama and Johnson (11). Egg yolk provided a mixture of phospholipids in which phosphatidyl ethanolamine was readily identified as a major component (12), and an extract of rat spleen served as a source of phosphatidyl serine (13).

Identification and Estimation of Individual Phospholipids. Thin layer (14), paper (15) and ion exchange (16) chromatography were used in the characterization of the fractions eluted from the adsorption columns, or for the inspection of the hydrolytic products derived from the individual lipids. Identification of the four major phospholipid fractions (as phosphatidyl ethanolamine, phosphatidyl serine, lecithin and sphingomyelin) was achieved by comparison of their behavior with that of reference compounds in the chromatographic systems listed above. Ethanolamine, serine, choline and plasmalogens were determined by standard methods (16–18).

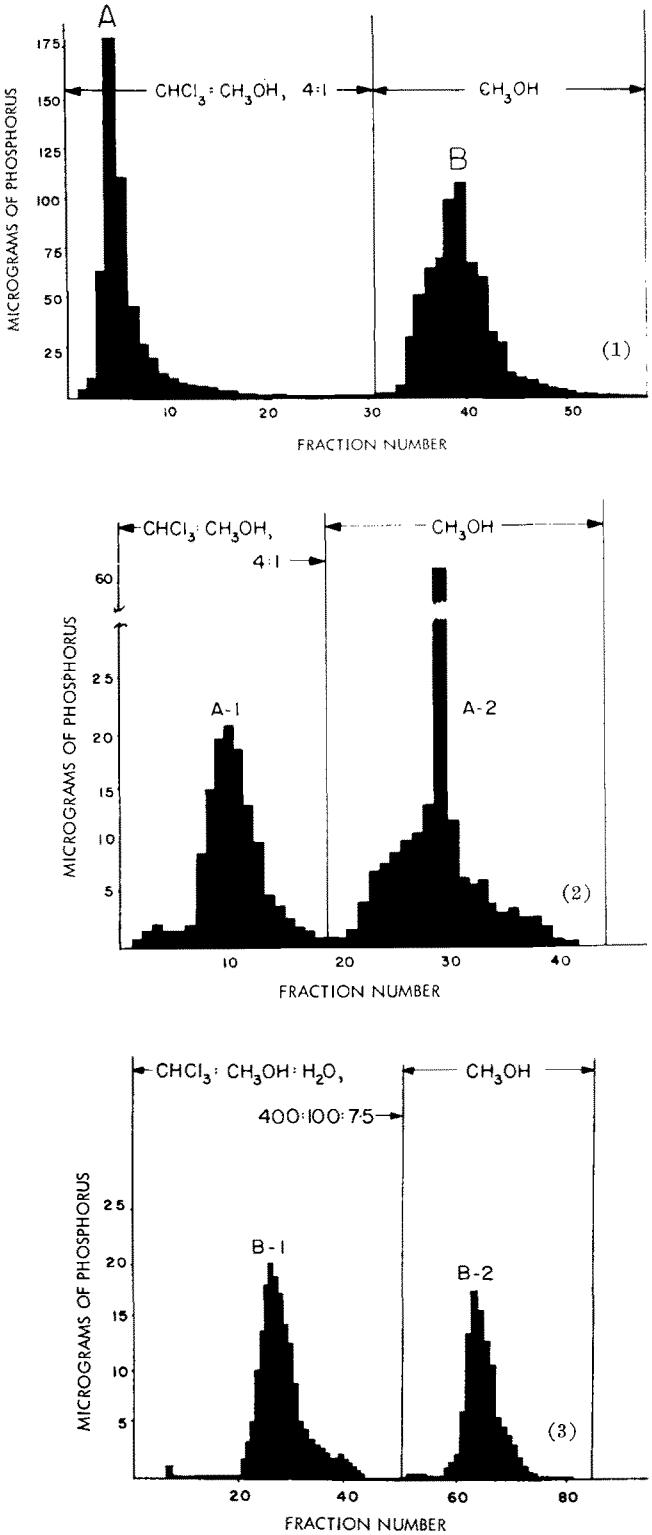

FIG. 2. Fractionation of red cell phospholipids by silicic acid column chromatography. 1) First stage: fraction A includes the amino-phospholipids, and fraction B, the choline-containing phospholipids. 2) Second stage: fraction A-1 represents phosphatidyl ethanolamine, and A-2, phosphatidyl serine. 3) Third stage: fraction B-1 represents lecithin, and B-2, sphingomyelin.

