牛の赤血球膜リン脂質及び構成脂肪酸の季節変化

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Seasonal Changes in the Phospholipids and Fatty Acid Composition of the Bovine Erythrocyte Membrane

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Phospholipids are the principal components of the lipid bilayer membrane [12]. These fatty acid composition is an important factor which affects membrane fluidity [3]. The proportion of unsaturated fatty acids of phospholipids and the phosphatidyletanolamine (PE) increases under environmental low temperatures in the biomembranes of fish to maintain the constant membrane fluidity [7, 11].

Even in homoiothermic animals, biomembrane lipid composition may change due to the changes of environmental temperature. The present study was done to clarify whether there is a seasonal change in phospholipid composition and/or fatty acid composition of bovine erythrocyte membrane. If there is a change, it should influence the erythrocyte osmotic fragility.

To the above aim, ten clinically healthy cows lactating were examined at March 3 and July 31, 1986. The averages of the daily highest and lowest temperatures in these months were -1.5 ± 2.0°C and -5.7 ± 1.7°C; 27.7 ± 4.5°C and 15.5 ± 2.0°C, respectively.

The blood was bled from the juglar vein and pored into tubes containing dipotassium salt of EDTA at a concentration 1 mg/m*l*. After centrifugation at 2,000 g, 4°C for 20 minutes, the erythrocytes were washed 7 times with physiologic saline. The packed cells were used for extraction of phospholipids and fatty acid analysis. Erythrocyte lipids were extracted with isopropyl alcohol and chloroform (7:11, v/v) according to the method of Broekhuyse [2]. Phospholipids were separated on a silica gel-coated thin layer rod (CHROMAROD-S III, Dia-iatoron Co., Ltd. Tokyo) by developing it with chloroform/methanol/water (60:35:4, v/v/v) and each separated phospholipid content was quantified by a flame-ionizing detecter (Thinchrography TH-10, Same company) [1].

Fatty acid composition of phospholipids and glycosphingo-lipids (complex lipids) was analyzed by Japanese Special Analysis Research Institute. Neutral lipids were separated by Kiesel Gel 60 coated thin-layer plate (Merck, A. G., Darmstadt Germany) by developing a solvent system of n-hexane-diethyl ether-acetic acid (90/10/0.025, v/v/v). The original position containing complex lipids were scraped and followed by methanolysis with 10% BF_3/methanol (Wako Pure Chemical Industries, Ltd. Osaka) at 80°C for 90 min. Fatty acid methyl esters were extracted with n-hexane and analyzed at 270°C oven temperature by a gas chromatograph apparatus of MODEL 263 (Hitachi, Ltd. Tokyo.) equipped with a 3 mm × 3 m glass column packed with 10% Silar 10C on Chromosorb W, HP (100~120 mesh). A mixture of authentic fatty acid methyl esters (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was used for identification of each component by its retention time. Osmotic fragility was also measured using the coil-planet centrifuge method (CPC) [5].

Sphingomyelin (SM) and phosphatidylethanolamine (PE) were major components of phospholipids in bovine erythrocyte, and they composed of approximately 90% phospholipids. In comparison between winter and summer, SM content was significantly higher and PE content was lower in winter (Table 1). Our results in summer were almost identical with those described by Takahashi, *et al.* [10] and Nelson, *et al.* [8].

In summer, lignoceric acid (C24:0), palmitic acid (C16:0) and oleic acid (C18:1) were detected as three major components of the complex lipid fraction in the order of more abundant content, whereas in winter the order was changed to C18:1, C24:0 and C16:0, respectively (Fig. 1, Table 2). A ratio of unsaturated fatty acids to saturated fatty acids were significantly higher in winter, however, polyunsaturated fatty acids contents showed no significant difference. Our results in summer and winter were similar to the

Table 1. Seasonal changes in erythrocyte membrane phospholipids in cows

Phospholipid		Summer	Winter
Phosphatidylethanolamine	(%)	32.1±3.2[a)]	22.9±2.2***[b)]
Phosphatidylserine + Phosphatidylinositol	(%)	8.5±3.0	7.5±1.0
Phosphatidylcholine	(%)	3.2±2.2	1.8±1.1
Sphingomyelin	(%)	56.0±5.4	67.3±3.6***

a) Mean and standard deviation of each phospholipid content(%) conputed from 10 different cow samples.
b) Significantly different between the levels in 2 groups at $p<0.001$.

