バルビタールカルシウム緩衝液による牛皿清蛋白質のセルロースアセテート膜電気泳動について

誌名	日本獣醫學雑誌 = The Japanese journal of veterinary science
ISSN	00215295
著者	川村, 清市 安田, 純夫 田山, 春實 茂木, 久佳 高瀬, 勝晤 小笠原, 成郎
巻/号	36巻4号
掲載ページ	p. 285-290
発行年月	1974年8月

Jap. J. vet. Sci., **36**, 285～290 (1974)

Cellulose Acetate Membrane Electrophoresis of Bovine Serum Proteins Using a Barbital Calcium Buffer

Seiichi KAWAMURA, Yoshio YASUDA, Harumi TAYAMA, Hisayoshi MOKI, Katsuaki TAKASE and Shigeo OGASAWARA

Faculty of Veterinary Medicine, School of Animal Sciences, Kitasato University, Towada-shi, Aomori 034

(Received for publication October 1, 1973)

Abstract. In order to improve the separativity of serum proteins of cattle in electrophoresis on cellulose acetate membrane, the constituents and the concentrations of buffer solution were investigated in this experiment.

Better results of fractionation were obtained from a barbital calcium buffer (pH 8.6) which consisted of 2.0 g of calcium lactate and 1.0 *l* of 0.06 M barbital buffer than from the standard barbital buffer. By using that buffer, α-globulin (gl) in the serum of cattle was divided into two fractions, α_1-gl and α_2-gl, and the separation of β-gl and γ-gl was clearly visible.

The percentages of the serum protein fractions in healthy dairy cattle were determined and the normal range of each fraction was calculated. A significant increase in α_1- and α_2-gl was recognized in various diseases, including liver disturbances, nephritis and some infectious diseases.

It is considered that in making the clinical diagnosis of some disease, the barbital calcium buffer is worthy of applying to the fractionation of serum proteins of cattle by electrophosesis with cellulose acetate membrane. By using this buffer, the serum protein fractions were separated distinctly, and α_1- and α_2-gl divided clearly and increased significantly in several diseases.

Today, the fractionation of serum proteins by means of zone electrophoresis with cellulose acetate membrane is performed as a routine work for clinical diagnosis. This electrophoresis is highly efficient in separating the serum proteins in a short time and dealing with many samples at ones.

The buffer solution generally used for the fractionation of animal serum proteins in electrophoresis are 0.05 M tris-solution barbital buffer (pH 8.8) [9] and 0.06 M barbital buffer (pH 8.6) [13]. The latter has often utilized for the fractionation of serum proteins in cattle.

Electrophoresis with cellulose acetate membrane make it possible to separate the serum proteins in animals. Excellent electrophorograms produced by it have been presented for dogs [4] and horses [1, 10], as well as for man [12]. The serum proteins of cattle, however, have not clearly been fractionated. Especially, α-gl has not been separated into two fractions, α_1-gl and α_2-gl, as yet. Furthermore, the diagnostic significance of changes in α_1- and α_2-gl has been clarified in human medicine [7]. Therefore, it considerably significant for a clinical diagnosis in cattle to divide α-gl into α_1- and α_2-gl.

In order to improve the separativity of

バルビタールカルシウム緩衝液による牛血清蛋白質のセルロースアセテート膜電気泳動について：川村清市・安田純夫・田山春實・茂木久佳(北里大学畜産学部家畜内科学教室)，高瀬勝晤・小笠原成郎(同家畜外科学教室)

serum proteins of cattle by electrophoresis with cellulose acetate membrane, studies were made on the fractionation of serum proteins with barbital buffer to which calcium lactate had been added. The results of the fractionation with the modified buffer are presented in this paper.

Materials and Methods

1. Bovine serum: Serum samples were collected from thirty healthy cattle of the Holstein breed 2 to 6 years of age. These cattle had been subjected to clinical and blood examinations to check for health. They did not include cows at three months or more of pregnancy or cows three months or less after parturition.

On the other hand, serum samples were collected from 13 dairy cattle, which were found to have an increased level of α-gl. These cattle had been suffering from endometritis, sunstroke, theileriosis, downer syndrome after parturition, secretion of milk of normal acidity and positive for the alcohol test, ketosis, acute hepatitis, bracken poisoning and glomerular nephritis.

2. Procedure of electrophoresis: Electrophoretic separation of serum proteins was carried out by the technique of Ogawa [13] as standard method. The microzone electrophoretic system with Separax Model 238, Johko Sangyo Company, Tokyo was used as supporting medium. Serum total protein levels were determined by using a refractometer (Hitachi Ltd., Tokyo).