The fatty acids were determined by gas chromatography of the methyl esters in an Aerograph Model 600B gas chromatograph, equipped with a hydrogen flame ionization detector. The stainless steel column (5ft x 1/8 in. OD) was packed with firebrick 60/80 mesh, coated with 20% (w/w) diethylene glycol succinate, and all runs were made at temp between 190 and 205C. The phosphoglyceride fatty acids were converted to their methyl esters by treating the intact lipid with 5% methanolic HCl in sealed ampoules at 90C for 2 hr (19). Sphingomyelin fatty acids were methylated by refluxing in 10% methanolic sulphuric acid for 6 hr (20). In all cases the methyl esters were recovered from the reaction mixtures by extraction with petroleum ether.

The percentage composition by wt was obtained by measuring the areas of the individual ester peaks, and relating them to the total area recorded for all the peaks. This was shown to be a true measure of fatty acid concn by calibrating the detector with standard mixtures C and F provided by the National Institutes of Health, Metabolism Study Section, Bethesda, Md. For ease of comparison, the primary data were converted to mole percentages by assigning appropriate mol wt to all components. Any error arising from this conversion was assumed to be small because the readily identifiable fatty acids made up 90% of the total.

Results and Discussion

Fractionation of Red Blood Cell Phospholipids

Typical elution patterns in the three-stage procedure for the fractionation of red cell phospholipids are illustrated in Figure 2, and the purity of the four end-products is indicated by the data given below.

The principal characteristics of the four fractions were as follows:

Fraction A-1. On TLC, material from this peak ran as a single spot with the staining properties of an amino-phospholipid, and the mobility of phosphatidyl ethanolamine. Chromatography of hydrolysis products on paper and ion exchange columns revealed the presence of ethanolamine and the absence of serine. The M ratio of phosphorus to ethanolamine was 1.00:0.91.

Fraction A-2. TLC of this fraction also revealed the presence of material running as a single spot, and with the staining properties of an amino-phospholipid. The mobility was the same as that of phosphatidyl serine. Serine, but no ethanolamine was found by chromatography of the products of hydrolysis on paper and ion exchange columns. The M ratio of phosphorus to serine was 1.00:0.92.

Fraction B-1. Chromatography of material from this fraction, on both "neutral" and "basic" chromatoplates, showed that it ran as a well-defined spot with the staining characteristics of a choline-containing phospholipid and the mobility of lecithin. The M ratio of phosphorus to choline was 1.00:0.90.

Fraction B-2. In both TLC systems, this fraction ran as a single spot with the staining properties of a choline-containing phospholipid, and the mobility of sphingomyelin. The M ratio of phosphorus to choline was 1.00:1.05.

On the basis of these results, it was concluded that Fractions A-1, A-2, B-1 and B-2 represented phosphatidyl ethanolamine, phosphatidyl serine, lecithin and sphingomyelin, respectively.

Effect of Diet on the Individual Phospholipid Classes

Twenty samples of cells from the three diets were carried through the analytical procedure outlined in Figure 1, and the results of the analyses are summarized in Table I. The total lipid phosphorus content average 12.6 ± 0.4 mg/100 ml of red cells. Lecithin was the predominant phospholipid and accounted for 34% of the total on all three diets, while sphingomyelin, phosphatidyl ethanolamine and phosphatidyl serine represented 25%, 25% and 16%, respectively. When these data are combined with the results of the fatty acid analyses (Table IV), the calculated total phospholipid content averages 315 ± 10 mg/100 ml of red cells.

