Antigenicities of Human and Bovine Raw Milk and Blood Sera, and these Comparative Analysis

By Fumisaburo TOKITA and Fujio TAKAHASHI

Laboratory of Animal Products Technology, Fac. Agric., Shinshu Univ.

INTRODUCTION

It has been studied on the chemical and physical properties of bovine and human milk components by various methods, and compared on the properties each other[1,2]. It is also well known that these results obtained were useful basic data for manufacturing of milk products. Immunological and immunochemical methods, on the other hand, were more sensitive and more specific detection methods for macromolecular components of milk than the chemical and physical methods; the methods were of great use as comparative examination on antigenicities of milk from various animals and as re-examination on fractions obtained by chemical and biological methods. It seems consequently that the fundamental data of milk by the immunochemical methods are placed on new problems.

A more extensive discussion of immunological and immunochemical experiments of milk components was found in 1935[3] and then in further references; in the sera of human infants and children hemagglutinating, complement-fixing, anaphylactic, nonprecipitating and precipitating antibodies to the milk proteins have all been demonstrated[4~12]. Ordinarily allergic symptoms developed in children about three weeks after the first feeding with bovine milk[12,13]. The other hand, Sood et al.[14] has mentioned that only one case of human milk allergy was found during the last six years. It seems then that the symptoms developed with bovine milk were observed more frequently than these with human milk. Although many diverse types of milk antibodies and allergenicities may be found, however, demonstrable pathologic significances were not always found in all the children [15]. In any cases, antigenicities of bovine milk components were apparently differed from these of human milk. In highly purified milk proteins were used for the Dual-ingestion Passive Transfer Test, it is then demonstrated that pasteurized milk resulted in frequent reactions at sites passively sensitized to alpha-casein, beta-lactoglobulin and alpha-lactalbumin [12,16], and that the vast majority of infants sensitive to bovine milk could tolerated heat-

denatured milk[16].

As a part of milk protein from immunological point of view, an investigation of the antigenic composition of milk by the diffusion-in-gel or immune electrophoretic techniques have recently been undertaken[17~35]. The methods also, if the components are mixture, can be detected by antigen-antibody precipitation reactions which are more specific and more sensitive than chemical detection methods. These experiments were shown that bovine milk contained twelve antigenic factors, and at least six of these substances were related to bovine blood serum proteins. In human milk similar factors were found and these were similarly related to human blood serum.

This paper reports preliminary observations on production of antisera against milk and blood serum with mature rabbits, and on various comparisons of antigen-antibody precipitation reaction by in-gel and immune electrophoresis.

MATERIALS

I. Preparation of antigens

1. Human milk and its protein fractions

Human mature milk (HM) was collected after 3 weeks of parturition from a healthy woman. The milk was immediately centrifuged at 3000 r.p.m. for 30 min to prepare the skim milk. Human casein was prepared from the skim milk by isoelectric precipitation method at pH 4.6[36]. Globulin, alpha-lactalbumin and red-protein were prepared by the method of Maeno et al.[37]. The skim milk, casein and whey protein fractions were dialyzed against cold distilled water for about 20 hours and lyophilized and then stored in a desiccator at 4°C.

2. Bovine milk and its protein fractions

Bovine mature milk (BM) was collected from mixed-herd milk of Holstein cows in our Farm attached to this Faculty, and its skim milk prepared by same method and used throughout the studies. Bovine casein was prepared from the fresh skim milk by isoelectric precipitation method at pH 4.6. Alpha- and beta-casein were prepared by the method of Hipp et al.[38]. Beta-lactoglobulin and alpha-lactalbumin were prepared from whey by the method of Aschaffenburg and Drewry[39]. Serum albumin and immune-globulin were also prepared by the methods of Polis et al.[40] and Smith[41] respectively.

3. Blood sera of human and bovine

Bovine blood serum was prepared from blood of a healthy Holstein cow. Human blood serum was also prepared from a healthy human adalts. The both sera were lyophilized and stored in a desiccator at 4°C.

Table 1.
Effects of immunization methods and antigens on production of antibodies

Antigens	Injection			Antibody values Age* (wks.)		Rabbits used
	Average amount of antigens (mg/wks.)	Adjuvant	Routes	4	7	(No.)
Skim milk						
Bovine	50	Non	Intra-peritoneal	4**	16	5
//	15	Absorption	Intra-venous	16	32	4
//	20	//	Intra-muscular	16	32	3
Human	15	//	Intra-venous	8	32	3
//	120	//	Intra-muscular	32	128	3
Milk whey						
Bovine	70	//	//	64	64	3
Human	70	//	//	64	128	3
Milk casein						
Bovine	60	//	//	8	16	3
//	60	Complete Freunds	//	16	64	3
Blood serum						
Bovine	50	Absorption	//	64	128	3
Human	50	//	//	64	128	3

* As calculated from the first immunization.
** The antibody values were expressed with average rate of dilution of the twofold diluting antisera method.

II. Preparation of antibodies

1. Immunization methods

Mature rabbits were immunized against bovine and human raw milk (anti-BM, anti-HM), bovine and human whey, bovine casein and bovine and human blood sera (anti-BS, anti-HS). As shown in Table 1, the rabbits were injected intraperitoneal, intravenously or intramuscularly three times a week for 7 – 9 weeks with each solution of various antigens respectively. After a rest period of 1 week, approximately 40 ml of blood was obtained from carotis artery of the rabbits, and then separated the antisera. About 0.01% methiolate as preservative was added to the antisera and stored at 4°C respectively.

