CHANGES IN THE MILK FAT GLOBULE MEMBRANE AND THEIR RELATION TO LIPASE ACTIVITY

By

GEORGE C. HSIEH

Bachelor of Science

Taiwan Provincial Chung-Hsing University

Taichung, Taiwan, Republic of China

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Thesis Approved:

J. Brickley
Thesis Adviser

Milton Wells

D. D. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

The milk fat globule membrane, is composed of phospholipids and protein, which act as an emulsifier between milk fat and the milk plasma. It also has been considered to be a natural barier which protects the fat from outside forces. Not until the membrane has been ruptured, can lipase or other enzymes in the milk plasma attack the fat. The lipase activity of milk may vary both within and among cows. This variability might be due to changes in the substrate, making it more easily available to the enzyme at some times than it is at others. Such a change in the substrate could possibly be due to a rupturing of the milk fat globule membrane or to a lack of sufficient membrane material to completely cover all the fat globules. The purpose of this work was to test this theory by determining whether any statistical relationship existed between the lipase activity of milk and the amount of milk fat globule membrane protein in the milk.

CHAPTER II

LITERATURE REVIEW

According to King (10), Ascherson in 1840 showed that milk fat globules were covered by a thin membrane, which acted as an emulsifier between milk fat and the aqueous phase (milk plasma). Accumulated studies in recent years have shown that the components of this membrane are: (a) membrane protein, which is a globulin-like, but different from any other known milk protein (4); (b) phospholipids; mainly lecithin, cephalin and sphingomylein (12); and (c) lipoprotein. The lipids occuring in lipoproteins were fatty acids, phospholipids, high-melting glycerides, or cholesterol and its esters (10). Several hypotheses of membrane structure have been proposed. The most detailed was published by King in 1955 (10). He depicted the membrane structure as a surface layer of polar phospholipids with other less polar lipids (cholesterol, high-melting triglycerides, etc.) extending into the peripheral area of the fat droplet. Attached to the polar phospholipid heads were proteins that extended into the plasma.

In order to isolate the membrane, fat globules have to be "washed" with water then the fat extracted with solvents, or by churning. Washing has generally been carried out in one of two ways: (a) by allowing the fat globules to rise through a water column, or (b) by repeated dilution of the cream with water, followed by centrifugal separation. Method (a) has been criticized because the ascending fat globules would

form clusters, entrapping a part of the plasma, which would thus escape the washing action (10). The second method has been widely used. Jenness and Palmer in 1945 (8) separated cream from fresh milk at 40°C, with four volumes of water. Brunner and Herald in 1957 (5) separated cream at 46°C and standardized it to 30% fat, then washed it six times at 40°C with three volumes of water. Alexander and Lusena in 1961 (1) separated the cream from fresh raw milk (not longer than three hours after milking) at a temperature higher than 30°C and adjusted it to 50% fat before washing. Chien and Richardson in 1967 (6) separated the cream and washed it three times with distilled water. The mixture was stirred and transferred into centrifuge tubes to separate the cream and remove the washings. This process was repeated until the cream churned; the membrane materials measured in the washings were counted as the outer part of the membrane, and those from melted butter were counted as inner part.

Generally, the membrane protein is measured by evaporating the washed cream from which the fat is first extracted with solvents (5,10); by micro-Kjeldahl nitrogen-determination (3), or by dioxan (8). Different amounts of membrane protein have been reported varying from 0.026 to 0.86 g of protein per 100 g fat (6,7,8,14). King explained that the variations were due to the loosely-bound outer parts of the membrane being removed to different degrees during the various washing procedures. As evidence of this, Chien and Richardson (6) reported that about 27% of the membrane was "desorbed" after six washings and 54% after 12 washings.