The small standard deviations recorded indicate the uniformity of composition of red cell phospholipids, and suggest that the levels of the individual phospholipids remain relatively constant despite marked changes in the type of dietary fat supplied. The data are in good agreement with those recently reported by Reed et al. (21), and Weed et al. (22) who performed their separations of filter paper impregnated with silicic acid, and by Farquhar (23), and Farquhar and Ahrens (5), who used silicic acid column chromatography. Comparable values for lecithin and sphingomyelin have also been obtained on adsorption chromatography by Phillips and Roome (24) and Balint et al. (25), although neither of these groups was able to achieve satisfactory resolution of phosphatidyl ethanolamine and phosphatidyl serine. Dawson et al. (26) and de Gier and Van Deenen (27) have reported somewhat different values, but differences in methodology make direct comparison difficult.

Table II lists the average mol wt of the four major red cell phospholipids (based on fatty acid analyses, Table IV) from which the theoretical phosphorus content has been computed as a percentage of the total wt of the phospholipid. The phosphorus amt to almost exactly 4% of the total wt of the phospholipid, confirming the validity of the conversion factor 25 in changing lipid phosphorus to wt of phospholipid, at least in so far as the red cell phospholipids are concerned.

Table III summarizes the data on the red cell plasmalogens. Irrespective of dietary history, ca. 36% of the phosphatidyl ethanolamine, 4% of the phosphatidyl serine and 6% of the lecithin fraction was present in the plasmalogen form. The corresponding values of 67%, 8% and 10%, quoted by Farquhar (23), are considerably higher. Of the total red cell phospholipid, 14% was in the plasmalogen form, while previous estimates have ranged from trace (10) to 22% (23). The discrepancies between these reports are probably more indicative of different analytical methods rather than any true variation.

TABLE I

Phospholipid Content of Red Blood Cells

Dietary regimen	Total lipid phosphorus mg/100 ml	Individual phospholipids as % of total			
		PE	PS	LEC	SPH
A[a]	12.4 ± 0.4[d]	25 ± 2	15 ± 2	34 ± 1	26 ± 3
BF[b]	12.7 ± 0.4	25 ± 2	17 ± 2	33 ± 2	24 ± 3
CO[c]	12.6 ± 0.4	24 ± 1	17 ± 1	34 ± 1	25 ± 2
Average	12.6 ± 0.4	25 ± 2	16 ± 2	34 ± 2	25 ± 3

[a] 10 subjects.
[b] 5 subjects.
[c] 5 subjects.
[d] Mean ± standard deviation.

Note abbreviations used in Tables I–IV: PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; LEC, lecithin; SPH, sphingomyelin; PL, phospholipid. Except where otherwise stated, terms phosphatidyl ethanolamine, phosphatidyl serine and lecithin are used to include both the diester and the plasmalogen forms of the corresponding phosphoglycerides.

TABLE II

Red Blood Cell Phospholipids: Average Mol Wt, Phosphorus Content and concn

Component	Average mol wt	Percentage phosphorus	Concn in red cells	
			mg/100 ml	moles × 10^4/100 ml
PE	719	4.31	72	1.00
PS	783	3.96	46	0.58
LEC	781	3.97	108	1.39
SPH	768	4.04	82	1.07
Total PL	770	4.03	315	4.04

Effect of Diet on the Phospholipid Fatty Acid Composition

The results of the fatty acid analyses are summarized in Table IV. The small standard deviation observed for all the fatty acids indicate the minor nature of the differences between individual subjects, and a rather uniform response to a dietary stress. Further, it is apparent that each phospholipid family has a distinctive fatty acid spectrum, which may be modified by dietary fatty acids to a limited extent only.