2. Making of antigen solutions

The solution of the antigens was prepared and used as follows, i) No adjuvant; about 13 mg of lyophilized antigens were suspended in 3 ml distilled water. ii) Adjuvant I (absorption); about 5 to 40 mg of antigens were suspended in 1 to 4 ml of 0.7% potassium alum solution. iii) Adjuvant II (complete Freund's); about 20 mg of antigens were suspended in 0.5 ml of distilled water, and the solutions

were emulsified with equal volumes of complete Freund's adjuvant*.

METHODS

III. Measurement of pH

The pH were measured with the glass electrode pH meter of Toa Denpa Kogyo Co, Model HM-5A pH meter equipped with standard buffer solutions at pH 4.00 and 6.90 at 15°C.

IV. DEAE-cellulose column chromatography

The method of chromatography on DEAE-cellulose column was made by the method of Yaguchi et al.[42]; the separation of the milk proteins was done by stepwise changes of NaCl concentration in phosphate buffer solution at room temperature, and the effluent was examined continously with Hitachi Model 101 spectrophotometer weared flow cell at 280 mμ.

V. Methods of antigen-antibody reaction

1. Test of antibody value

About 1.5 ml of blood sample was drawn from ear vein of rabbits during immunization, after clotting antisera were separated and antibody values were tested by the method of twofold serial dilution tube tests lie 0.2 ml of 0.05% antigen solution upon 0.5 ml of the serum. Antibody value was expressed with diluent magnifications of the antisera.

The antisera were diluted with 1.5% arabic gum solution which was dissolved in 0.85 g of sodium chloride and 0.1 g of methiolate per 100 ml of distilled water.

2. Double diffusion-in-gel technique

The double diffusion-in-gel technique of Oudin[43] and Ouchterlony[44,45] slightly modified by Hanson[46] was used; petri dishes (diameter; 10 cm) were coated with a layer (thickness; 2 mm) of 1.5% agar in phosphate buffer solution (pH; 7.4, μ; 0.05) containing 0.01% methiolate as a preservative. After the agar gelled five small wells, 15-20 mm apart, were punched in the agar, and 0.05 to 0.10 ml of the various antigens was placed in the around wells, and a similar quantity of antisera was also placed in the central well. Diffusion of the antigens and antisera occured in the agar at 30°C and bands of precipitation were observed at 24 hours intervals.

About 10 mg of the lyophilized antigens were dissolved in 1 ml of 0.9% sodium chloride solution, and antisera were dissolved in water to the original volume of the antisera. Then the samples were used for the examinations.

3. The agar immune electrophoretic technique

The technique of Grabar and williams[47] modified to micro method by

* Supplied by the Difco Laboratories, Michigan, U.S.A.

Scheidegger[48] was used. The electrophoresis was made 2 mm layer on glass plate 10 x 7 cm with 1.3% agar in veronal acetate buffer solution (pH; 8.6, u; 0.05). The central well 1.5 mm was filled with the antigen about 0.004 ml, and electrophoretic separation was done at 1.5 mA/cm in the agar for 150 min at room temperature. After the electrophoretic run a longitudinal through (1 x 70 mm) parallel to the direction of the migration of the antigen was cut at a distance of 4 mm from the well. It was filled with about 0.02 ml of the antiserum. The plate kept in humid chamber at 30°C. The resulting precipitation bands were recorded by free-hand drawing and photography. If necessary, the plates were then washed, dried and stained with 0.5% Amidoschwarts in methanol-water-acetic acid solution (5:5:1).

Antigen and antibody solution were prepared as the same method as the case of Double diffusion technique, especialy the former then was dialyzed with the veronal acetate buffer solution for about 12 hours and used.

RESULTS AND DISCUSSION

VI. Production of antibody

As a preliminary investigation, production of antibody was observed under the various conditions by the test of antibody value of antisera. The results were summarized in Table 1.

1. Differences in adjuvant methods

The rabbits were immunized with the antigens by three kinds of adjuvant methods. Antibody value of antisera immunizing with the adjuvant I method was higher than that of the no adjuvant method, and that of the adjuvant II method showed the highest antibody value among the other methods under the same condition of immunization. The adjuvant II method, in other words, was devoted to achieve a maximal antibody responses with a minimum quantity of antigen and the fewest number of injections.

2. Differences in injecting routes

Antibody against the antigens was produced in rabbit's blood by the method of various injecting routes which are intraperitoneal, intravenous and intramuscular methods. On the results, intravenous and intramuscular injections showed a similar antibody values, and intraperitoneal showed the lowest value. On the case of intraperitoneal injection, a few of rabbits exhibited an arthus phenomenon during hyperimmunizing, and the case of intravenous exhibited an anaphilactic phenomenon.

From these it is quite possible that intramuscular injection to rabbit was most safety and effective immunizing method for producing the highest antibody

value.