In Jenness and Palmer's work (8), there seemed to be no relation between membrane protein content and breed. However, they suggested the

possibility of differences between individual cows. They further postulated that this difference might be due to differences in the size of fat globules. Thus, they expected any of the well-known conditions affecting fat globule size to influence the quantity of membrane materials. The fat globule membrane has been considered a natural barrier to lipid hydrolysis, but how it functions as a barrier, is still not known. Keeney et al. (9) in 1968 reported that the addition to milk of a small amount (0.005 to 0.100%) of phospholipid would greatly decrease the acid degree value of that milk. He theorized, however, that these phospholipids deactivated the lipase in the milk rather than forming a membrane which prevented lipase attack.

CHAPTER III

PROCEDURE

Representative samples (4-7 liters) of the complete afternoon milking were collected from Ayshire cow 488 at irregular intervals from March through July. This cow was in the latter half of her lactation. During the experiment she was healthy, fed the same ration; and housed under the same conditions as the other cows in the Oklahoma State University milking herd. When taking samples, 200 ml aliquots were cooled to 0-5°C in less than 5 minutes, then held at that temperature until analyzed for lipase activity 2 - 3 hours later. The remainder of the milk sample was separated into cream and skim milk with a DeLaval cream separator model 9; this machine was preadjusted in a manner so the fat content of the separated cream would be about 30-35%. The skim was discarded, and the separated cream was collected to be washed. The cream washing procedure followed the pattern of Jenness and Palmer (8). To the separated cream, four volumes of tap water (40-45°C) were added and the cream was reseparated. The water from the skim spout was discarded, the washed cream was collected from the cream spout and rediluted; this procedure was repeated six times. The washed cream was finally stored in the refrigerator (0-5°C) overnight.

The washed cream obtained from the above procedure was churned in a Waring blender. The butter was collected in a beaker, and the buttermilk was filtered through a Kendall 7" non-gauze milk filter disk to

remove any fat particles and then saved. The churned butter was melted on a steam bath, and the butter fat decanted as completely as possible.

The remaining fat in the butter plasma was extracted with 95% ethyl alcohol and an ether mixture (50% ethyl ether, and 50% petroleum ether), in a ratio of 1 : 1 : 4 of sample : alcohol : ether, respectively. This extraction procedure was repeated three times after which the butter fat was discarded. The remaining butter plasma was heated on a steam bath to evaporate the solvents, then homogenized through a "laboratory hand homogenizer"[1] three times to re-emulsify it. The fat-free plasma then was combined with the buttermilk from the original churning and the total volume recorded.

Total solids, total fat, and total protein were determined on both buttermilk and the original milk using Mojonnier (2), Babcock (11) and Kjeldahl (3) techniques, respectively. The nitrogen contents of the milk and buttermilk were converted into protein percentages by multiplying by 6.38 or 8.58, respectively; assuming 15.67% nitrogen in the milk protein (3) and 11.65% in the buttermilk (membrane) protein (16).

The procedure for determining milk lipase activity was first used by Pickett (13). In this procedure, 25 ml of skim milk (obtained by centrifuging the whole raw milk) were added to 25 ml of 0.2 M "Tham" buffer at pH 8.5 and 2 ml of tributyrin substrate.[2] Duplicate determinations were prepared for each milk sample. The lipase in one duplicate, marked "blank", was inactivated immediately by adding HCl to reduce the

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[1]Manufactured by Fisher Scientific Co.

[2]This substrate contained 15% tributyrin and 30% bovin albumin (Fraction V from Sigma Chemical Co.). The mixture was homogenized until a stable emulsion was formed.

pH to 2.0 or less. The other duplicate, marked "test," was incubated one hour at 37°C, after which it was also inactivated with HCl. The fatty acids in both determinations (blank and test) were extracted with ether, then titrated with KOH to a Thymolphthalein end-point. The results were corrected for the blank, then expressed as grams of butyric acid released per 100 ml of skim milk.