Both of the cephalins contain large amt of arachidonic acid, but can be differentiated on the basis of the high palmitic acid content in the ethanolamine, and high stearic acid content in the phosphatidyl serine fraction. These differences are interesting in view of the suggestion (28) that the ethanolamine phosphatide arises from the decarboxylation of phosphatidyl serine. The fatty acids of lecithin present another pattern: although somewhat similar to the fatty acids of phosphatidyl ethanolamine, they are easily distinguished because of the large differences in the amt of arachidonic and linoleic acids. The M ratio of saturated to unsaturated fatty acids in the three phosphoglycerides was very nearly 1:1, indicating that there was available for each phosphoglyceride molecule one saturated and one unsaturated acid. The sphingomyelin fatty acids differ radically from those of the other phospholipids, in that no other fraction had such a high proportion of saturated acids (M ratio of saturated to unsaturated fatty acids of 3:1), or of fatty acids of chain length in excess of 20 carbons.

It is of interest to compare these results with those presented by the two other investigators who have studied the fatty acids of the individual phospholipids of the red cells of man. Farquhar (23) and Farquhar and Ahrens (5) analyzed a pooled sample of red cells from three normal adults, and obtained results for phosphatidyl serine, lecithin and sphingomyelin fatty acids similar to the average values reported here.

TABLE III

Red Blood Cell Plasmalogens

Phospholipid	Percentage plasmalogen		
	Diet A[a]	Diet BF[b]	Diet CO[c]
PE	34 ± 9[d]	37 ± 6	36 ± 5
PS	5 ± 3	4 ± 3	3 ± 3
LEC	6 ± 2	7 ± 1	6 ± 4

[a] 10 subjects.
[b] 5 subjects.
[c] 5 subjects.
[d] Mean ± standard deviation.

TABLE IV

Red Blood Cell Phospholipid Fatty Acids Following Different Dietary Regimens (Expressed in mole %)

Fatty acid	Diet A				Diet BF[a]				Diet CO[b]			
	PE	PS	LEC	SPH	PE	PS	LEC	SPH	PE	PS	LEC	SPH
16:0	29 ± 2[b]	14 ± 1	34 ± 1	28 ± 5	30 ± 1	15 ± 1	34 ± 1	29 ± 6	28 ± 1	14 ± 2	32 ± 1	30 ± 3
16:1	3 ± 1	2 ± 1	2 ± 1	3 ± 2	3 ± 1	3 ± 1	3 ± 1	2 ± 1	2 ± 1	1 ± 1	2 ± 1	1 ± 1
18:0	9 ± 1	36 ± 2	13 ± 1	7 ± 2	9 ± 1	38 ± 1	13 ± 1	6 ± 1	8 ± 1	37 ± 2	13 ± 1	7 ± 1
18:1	22 ± 2	15 ± 1	22 ± 1	6 ± 2	22 ± 2	15 ± 1	25 ± 1	5 ± 1	21 ± 1	14 ± 1	17 ± 1	4 ± 1
18:2	6 ± 1	7 ± 1	18 ± 2	2 ± 1	5 ± 1	4 ± 1	12 ± 1	2 ± 1	7 ± 1	9 ± 1	25 ± 3	2 ± 1
20:4	18 ± 1	21 ± 1	6 ± 1	.....[c]	19 ± 1	20 ± 1	5 ± 1	.....	18 ± 1	21 ± 1	7 ± 1	.....
20:0	.....	.....	.....	2 ± 1	.....	.....	.....	2 ± 1	.....	.....	.....	2 ± 1
22:0	.....	.....	.....	8 ± 2	.....	.....	.....	9 ± 1	.....	.....	.....	8 ± 1
24:0	.....	.....	.....	20 ± 7	.....	.....	.....	18 ± 4	.....	.....	.....	21 ± 4
24:1	.....	.....	.....	14 ± 3	.....	.....	.....	13 ± 3	.....	.....	.....	16 ± 3
d	51/49	55/45	52/48	75/25								

[a] Principal fatty acids; 16:0, 13%; 18:0, 2%; 18:1, 28%; 18:2, 55%.
[b] Principal fatty acids; under 16:0, 32%; 16:0, 23%; 18:0, 10%; 18:1, 29%; 18:2, 1%.
[c] Under 1%.
[d] Ratio saturated to unsaturated fatty acid.
[e] Mean ± standard deviation.