3. Differences in antigens

The rate of antibody production against the various antigens under the same condition was also observed. The value of antiserum against milk whey was similar to that against blood serum, and higher than that against casein. The value of anti-milk whey serum was similar to that of blood serum under the same immunizing condition. As to the antibody values, further more, no differences were observed between the same kinds of human and bovine source antigens. As stated above, the antisera of various antibody values were prepared in this examination. In these, the antisera showing the highest antibody values against the same antigens were pooled and used in later desired reacting tests respectively.

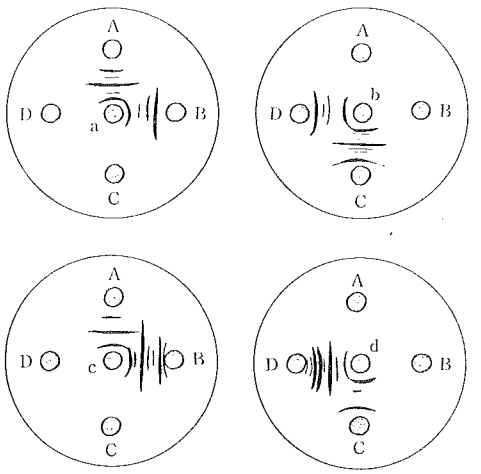

Fig. 1. Diagrams of double diffusion in-gel patterns of antigens diffused against antisera.
Antigens. A; Bovine milk (BM).
B; Bovine blood sera (BS).
C; Human milk (HM).
D; Human blood sera (HS).
Antibodies. a; Anti-BM. b; Anti-HM.
c; Anti-BS. d; Anti-HS.

VII. Precipitation patterns obtained by double diffusion-in-gel analysis

Precipitation patterns were obtained by double diffusion-in-gel analysis of human and bovine milk (HM and BM) and human and bovine blood sera (HS and BS) diffused against each antiserum. The results were shown in Figure 1.

1. Reaction of antigens-anti-BM serum

Figure 1a was representative diagrams when anti-BM serum diffused against the several antigens. Larger number of precipitation lines were formed with BM than with the other antigens, and no precipitation lines were formed with HM and HS. In the precipitation pattern of BM-anti-BM serum and BS-anti-BM serum reaction, at least six and four lines were recognized respectively, and one line of them was fused each other.

2. Reaction of antigens-anti-HM serum

Figure 1b was the diagrams of anti-HM serum diffused against the several antigens. Larger number of precipitation lines were formed with HM, and no precipitation lines were formed with BM and BS. In the pattern seven and four

lines, at least, were recognized in HM-anti-HM serum and HS-anti-HM serum spectra respectively, and one line of them was fused.

3. Reaction of antigens-anti-BS serum

Figure 1c was the diagrams of anti-BS serum diffused against the antigens. Larger number of precipitation lines were recognized in BS-anti-BS serum reaction, and a few lines were recognized in BM-anti-BS spectrum. No precipitation lines were formed in HM-anti-BS and HS-anti-BS sera reactions. One of the lines between BM and BS against anti-BS, at least, was fused as shown in Figure 1c.

4. Reaction of antigens-anti-HS serum

Figure 1d was the diagrams of anti-HS serum diffused against the antigens. Larger number of precipitation lines were recognized in HS-anti-HS serum reaction, and a few lines were recognized in HM-anti-HS serum reaction. No precipitation lines also were formed in BM-anti-HS and BS-anti-HS serum reactions. One of the lines between HM and HS against anti-HS serum, at least, was fused as shown in Figure 1d.

It may be suggested from above the results that immunological analysis by double diffusion method had revealed the several kinds of antigenic substances in BM, BS, HM and HS. Some of these milk substances were found to be immunologically related to blood serum substances, and at least one of identifiable substances were found between BM and BS or HM and HS respectively. No immunologically relations, however, were found between BM and HM, BM and HS, HM and BS or HS and BS by the methods.

VIII. Immune electrophoretical precipitation patterns

Precipitation patterns were obtained by immune electrophoretic analysis of BM, HM, BS and HS diffused against each antisera after electrophorezed antigen in agar gel.

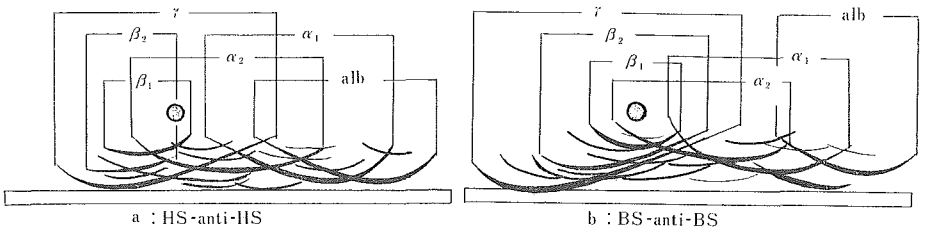

Fig. 2. Diagrams of immune electrophoretic patterns of HS and BS diffused against anti-HS and anti-BS sera.
Barbital buffer pH 8.6, $\mu = 0.05$, Ionagar 1.3%, Agar gel slide 7 x 10 cm, for 150 min at electric current 3 mA/cm.