3. Statistical analysis: Mean values, standard deviations, confidence limits and rejection limits were calculated from the formulae of Torii et al. [19]. The confidence limits and rejection limits were studied in comparison with the physiological range and the borderline of normal values, respectively.

Results

1. Effect of addition of calcium to barbital buffer on separativity of serum proteins

In order to improve the separativity of serum proteins in cattle by electrophoresis with cellulose acetate membrane, modified barbital buffer solutions were prepared by dissolving 1.0 g to 4.0 g of calcium lactate in 1.0 *l* of 0.06 M barbital buffer solution and adjusting the resulting solution to pH 8.6. Five bovine serum samples were fractionated by using 0.06 M barbital buffer solution containing or not containing calcium to compare the electrophoretic pattern and percentage of each protein fraction obtained (Fig. 1 and Table 1).

By using the modified buffer solution containing 1.0 g of calcium lactate, α-gl was separated into two fractions, α_1-gl and α_2-gl. As seen in Table 1, albumin was higher and α- and β-gl were little lower in level when the barbital solution containing calcium was used than when the plain barbital buffer solution was used.

When the modified buffer solution containing 2.0 g of calcium lactate was applied, α-gl was also separated into two fractions, α_1-gl and α_2-gl, in all the 5 serum samples. These was also a slight decrease in β-gl level and a slight increase in albumin, α-gl and γ-gl levels.

When the buffer solutions containing more than 3.0 g of calcium lactate were used, however, there were a marked decline in the separativity of α-gl, a considerable decrease in albumin level, and a considerable in-

Fig. 1. Electrophoretic patterns of serum proteins in cattle determined by using barbital buffer (L) and modified barital buffer (R)

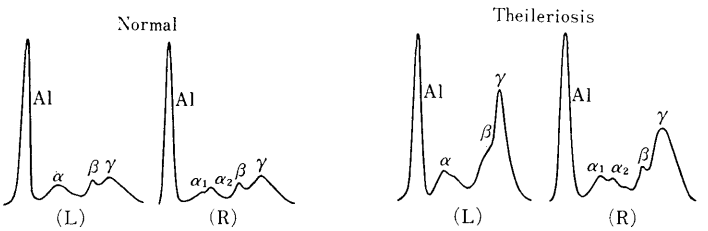

Table 1. Percentages of serum protein fractions in cattle isolated with barbital buffer and modified barbital buffer containing calcium lactate (CL)

Buffer		Animal No.	Albumin	α-globulin			β-gl	γ-gl
				total	α_1	α_2		
Barbital buffer solution		1	42.7	13.7			11.4	32.2
		2	41.3	15.0			14.7	29.0
		3	40.2	16.0			11.6	32.2
		4	39.1	18.7			13.2	28.8
		5	39.3	16.6			14.0	30.1
		Mean	40.5	16.0			13.0	30.5
Modified buffer solution containing	1.0 g* of CL	1	42.8	14.0	5.3	8.7	10.1	33.1
		2	42.0	15.0	6.6	8.4	14.0	29.0
		3	41.8	15.2	5.4	9.8	11.0	32.0
		4	45.4	13.2	6.0	7.2	12.4	30.0
		5	39.8	16.1	7.3	8.8	12.6	31.5
		Mean	42.2	14.7	6.1	8.6	12.0	30.9
	2.0 g of CL	1	41.3	16.6	8.3	8.3	9.1	33.0
		2	43.1	14.8	2.8	12.0	14.6	27.5
		3	39.6	16.9	9.4	7.5	9.4	34.1
		4	45.1	15.6	8.8	6.8	10.9	28.4
		5	36.5	19.2	7.7	11.5	11.6	32.7
		Mean	41.1	16.6	7.3	9.3	11.1	31.2
	3.0 g of CL	1	41.4	16.6	7.8	8.8	11.0	31.0
		2	40.4	15.1	4.5	10.6	14.9	29.6
		3	34.1	16.8			11.7	37.8
		4	39.3	17.9			13.6	29.2
		5	35.2	17.6			13.9	33.3
		Mean	38.0	16.9			13.0	32.1

Remarks.
*: Amount of calcium lactate contained in 1.0 *l* of 0.06 M barbital buffer solution.

crease in α-gl and γ-gl levels. From these observations, it was found that the barbital buffer solution containing 2.0 g of calcium lactate had brought about the best results of the fractionation of serum proteins in cattle.