CHAPTER IV

RESULTS AND DISCUSSION

The data are shown in Table I and the amount of membrane protein per 100 g of milk fat[1] is graphed (Figure I) by sampling date. This graph shows large day-to-day variations in the amount of membrane protein in the milk of this cow. The range of this variation was 0.38-1.00 g membrane protein per 100 g milk fat. This range agrees reasonably well with the data of others (6, 8, 14) for example, the data of Jenness and Palmer had a range of 0.38-0.86 g per 100 g fat (8). Statistical analysis of the day-to-day variations in the present study showed that this variance was significantly larger ($P < 0.05$) than the variance of duplicate milk samples (Table I). Thus, in this animal much of the variance in the amount of milk fat globule membrane protein was caused by day-to-day variations within the cow. In the literature, variances of a similar magnitude in the amount of membrane protein were attributed to different isolation techniques.

The amount of membrane protein in the samples was compared to all measurements made on the milk. This was done in sets of two (membrane protein vs milk fat etc.) by means of scatter diagrams. The random distribution of dots in all but one of these diagrams indicated that there was no statistical relationship between milk lipase activity and

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[1] Calculated as: $\frac{\text{Protein in washings x g washings}}{\text{Milk fat x g milk sample}}$.

the pounds of milk produced, the percent fat, the percent of solids or the percent of protein in the milk.

There did seem to be a little relationship between lipase activity and the amount of membrane protein in the milk. These data are shown as a scatter diagram in Figure 2. The correlation coefficient for these two variables was 0.48 and the regression line calculated for the data was $Y = 0.11X + 0.16$, where Y = lipase activity and X = the amount of membrane protein. The lipase activity increased as the amount of membrane protein increased. Thus, cow 488 apparently always produced enough membrane material to coat all the fat globules. If she had not produced enough membrane material, one would have expected an increased lipase activity to be associated with decreased membrane protein contents after the point was reached where the fat globules were not all completely covered. These data also indicated that the lipase enzyme is at least partially associated with the membrane protein, which is in agreement with the findings of Tarassuk and Frankel (15) and others.