1. Reaction of HS-anti-HS and BS-anti-BS sera

The immune electrophoretical precipitation spectra of HS-anti-HS serum or BS-anti-BS serum reactions have been examined and discussed by many workers. In this examination the spectrum of HS-anti-HS serum reaction was consisted of large number of precipitation lines. Agar gel electrophoresis of the human blood serum showed after stainning with Aminoschwarz 10B, six fractions that were suggested to be; albumin, alpha$_1$-, alpha$_2$-, beta$_1$-, beta$_2$- and gamma-globulin. From the immune electrophoretic pattern, the about fifteen or more separate precipitation lines of HS-anti-HS serum reaction were localized at the albumin, alpha$_1$-, alpha$_2$-, beta$_1$-, beta$_2$- and gamma-globulin regions as shown in Figure 2a. The spectrum of BS-anti-BS serum reaction also was consisted at least of sixteen or more precipitation lines. The separate lines were then localized at the regions with the electrophoretic pattern of bovine blood serum as shown in Figure 2b.

From these results, it has been found that a large number of antigenic substances is contained in both human blood serum and bovine blood serum, and that the spectrum of HS-anti-HS serum reaction is nearly similar to that of BS-anti-BS serum reaction. It has not been cleared, however, whether both of them are serologically identical or not.

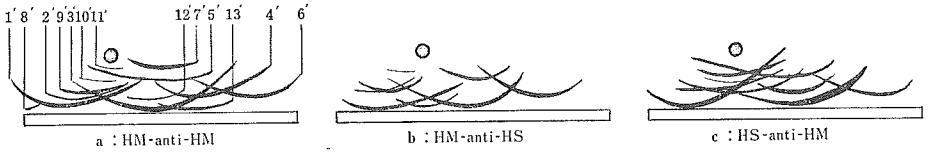

Fig. 3. Diagrams of immune electrophoretic patterns of HM and HS diffused against anti-HM and anti-HS sera.

2. Reaction of HM-anti-HS, HS-anti-HM, and HM-anti-HM sera

Of the precipitation pattern of HM-anti-HS serum reaction eight lines were recognized as shown in Figure 3b. One of these was dense and situated in the albumin, one line in the alpha$_1$-, one in the alpha$_2$-, two lines in the beta$_1$- and three lines in the beta$_2$-globulin region respectively. The spectrum of HS-anti-HM serum was consisted nine lines as shown in Figure 3c. In these one line was situated in the albumin, five lines in the alpha$_1$- to beta$_1$-, two lines in the beta$_2$- and one line in the gamma-globulin region. The precipitation pattern of HM-anti-HM serum reaction consisted of thirteen separate lines as shown in

Figure 3a. In these one line was situated in the albumin region, nine lines were situated in the region of the $alpha_1$– to $beta_1$–globulin, of which one dense line was situated with its maximum in the $beta_1$– or $beta_2$– and one line in the gamma–globulin region when compared with the spectrum of HS–anti–HS serum (Figure 2a).

From the precipitation pattern of HM–anti–HM serum reaction by immune electrophoresis, twelve lines are recognized, among which at least eight or nine precipitation lines are found to be closely related or identical to antigenic substances in human serum[17, 20, 49]. This precipitation pattern obtained tends larger in number of precipitation lines, compared to twelve lines in the same spectrum which recently have been published by Hanson[20].

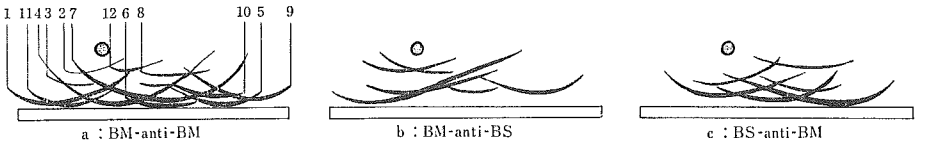

Fig. 4. Diagrams of immune electrophoretic patterns of BM and BS diffused against anti–BM and anti–BS sera.

3. Reaction of BM–anti–BS, BS–anti–BM and BM–anti–BM sera

The spectrum of BM–anti–BS serum reaction consisted of six precipitation lines as shown in Figure 4b. Of these one dense line was situated in the albumin, one line in the $alpha_1$–, three lines in the $beta_1$– to the $beta_2$–globulin and one line in the gamma– to $alpha_2$–globulin locales comparing with the pattern of BS–anti–BS serum reaction (Figure 2b). The precipitation pattern of BS–anti–BM serum reaction showed at least six separate lines as shown in Figure 4c. These were situated throughout the regions of gamma–globulin to serum albumin. In the precipitation pattern of BM–anti–BM serum reaction twelve separate lines were recognized as shown in Figure 4a. It seems from the diagram that relative mobilities and separable pattern of the antigenic proteins of milk in agar gel varied that in filter paper and starch gel electrophoresis at same buffer solution.

As illustrated in Figure 4b and 4c, the relationship between the antigenic factors in milk and blood serum would be suggested that at least six separate antigenic factors in bovine milk were to be serologically identical or related to bovine blood serum proteins. However, it has been unknown as to the structural similarity of these antigenic substances. The fact that bovine milk is consisted

of a number of antigenic factors was in agreement with the results by Hanson et al. [19,50].

4. Other antigen-antibody precipitation reaction

Immune electrophoretic analysis on other antigen-antibody reaction, that are HM-anti-BM, HM-anti-BS, HS-anti-BM, HS-anti-BS, BM-anti-HM, BM-anti-HS, BS-anti-HM and BS-anti-HS, were carried out under the same condition as stated above. No precipitation lines were found in the all the pairing of antigen-antibody reactions. Then no immunological relations were recognized between human source antigens and bovine source antigens under the immune electrophoretic analysis.