Farquhar's data (23) for phosphatidyl ethanolamine fatty acids differ slightly, presumably because his values were corrected for the presence in the methyl ester mixture of dimethyl acetals arising from the phosphatidal ethanolamine. Hanahan et al. (10) also investigated red cell lecithin fatty acids, and reported values almost identical to the average values reported here. Their values apparently represent the average (or composite) values of at least six different samples of blood, and the deviations between samples were said, in general, not to exceed ±10%. In the sphingomyelin fraction Hanahan et al. (10) detected substantial amt of palmitic, oleic and behenic acids, but no lignoceric or nervonic. In contrast, in the analyses reported here, and by Farquhar and Ahrens (5), fatty acids of chain length greater than 20 carbons account for over one-third of the sphingomyelin fatty acids. Although there is no obvious explanation for this discrepancy, it should be noted that these long-chain fatty acids have been known for many years to be a part of the sphingomyelin of spleen, lung and brain (29).

The samples obtained after the 16-day dietary periods reveal statistically significant differences ($p < 0.02$) in three instances. When compared with the subjects consuming the diet with the high butter fat content, the subjects on the diet rich in corn oil showed higher levels of linoleic acid in lecithin and phosphatidyl serine, and lower levels of oleic acid in lecithin. As previously suggested (3) such rapid changes are probably the result of exchange of fatty acid between plasma and red cells, rather than of maturation and release of a new generation of red cells. Because of the relatively small amt of linoleic acid in phosphatidyl serine it is not possible to determine whether the increase has been at the expense of a specific acid. However, the situation is quite different in the lecithin fraction, which contains over 90% of the newly incorporated linoleic acid, and where the increase in this acid has been largely compensated for by a decrease in oleic acid.

These observations may be summarized as follows. First, the changes in fatty acid distribution were largely confined to a single family of phospholipids, the lecithins, and no major changes occurred in the sphingomyelin or cephalins. Second, the fatty acid exchange involved unsaturated fatty acids almost exclusively: the increased incorporation of unsaturated fatty acid was at the expense of unsaturated fatty acid already present, and did not alter the amt of saturated fatty acid. Third, within the group of unsaturated fatty acids, a specific pair was involved: linoleic replaced oleic, but not arachidonic or palmitoleic acids. Hanahan et al. (10) have reported that red cell lecithins usually have a saturated fatty acid in the α-position, and an unsaturated fatty acid in the β-position, so that unless abnormal red cells were produced during this dietary experiment(which is unlikely) the observed exchange is assumed to have taken place at the latter position.

The high degree of specificity implied by these observations is supported by the data of others. Mulder et al. (4), working with rabbits, and Farquhar and Ahrens (5), working with a single human subject, found that diet induced changes in red cell fatty acids were greatest in the lecithin fraction, although lesser changes were observed in other phospholipids as well. Oliveira and Vaughan (30) incubated red cells with labelled fatty acids, in the presence of ATP and coenzyme A, and recovered most of the label in the lecithin, very small amt in the cephalins, and none in the sphingomyelin. Under similar conditions, they found that sheep red cells, which in common with the red cells of other ruminants contain very little lecithin, incorporated almost no labelled fatty acid. On the other hand, rat red cells, which contain more lecithin than do the red cells of man, incorporated the highest amt of label. That this type of specificity is not peculiar to erythrocytes is suggested by Leat (31), who noted that the plasma lecithin of a pig incorporated almost twice as much linoleic acid as did plasma cephalins and sphingomyelin. It appears well established, therefore, that lecithin fatty acids are more susceptible to diet-induced alterations than are the fatty acids of other phospholipids, although the reasons for this susceptibility are not yet known.