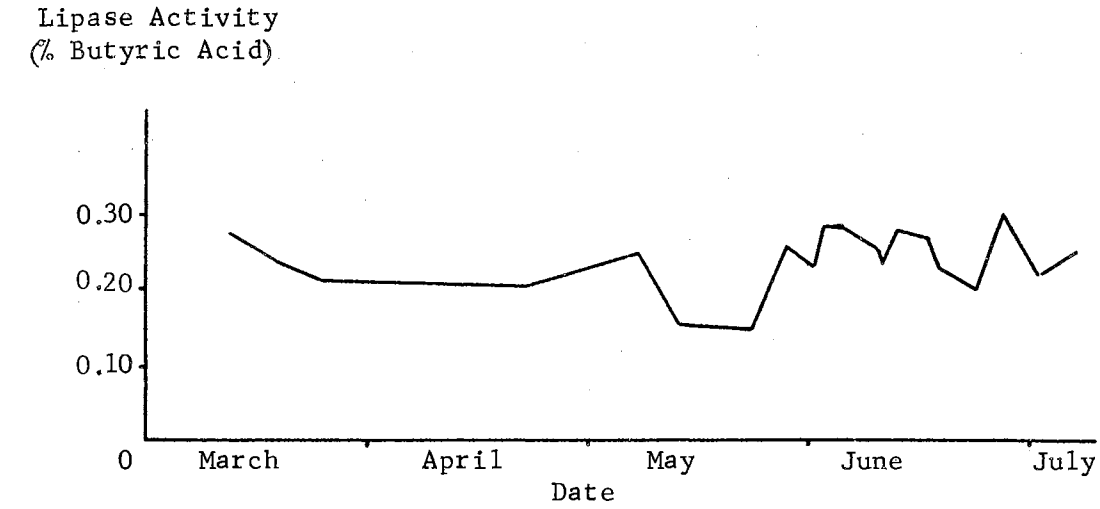
TABLE I

ANALYSIS OF MILK AND FAT GLOBULE WASHINGS
FROM COW 488

Sampling Date Mo-Day	Milk						Washings				Membrane
	Milk Produced lb.	Sample Volume Liters	Fat	T.S.	Protein	Lipase Activity	Volume Liters	Fat	T.S.	Protein	g Washed Protein per 100g Milk Fat
			--------%		--------(% Butyric Acid)			---------%		---------	
3-11	20.5	7.0	4.1	12.5	3.2	0.28	1.60	0.28	0.41	0.09	0.48
3-18	20.0	7.0	3.5	12.4	3.2	0.25	1.60	0.38	0.57	0.14	0.91
3-23	20.0	7.0	4.1	12.4	3.1	0.22	2.20	0.36	0.42	0.10	0.73
4-22	21.5	5.5	4.3	13.0	2.8	0.21	0.90	0.60	0.83	0.15	0.57
5- 7	20.0	5.5	4.8	13.7	3.2	0.26	1.60	0.35	0.43	0.10	0.55
5-13	15.0	5.5	4.4	12.9	3.1	0.16	1.90	0.24	0.25	0.05	0.40
5-23	16.0	5.5	4.4	13.3	3.5	0.15	0.80	0.40	0.49	0.12	0.38
5-28	15.0	5.5	3.8	12.7	3.5	0.26	1.00	0.34	0.44	0.13	0.58
6- 2	17.0	5.5	3.8	12.8	3.3	0.23	2.00	0.30	0.41	0.09	0.79
6- 3	20.0	5.5	3.9	12.8	3.3	0.29	2.05	0.32	0.39	0.08	0.74
6- 5	17.0	5.5	3.8	12.3	3.3	0.29	2.00	0.36	0.43	0.09	0.87
6-10	17.0	4.0	3.6	12.0	3.4	0.26	1.60	0.20	0.25	0.09	0.94
6-11	17.0	3.5	4.0	13.0	3.6	0.24	1.60	0.22	0.33	0.09	0.94
6-13	20.0	4.0	4.1	12.7	3.5	0.28	1.45	0.28	0.35	0.12	1.00
6-17	21.0	4.0	4.3	13.2	3.4	0.27	1.35	0.34	0.44	0.10	0.76
6-19	17.0	4.0	4.3	13.0	3.4	0.23	1.40	0.32	0.43	0.11	0.89
6-20	17.0	4.0	4.7	13.6	3.6	0.22	1.40	0.32	0.39	0.11	0.82
6-24	13.5	4.0	4.8	14.2	3.6	0.20	1.40	0.24	0.34	0.11	0.76
6-27	13.5	4.0	3.8	13.0	3.7	0.30	1.45	0.22	0.35	0.10	0.88
7- 2	15.0	4.0	3.3	12.3	3.6	0.22	1.42	0.30	0.34	0.08	0.82
7- 8	15.0	4.0	4.4	13.5	3.8	0.26	1.60	0.28	0.34	0.07	0.60

Table I - Continued

Sampling Date Mo-Day	Milk					Washings				Membrane
	Milk Produced lb.	Sample Volume Liters	Fat %	T.S. %	Protein %	Lipase Activity Butyric Acid	Volume Liters	Fat %	T.S. %	Protein %	g Washed Protein per 100g Milk Fat
Duplicate Determinations											
4-22-a		5.5	4.3	13.0	2.8		0.92	0.60	0.83	0.15	0.57
4-22-b		5.5	4.3	13.0	2.8		0.92	0.68	0.77	0.15	0.58
5- 7-a		5.5	4.8	13.7	3.2		1.60	0.36	0.42	0.10	0.58
5- 7-b		5.5	4.7	13.7	3.1		1.60	0.34	0.45	0.09	0.52
6- 6-a		5.5	3.7	11.6	3.2		1.40	0.34	0.42	0.09	0.61
6- 6-b		5.5	3.6	11.5	3.2		1.40	0.34	0.40	0.09	0.61
6-20-a		4.0	4.7	13.6	3.6		1.40	0.28	0.35	0.12	0.81
6-20-b		4.0	4.8	13.6	3.6		1.40	0.36	0.43	0.10	0.83
7- 8-a		4.0	3.0	12.2	3.8		1.60	0.36	0.35	0.06	0.75
7- 8-b		4.0	3.0	12.1	3.9		1.60	0.36	0.40	0.07	0.93