These differences of antigenic factor probably related to that the allergic symptoms developed more often in children with bovine milk, but not developed with human milk as stated in this introduction[4,12,13].

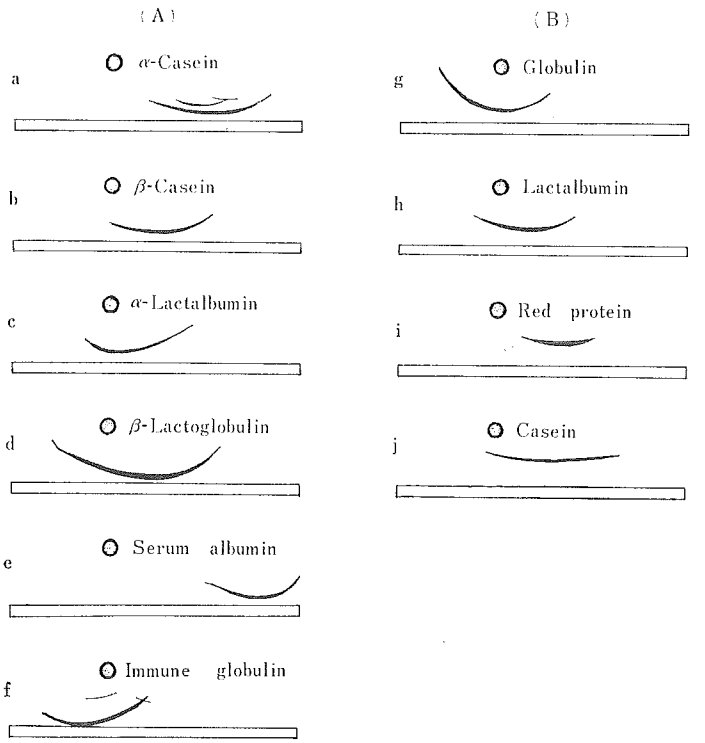

Fig. 5. Diagrams of immune electorophoretic patterns of various milk protein fractions diffused against anti-BM and anti-HM sera.
A; BM fractions-anti-BM.
B; HM fractions-anti-HM.

IX. Immune electrophoretic analysis of milk protein fractions

1. Milk protein fractions against anti-milk sera

Representative diagrams showing the use of anti-milk sera for resolving the milk protein fractions are shown in Figure 5. The diagrams of a) to f) in Figure 5 were immune electrophoretic analysis of BM protein fractions against anti-BM serum, and g) to j) were of HM protein fractions against anti-HM serum.

Table 2.
Identification of BM-anti-BM and HM-anti-HM spectra by comparative immune electrophoresis

Bovine milk		Human milk	
Spectra No.*	Component	Spectra No.**	Component
1:	Immune globulin	1′:	Immune globulin
2:		2′:	
3:		3′:	Milk beta-globulin
4:	Alpha-lactalbumin	4′:	Lactalbumin
5:	Alpha-casein	5′:	Red protein
6:	Beta-casein	6′:	Serum albumin
7:	Beta-lactoglobulin	7′:	Casein
8:		8′:	
9:	Serum albumin	9′:	
10:		10′:	
11:		11′:	
12:		12′:	
		13′:	

* Spectra No. in Fig. 4a.
** Spectra No. in Fig. 3a.

The Precipitation lines for bovine alpha-casein were observed on the anode side of the origin as the Figure 5a, and three lines, in which one was dense, were formed in the pattern. It seems that three different antigenic factors were recognized in bovine alpha-casein prepared by the Hipp's method. For immune globulin, three different lines also were observed as shown in Figure 5f. The precipitation lines for bovine beta-casein, alpha-lactalbumin, beta-lactoglobulin and serum albumin were observed one dense precipitation line respectively as shown in Figure 5b, c, d and e. From the lines and comparative immune electrophoretic analysis, the precipitation lines of BM-anti-BM serum reaction (Figure 4a) were recognized locally as shown in Table 2. However, other remaining precipitation lines were unknown. The many antigenic substances were contained

in milk, and many of these were recognized the proteins. The investigation of this antigenic factors and these active structures must be the subject of future research.

On human milk protein fractions, one dense precipitation line was observed on the each fraction against anti-HM serum reaction respectively. From the same analysis the precipitation spectrum of HM-anti-HM serum (Figure 3a) was also recognized locally as shown in Table 2. Further investigations of chemical and immunological analysis on human milk proteins are more necessary than the bovine milk proteins, and being carried out.