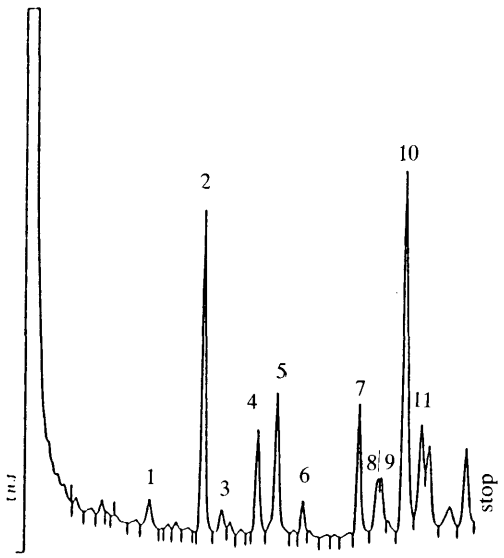

Fig. 1. Analysis of fatty acid methyl esters derived from cattle erythrocyte membrane lipids by gas-liquid chromatography. Each fatty acid of 1: $C_{14:0}$,2: $C_{16:0}$, 3: $C_{16:1}$, 4: $C_{18:0}$, 5: $C_{18:1}$, 6: $C_{18:2}$, 7: $C_{22:0}$, 8: $C_{20:3}\omega 6$, 10: $C_{24:0}$ and 11:$C_{24:1}$ was indentified from its retention time determined by authentic fatty acid mixture.

Table 2. Seasonal changes in fatty acid fractions of the erythrocyte membrane complex lipids in cows

Fatty acid	Summer	Winter
C14:0	3.0±0.79[a)]	2.8±0.55
C16:0	18.7±2.51	17.3±2.03
C16:1	1.8±0.43	3.0±1.09**[c)]
C18:0	6.1±0.81	5.6±0.49
C18:1	13.2±4.17	30.3±3.84**
C18:2	8.3±3.76	7.6±2.79
C20:3	5.3±2.08	3.4±0.29*[b)]
C20:4	1.8±0.86	1.9±1.14
C22:0	7.0±1.17	4.7±0.76**
C24:0	24.4±3.66	18.5±2.71**
C24:1	10.4±1.98	5.0±0.97**
Saturated	59.2±4.53	48.8±3.96**
Unsaturated	40.8±4.53	51.2±3.96**
Polyunsaturated	15.4±2.66	12.9±2.98

a) Mean and standard deviation of each fatty acid content(%) conputed from 10 different cow samples.
b) Significantly different between the levels in 2 groups at $p<0.05$.
c) Significantly different between the levels in 2 groups at $p<0.01$.

results in summer and winter were similar to the data published by Gillis, *et al.* [4]. A significant correlation was observed between erythrocyte membrane PE content and polyunsaturated fatty acid content ($n=20$, $r=0.558$, $P<0.05$), or saturated fatty acid content ($n=20$, $r=0.637$, $P<0.01$), indicating that the changes of polyunsaturated and saturated fatty acid contents were derived from the change of PE content.

In the osmotic fragility test, hemolysis-starting point (HSP) was significantly lower in winter (135.9±7.9 mOsm) than in summer (156.5±11.4 mOsm). And hemolysis-maximum point (HMP) shifted to the hypotonic side in winter (Table 3).

The change of environmental temperature has been considered so far to not so much effect on cell membrane lipid components in mammals, because their body temperature is always constant in mammals. Nevertheless, the results of the present study demonstrated that there are seasonal changes in erythrocyte membrane phospholipids, fatty acid composition and erythrocyte fragility. These results suggested that even

Table 3. Seasonal changes in the osmotic fragility of erythrocytes in cows measured by the CPC

Item		Summer	Winter
HSP	mOsM	156.5±11.4[a)]	135.9±7.9***[c)]
HMP	:	117.2±7.8	108.2±8.5*[b)]
HEP	:	84.9±9.7	87.4±9.7
HW	:	71.7±9.7	48.6±11.0***

Abbreviations were use as follows: CPC, Coilplanet centrifge method; HSP, Hemolysis starting point; HMP, Hemolysis maximum point; HEP, Hemolysis end point; HW, Hemolysis width.

a) Mean and standard deviation of each hemolysis point (mOsM) conputed from 10 different cow samples.

b) Significantly different between the levels in 2 groups at $p<0.001$.

homoiothermic animals maintain a constant membrane fluidity by modulating cell lipid composition depending on the environmental temperature.

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要　　約

牛の赤血球膜リン脂質及び構成脂肪酸の季節変化（短報）：左向敏紀・武田信一・渋谷正行[1)]・小山秀一・内野富弥・本好茂一（日本獣医畜産大学獣医内科学教室，[1)]多摩老人病研究所）——牛において赤血球膜リン脂質分画，構成脂肪酸の冬期および夏期の季節変化を測定した．リン脂質では，Sphingomyelin（SM），Phosphatidylethanolamine（PE）が主成分で，SM と PE の和は，夏期，冬期とも約90%をしめ，冬期に SM が有意な高値，PE が低値であった．また，構成脂肪酸分画は，冬期に飽和脂肪酸の低値，不飽和脂肪酸の高値が認められた．牛の赤血球膜脂質も変温動物同様環境温度の変化により，その構成比に変化が起こると考えられた．