In contrast to the rapidity with which the appropriate modification in diet can lead to an increase in red cell linoleic acid, it is apparently difficult to increase the red cell content of certain other fatty acids. Mulder et al. (4) found that a diet rich in lauric acid failed to increase the red cell content of that acid, despite the fact that the lauric acid level of plasma increased substantially. Similarly, Farquhar and Ahrens (5) reported that a diet rich in linolenic acid increased the red cell content of that acid only from 0.1–1.5%, while the adipose tissue level increased from 0.2–17%. Obviously, there are limits to the type and extent of the exchanges that can be induced by diet in the red cells, suggesting that factors other than the relative abundance of any component in the diet, plasma or adipose tissue govern the fatty acid composition of the red cell phospholipids.

ACKNOWLEDGMENT

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REFERENCES

1. Hill, J. G., A. Kuksis and J. M. R. Beveridge, JAOCS *41*, 393 (1964).
2. Horwitt, M. K., C. C. Harvey and B. Century, Science *130*, 917 (1955).

3. Leibetseder, F., and E. H. Ahrens, Jr., Brit. J .Haemat. *5*, 356 (1959).
4. Mulder, E., J. de Gier and L. L. M. van Deenen, Biochim. Biophys. Acta *70*, 94 (1963).
5. Farquhar, J. W., and E. H. Ahrens, Jr., J. Clin. Invest. *42*, 675 (1963).
6. Notham, M. M., and S. Proger, J. Am. Med. Assoc. *179*, 40 (1962).
7. Hurst, R. O., Can. J. Biochem. Physiol. *36*, 1251 (1958).
8. Rouser, G., A. J. Bauman, G. Kritchevsky, D. Heller and J. S. O'Brien, JAOCS *38*, 544 (1961).
9. Rouser, G., J. S. O'Brien and D. Heller, *Ibid. 38*, 1 (1961).
10. Hanahan, D. J., R. M. Watts and P. Pappajohn, J. Lipid Res. *1*, 421 (1960).
11. Nakayama, F., and C. G. Johnson, J. Lab. Clin. Med. *59*, 364 (1962).
12. Rhodes, G. M., and R. M. C. Dawson, "Chromatographic and Electrophoretic Technique," Ivan Smith, ed., Interscience Publishers, New York, 1958.
13. Cornatzer, W. E., W. A. Sandstrom and J. H. Reiter, Biochim. Biophys. Acta *57*, 568 (1962).
14. Skipski, V. P., R. F. Peterson and M. Barclay, J. Lipid Res. *3*, 467 (1962).
15. Block, R. J., E. L. Durrum and G. Zweig, "Paper Chromatography and Paper Electrophoresis," Academic Press, Inc., New York, 1958.
16. Slotta, K. H., and J. K. Powers, Anal. Biochem. *4*, 69 (1962).
17. Bottcher, C. J. F., C. Pries and C. M. van Gent, Rec trav. chim. *80*, 1169 (1961).
18. Williams, J. N., C. E. Anderson and A. D. Jasik, J. Lipid Res. *3*, 378 (1962).
19. Farquhar, J. W., *Ibid. 3*, 31 (1962).
20. Nelson, G. J., *Ibid. 3*, 71 (1962).
21. Reed, C. F., S. N. Swisher, G. V. Marinetti and E. G. Eden, J. Lab. Clin. Med. *56*, 281 (1960).
22. Weed, R. I., C. F. Reed and G. Berg, J. Clin. Invest. *42*, 581 (1963).
23. Farquhar, J. W., Biochim. Biophys. Acta *60*, 80 (1962).
24. Phillips, G. B., and N. S. Roome, Proc. Soc. Exptl. Biol. Med. *109*, 360 (1962)
25. Balint, J. A., H. L. Spitzer and E. C. Kyriakides, J. Clin. Invest. *42*, 1661 (1963).
26. Dawson, R. M. C., N. Hemington and D. B. Lindsay, Biochem. J. *77*, 226 (1960).
27. de Gier, J., and L. L. M. van Deenen, Biochim. Biophys. Acta *49*, 286 (1961).
28. Borkenhagen, L. F., E. P. Kennedy and L. Fielding, J. Biol. Chem. *236 PC*, 28 (1961).
29. Thannhauser, S. J., and N. F. Boncoddo, *Ibid. 172*, 141 (1948).
30. Oliveira, M. M., and M. Vaughan, Fed. Proc. *21*, 296 (1962).
31. Leat, W. M. F. Biochem. J. *84*, 85P (1962).