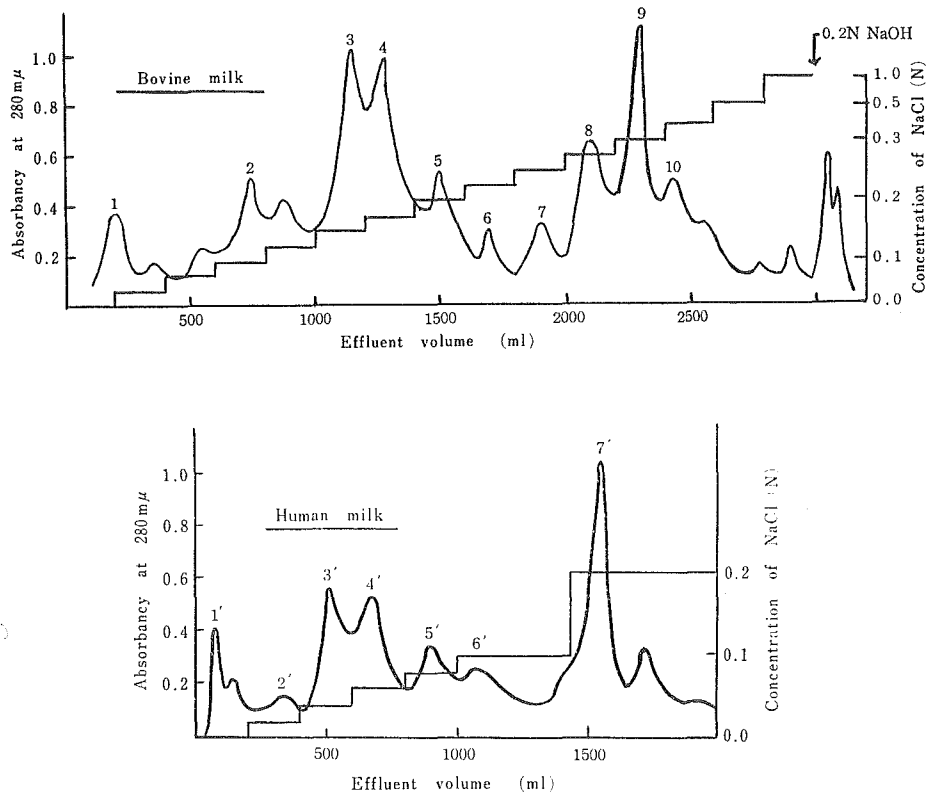

Fig. 6. DEAE-cellulose column chromatograms of milk protein by stepwise elution method. Column 6 x 5.5 cm, room temp., Flow rate 800ml/hr., Collect. 20ml.

2. Relation between precipitation spectra and DEAE-cellulose column chromatographic fractions

DEAE-cellulose column chromatogram of bovine milk was shown in Figure

6a. The chromatogram has been obtained by many workers, and its each peaks were examined: No 1, 2, 3–4, 5–6 and 8–10 were recognized as immune globulin, alpha-lactalbumin, beta-lactoglobulin, beta-casein and alpha-casein respectively[42,51]. The pattern of bovine milk produced in Japan differed from that of the milk produced in U. S. A.[42]. The chromatogram of human milk was also apparently differed from that of bovine milk and its peaks were marked with No 1′, 2′, 3′, 4′, 5′, 6′ and 7′ as shown in Figure 6b. Of these fractions peaks were separated respectively and immune electrophoretically analyzed against anti-BM and anti-HM sera. The representative diagrams were shown in Figure 7. From the results, it appears that casein of bovine milk was not so clearly separated same as the results of previous examinations[44]. Casein, globulin and lactalbumin on human milk protein were also not so clearly separated than the case of

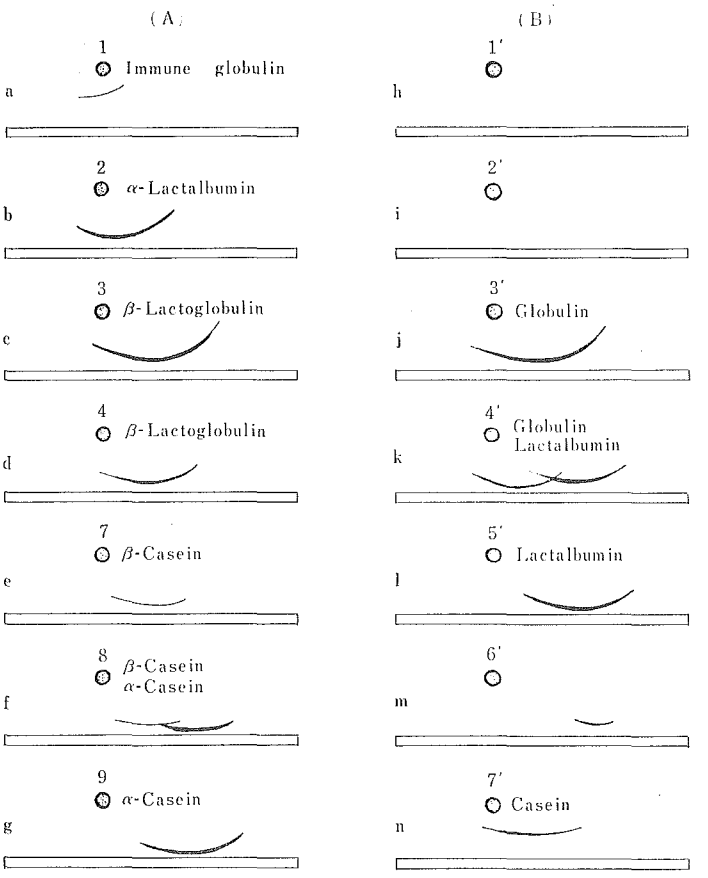

Fig. 7. Immune electrophoretic analysis of the fractions separated by the DEAE-cellulose column chromatography showing in Fig. 8.
A; BM fractions-anti-BM. B; HM fractions-anti-HM.

bovine milk protein. In view of the experiments described above, it can be suggested that the characters on hydration and zwitter ion structure of human milk proteins were different from these of bovine milk proteins. It is an interesting phenomenon with the different of antigenic factors between human and bovine milk protein. This point is investigating.