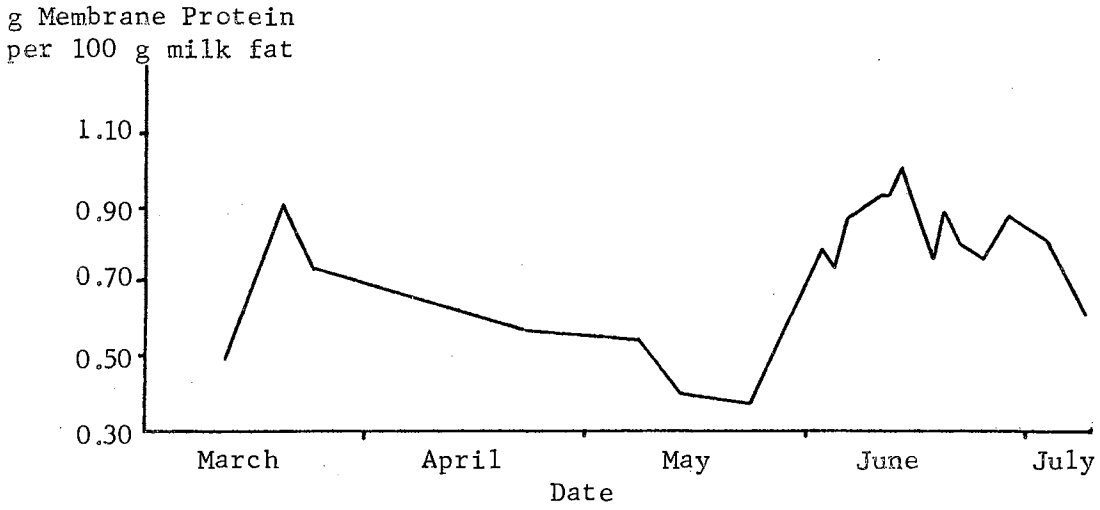


Figure 1. Changes in the Lipase Activity and Fat Globule Membrane Content in Milk of Cow 488 Over a Five Month Period of Time