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Fatty Acid Composition Changes in Meat Lipids During Low Temperature Storage[1]

C. K. HUSTON,[2] J. D. SINK, R. C. MILLER and J. W. SHIGLEY, Departments of Animal Industry and Nutrition and Biochemistry, The Pennsylvania State University, University Park, Pennsylvania

Abstract

The effect of low-temp storage on the fatty acid composition of meat lipids was studied. Fat samples were taken from the perirenal and subcutaneous depots of 12 beef carcasses. The samples were divided and one-half from each carcass were stored at −37C for four months. The fat samples (before and after the low-temp storage period) were then extracted, the glyceride fractions separated by low-temp solvent crystallization, and subsequently analyzed by gas-liquid chromatographic techniques. The data obtained in this study and the changes in fatty acid composition noted were not consistent with the pattern of oxidative deterioration but would appear to indicate that non-oxidative mechanisms were responsible for the changes in fatty acid composition observed.

Introduction

THE OXIDATIVE DECOMPOSITION PRODUCTS of meat lipids are prime contributors to the development of undesirable odor and flavor characteristics of meat and meat products (1), and their possible hazard to human health is of current interest (2).

When fats take up oxygen, rancid or off-flavor components are formed. This oxygen uptake and the onset of oxidative deterioration have been shown to be related to the unsaturation of the fat although great variations in natural fats, due to the presence of antioxidants, have been observed (3,4). In an extensive review, Lundberg (5) reported that the oxidative deterioration of food lipids involves autoxidation reactions affecting, primarily, the unsaturated acyl groups. The rate of autoxidation increases markedly with time, and in an exponential manner, with increasing unsaturation.

This problem of flavor changes related to meat lipids has been the subject of extensive research (4,6). Numerous research studies on frozen meats have emphasized the importance of low-temp in retarding rancidity (7–9). Hiner et al. (10) found deterioration in beef and lamb as well as pork in freezer storage to be due primarily to oxidation of the fat. Species differences in susceptibility to oxidation have been observed and have been attributed to the differences in fatty acid composition (11).

It was the purpose of this research to investigate, qualitatively and quantitatively by means of gas-liquid chromatography, the fatty acid composition changes in meat lipids during low temperature storage.

Experimental Procedure

Sampling, Extraction and Storage. Samples of the perirenal and subcutaneous depot fats were taken from the carcasses of 12 steers after the animals had been slaughtered and chilled for 24 hr. Four hundred-g samples were taken from the tip of the kidney knob fat and from the subcutaneous backfat at the 10–13 rib area of each carcass. These samples were stored (less than 24 hr) in a nitrogen atmosphere at −37C in labelled, paraffin-sealed, screw-cap jars until time for analysis. One-half of the samples from each carcass were stored at the same temp for a period of four months.

Eighty-g portions of the fat were cut into small pieces and extracted three times in a Waring blendor with a total of 500 ml of diethyl ether. The ether solution was filtered and dried over anhydrous sodium sulfate. The solvent was removed from the extract by means of a Rinco Evaporator, and the extract was stored in a nitrogen atmosphere. As a check on the completeness of extraction before and after freezer

[1] Journal Series of the Pennsylvania Agricultural Experiment Station, Paper No. 2900.
[2] Present address: U.S. Army Biological Laboratories, Frederick, Md.