SUMMARY

It is quite possible that immunological and immunochemical analysis, which are more sensitive and more specific detection methods for macromolecular substances, were of great use as comparative re-examination of the fractions of milk obtained by chemical methods. This paper reports preliminary observations on the antigenicities and their relationship of human and bovine milk and blood sera by means of in-gel and immune electrophoresis.

1). It is the most effective condition for the production of antibodies that the rabbits were hyperimmunized intramuscularly with milk whey protein and blood serum which were prepared with Complete Freund's Adjuvant (Adjuvant II).

2). Immunological analysis by double diffusion method had revealed the several kinds of antigenic substances in bovine milk (BM), bovine serum (BS), human milk (HM) and human serum (HS). Some of these milk substances were found to be immunologically related to blood serum substances, especially, at least one of identifiable substances was found between BM and BS or HM and HS respectively. No immunologically relations, however, were found between BM and HM, BM and HS, HM and BS or HS and BS by the method.

3). From the immune electrophoretical precipitation patterns, it has been found that large number of antigenic substances are contained in both human blood serum and bovine blood serum, and that the spectrum of human-serum-anti-human serum is nearly similar to that of bovine serum-anti-bovine serum. It has not been cleared, however, whether both of them are serologically identical or not.

4). In human milk, thirteen separate antigenic substances were recognized, at least eight or nine of which were found to be identical or closely related to these in human blood serum proteins. On the other hand, twelve separate antigenic substances in bovine milk were recognized, at least six of these were to be serologically related to bovine blood serum proteins.

5). No precipitation lines were also found in the human milk-anti-bovine milk, human milk-anti-bovine serum, human serum-anti-bovine milk, human serum-anti-bovine serum, bovine milk-anti-human milk, bovine milk-anti-human serum, bovine serum-anti-human milk and bovine serum-anti-human serum

spectra by immune electrophoresis. No immunological relations, in other words, were recognized between human source antigens and bovine source antigens.

6). Bovine milk protein fractions which were prepared with various chemical methods were immunologically examined against anti-bovine milk serum. The three different antigenic factors were observed in alpha-casein and immune globulin. On human milk protein fractions, however, one dense precipitation lines were recognized on the each fractions against anti-human milk serum.

7). DEAE-cellulose column chromatogram of human milk were apparently differed from these of bovine milk. It was found that the each fraction of the chromatograms was not always one antigenic factor by immune electrophoresis.

REFERENCES

1. Tsugo, T. et al.: Milk (Chair of Dairy Tech.) 1: 150, 272 (1963)
2. Tsugo, T. et al.: Principle of Dairy Sci., 1-176 (1967)
3. Ratner, B. and Gruehl, H.L.: Amer. J. Dis. Child., 49: 287, 289 (1935)
4. Anderson, A.F. and Schloss, O.M.: ibid., 76: 451 (1923)
5. Farr, R.S.: J. Infect. Dis., 103: 239 (1958)
6. Lippard, J.W.: Amer. J. Dis. Child., 57: 524 (1939)
7. Gunther, M. et al.: Immunol., 3: 296 (1960)
8. Peterson, R.A. and Good, R.A.: Pediat., 31: 209 (1963)
9. Holland, N.H. et al.: J. Pediat., 61: 181 (1962)
10. Raymond, D.A. et al.: Pediat., 31: 209 (1963)
11. Parish, W.E. et al.: Immunol., 3: 307 (1960)
12. Ratner, B.: Pediat., 22: 449, 648, 653 (1958)
13. Wilken-Jensen, K.: North. Pediat. Cong. II., 14th. 101 (1965)
14. Sood, S.C. and Singh, H.: Indian J. Pediat., 32: 163 (1965)
15. Crawford, L.V. et al.: South. Med, J., 58: 1140 (1965)
16. Crawford, L.V.: Pediat., 25: 432 (1960)
17. Johansson, B.: Nature., 181: 996 (1958)
18. Grabar, P. and Williams, C.A.: Biochim. Biophy. Acta., 17: 67 (1955)
19. Hanson, L.A. et al.: Exper., 15: 377, 473, 477 (1959)
20. Hanson, L.A. et al.: Int. Arch. Allergy., 15: 245, 257 (1959)
21. Hanson, L.A. et al.: ibid., 18: 241 (1962)
22. Grawford, L.V. and Grogan, F.T.: Pediat., 28: 362 (1961)
23. Grogan, F.T. and Grawford, L.V.: J. Immunol., 87: 240 (1961)
24. Grawford, L.V. and Grogan, F.T.: J. Pediat., 59: 347 (1961)
25. Hanson, L.A.: Prot. Biol. Fluids., B: 133 (1961)
26. Aurand, L.W. et al.: J. Dairy Sci., 46: 1177 (1963)
27. Hanson, L.A. and Manson. I.: Acta Pediat. 50: 484 (1961)
28. Saperstein, S. and Anderson, D.W.: J. Pediat., 61: 196 (1962)