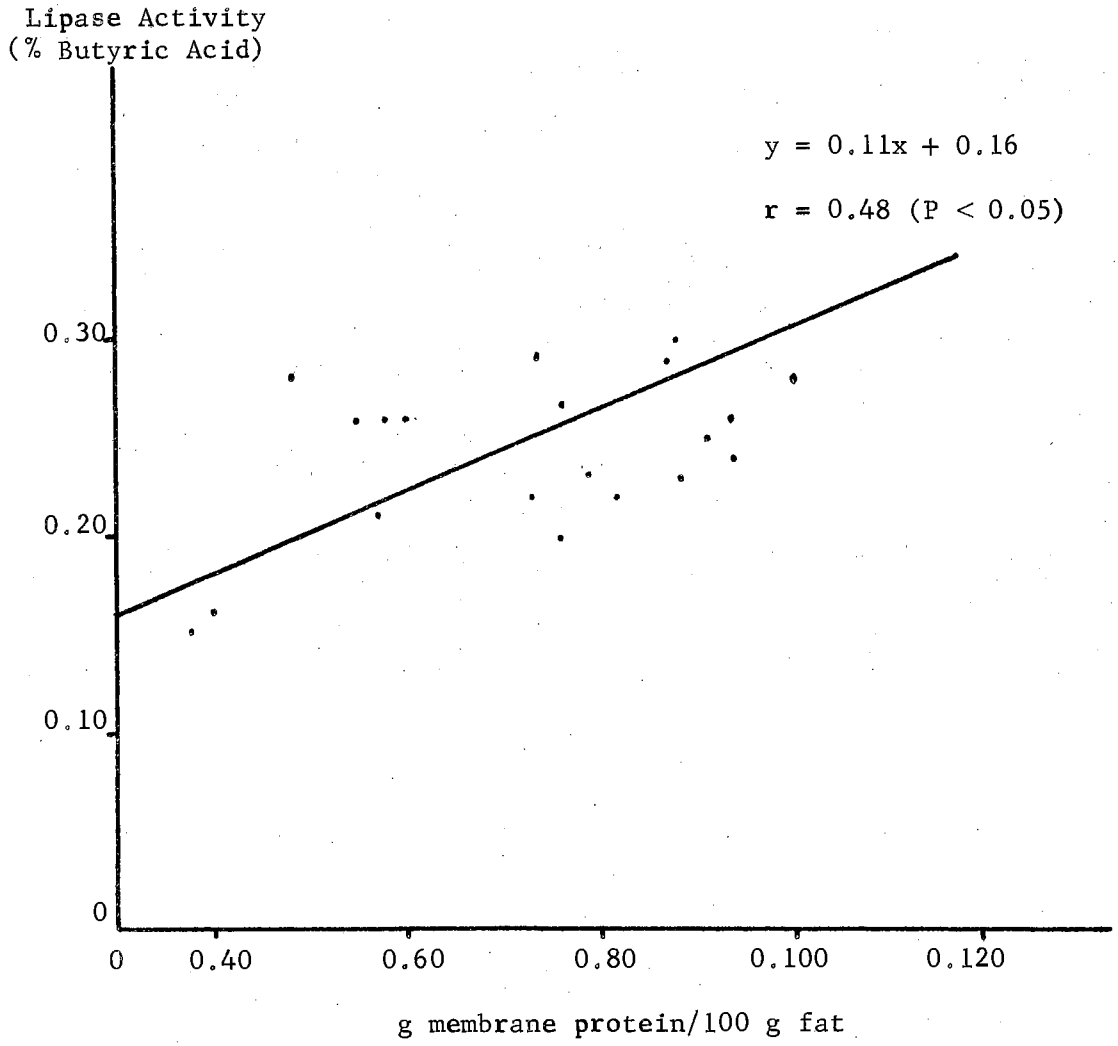


Figure 2. Scatter Diagram of Lipase Activity vs. Membrane Protein Content in Milk of Cow 488 Over a Five Month Period of Time

CHAPTER V

SUMMARY AND CONCLUSIONS

Samples from the afternoon milking of a single cow were collected periodically over a five month period. The cream separated from these milk samples was washed to collect the fat globule membrane. Fat, protein and total solids contents were measured in the milk and the washings. In addition, the lipase activity of the original milk was determined and the amount of membrane protein per 100 g of fat was calculated. There were statistically significant ($P < 0.05$) day-to-day variations in the membrane protein content of the milk of this cow--ranging from 0.38 to 1.00 g protein/100 g fat. Increased amounts of membrane protein were related to increased lipase activities in the milk--the correlation coefficient between these two variables was 0.48.

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VITA

George C. Hsieh

Candidate for the Degree of

Master of Science

Thesis: CHANGES IN THE MILK FAT GLOBULE MEMBRANE AND THEIR RELATION TO LIPASE ACTIVITY

Major Field: Food Science

Biographical:

Personal Data: Born on January 25, 1941, in Kwei-lin, China, the son of Lt. General and Mrs. Mang Hsieh.

Education: Graduated from High School of Taiwan Normal University at Taipei, Taiwan, in May, 1961; received the Bachelor of Science degree from Taiwan Provincial Chung-Hsing University, Taichung, Taiwan, with a major in Chemistry, in May, 1965; completed the requirements for the Master of Science degree at Oklahoma State University in May, 1969.

Professional Experience: 2nd Lt. in Nationalist Chinese Army, 1965-1966; graduate research assistant, Oklahoma State University, Dairy Department, 1967-1968; President of the Chinese Student Association, Oklahoma State University, 1967-1968.

Professional Organization: American Dairy Science Association.