29. Yoshimatsu, A.: Acta Pediat. Jap., 67: 427 (1963)
30. Todd, R.H. et al.: Amer. J. Dis. Child., 108: 479 (1964)
31. Dumas, B.R. et al.: Biochim. Biophy. Acta., 82: 494 (1964)
32. Hanson, L.A. and Johansson, B.: Int. Arch. Allergy., 20: 65 (1962)
33. Gugler, E. et al.: Schweig. Med. Wschr., 89: 1172 (1959)
34. Fey, H.: Milchwiss., 15: 105 (1960)
35. Roulet, D.L.A. et al.: idid., 16: 415 (1961)
36. Maeno, M. and Kiyosawa, I.: J. Agri. Chem. Soc. Jap., 37: 362 (1963)
37. Maeno, M. and Kiyosawa, I.: ibid., 37: 729 (1963)
38. Hipp, N.J. et al.: J. Dairy Sci., 35: 272 (1952)
39. Aschaffenburg, R. and Drewry, J.: Biochem. J., 65: 273 (1957)
40. Polis, B.D.: J. Biol. Chem., 187: 349 (1950)
41. Smith, E.L.: ibid., 164: 345 (1946)
42. Yaguchi, M. et al.: J. Dairy Sci., 44: 589 (1961)
43. Oudin, J.: Ann. Inst. Pasteur., 75: 30, 109 (1948)
44. Ouchterlony, Ö.: Progr. Allergy., 5: 1 (1958)
45. Ouchterlony, Ö.: Ark. Kemi. Min. Geol., 826: 1 (1949)
46. Hanson, L.A.: Int. Arch. Allergy., 17: 45 (1960)
47. Grabar, P. and Williams, C.A.: Biochim. Biophy. Acta., 10: 193 (1953)
48. Scheidegger, J.J.: Int. Arch. Allergy., 7: 103 (1955)
49. Gugler, E.: Schweiz. Med. Wechr., 88: 1264 (1958)
50. Hanson, L.A.: Exper., 15: 471 (1959)
51. Yaguchi, M. et al.: J. Dairy Sci., 45: 253 (1962)

乳汁および血清成分の抗原性およびその比較検討

鴇田文三郎・高橋富士雄

信州大学農学部　畜産製造学研究室

要　　約

高分子性物質の微量確認，あるいは活性構造の比較検討などの方法として，免疫化学的手法はより鋭敏でかつ特異的であることから，近年乳汁成分の乳腺内生合成機構の解明にも利用されつつある。著者らは乳汁利用の観点から，その種属間の比較および加工過程の諸変化の検討に，この免疫化学的反応を応用した一連の実験を行なつている。本報はその初報で，実験の方法も含めて人乳，牛乳および人血清，牛血清の抗原性およびその関連性について実験した結果である。

1.　免疫動物として成熟家兎を用い，抗原は Freund の完全 Adjuvant に調製し，筋肉内に注射する方法が最も効率のよい，また安全な高抗体を産生する方法である。なお，乳清および血清成分が抗原として高い抗体価を与える傾向を示した（Table 1.）。

2.　寒天内二重拡散法を用いた抗原—抗体沈降反応により，人および牛の乳汁と血清中に，それぞれ数種の抗原性物質の存在が認められた。また，これら乳汁成分の内のいくつかは血清成分と免疫学的に関連性があり，その１つは同一物質であることも認めた。しかし，牛乳と人乳，牛乳と人血清，人乳と牛血清，あるいは人血清と牛血清との間の成分には免疫学的な関連性が認められなかつた（Fig. 1）。

3.　免疫電気泳動法による沈降図から，人血清および牛血清中に非常に多くの抗原性物質が認められ，また，この両者のパターンは比較的よく類似している（Fig. 2）。この種の実験は古くから行なわれてきたもので，本実験の結果もよくそれと一致する。なお，この結果を基礎的沈降図として以下の実験の検討に用いた。

4.　人乳中に約13種類の抗原性物質が認められ，その内少なくとも８～９種類の成分は人血清のそれと抗原性において関連性がある（Fig. 3）。また，牛乳中にも約12種類の抗原性物質があり，その内の６種類が牛血清のそれと関連性がある（Fig. 4）。すなわち，血清内物質の６～７種類の構造はそのまま乳腺を通過して，あるいは同一構造に再合成されて牛乳成分になると考えられる。

5.　人より得た抗原，すなわち，人乳および人血清中の成分と牛より得た抗原，すなわち，牛乳および牛血清中の成分との間には，免疫電気泳動法によつても沈降線が認められなかつた。すなわち，この結果は二重拡散法と全く等しい結果である。換言すれば，免疫学的に関連性がないと云うことになる。

6.　種々の化学的方法により調整した牛乳成分と抗牛乳血清に対する免疫反応により実験

した。その結果，各画分の沈降線の位置が確認された（Fig. 5, Table 2.）。また，α-カゼイン，免疫グロブリンの精製は非常に難しいことも認められた。人乳についても同様の実験を行なつたが，その成分の精製法は検討されるべき今後の課題であるように思う。

7. 人乳および牛乳のＤＥＡＥ—セルローズカラムクロマトグラムを求めた（Fig. 6, 7）。その結果，人乳成分は比較的低食塩濃度部分に流出することが，牛乳成分と比較して確認された。さらに，これら各ピーク画分は必ずしも単一の抗原性を示さなかつた。