In addition to this experiment, examination was made on the concentration of buffer solution and the conditions of electrophoresis to induce migration at an interval of 3–4 cm between the albumin and γ-gl fractions. In it, a modified buffer (barbital calcium buffer) solution was prepared in the following manner: 2.56 g of diethylbarbituric acid, 12.00 g of sodium diethylbarbituric acid and 2.00 g of calcium lactate were dissolved in 1.0 *l* of distilled water. The pH of resulting solution was adjusted to 8.6. The ionic strength of the solution was 0.08 μ. An electrophorogram was recorded in the following conditions: 0.8 to 1.2 μ*l* of serum was smeared to a width of one cm on a membrane; the power of electric current was 1.0 mA/cm and the running time 55 to 65 minutes.

2. Percentages of serum protein fractions in healthy dairy cattle

The percentages of the serum protein fractions determined by using the barbital calcium buffer solution in thirty-five healthy

Table 2. Total protein levels and percentages of protein fractions in sera of healthy dairy cattle

	Total protein g/100 ml	Albumin	Percentage of protein fraction Globulin α_1	α_2	β	γ	A/G ratio
Mean value	7.5	44.9	6.8	11.3	10.5	24.9	0.86
Standard deviation	0.58	2.50	2.25	2.18	1.90	2.23	0.075
Confidence limit	7.2–7.8	44.0–45.8	6.0– 7.6	10.5–12.1	9.0–11.1	24.1–25.7	0.83–0.89
Rejection	6.2–8.8	39.0–50.2	2.1–11.5	6.7–15.9	7.0–14.0	20.3–29.5	0.65–1.07

Remarks. A total of 35 cattle were used.

dairy cattle are given in Table 2. This table shows that α-gl was separated into α_1- and α_2-gl in 83% of the serum samples used. The rejection limits of each fraction were 39.0–50.2% for albumin, 2.1–11.5% for α_1-gl, 6.7–15.9% for α_2-gl, 7.0–14.0% for β-gl, and 20.3–29.5% for γ-gl when abnormal values were rejected out by the rejection test of Smirnoff [19]. The A/G ratio ranged from 0.65 to 1.07.

3. Changes of α-globulin fractions in keeping with the fluctuation of clinical conditions

Changes in α_1- and α_2-gl levels during the clinical course were followed in 13 cases consisting of three cases of endometritis, two cases each of sunstroke and theileriosis, and one cases each of downer syndrome after parturition, secretion of milk of normal acidity and positive for the alcohol test, ketosis, acute hepatitis, bracken poisoning and glomeruler nephritis (Table 3).

During the clinical course an increase in α_1-gl was recognized in the cases of acute endometritis, secretion of milk of normal acidity and positive for the alcohol test, acute hepatitis and downer syndrome after parturition, and an increase in α_2-gl in the cases of incipient theileriosis, sunstroke, glomerular nephritis, ketosis, bracken poisoning and chronic endometritis. The percentages of the β- and γ-gl fraction, however, were within the range of rejection limits of each fraction in all the animals examined, except one, No. 418, with regard to that of the γ-gl fraction.

Table 3. Serum protein fractions in cattle showing increase in α-globulin

Animal No.	Total protein g/100 ml	Albumin	Percentage of protein fraction Globulin total	α α_1	α_2	β	γ	A/G ratio	Diagnosis
82	7.5	48.2	19.9	12.1	7.8	8.5	23.4	0.93	Acute endometritis
304	7.5	53.5	21.6	12.5	9.1	9.1	19.4	1.15	Secretion of milk of normal acidity and positive for the alcohol test
308	7.8	36.2	26.9	13.4	13.5	9.4	27.5	0.57	Acute hepatitis
319	6.6	53.8	21.4	17.1	4.3	7.7	17.1	1.16	Acute endometritis
418	5.7	19.1	29.4	21.5	8.2	9.5	36.3	0.25	Downer syndrome after parturition
11	8.1	43.8	20.2	3.6	16.6	13.6	22.4	0.78	Incipient theileriosis
16	7.6	47.8	19.6	3.0	16.6	12.6	20.0	0.92	Incipient theileriosis
19	7.5	37.8	16.6	1.9	14.7	11.6	34.0	0.61	Sunstroke
20	6.5	43.8	25.2	3.6	21.6	13.5	17.5	0.78	Glomerular nephritis
30	8.0	49.5	19.1	4.8	14.3	10.4	21.0	0.98	Ketosis
257	7.6	45.5	20.6	6.6	14.0	9.1	24.8	0.84	Bracken poisoning
405	8.6	46.7	21.1	4.7	16.4	8.2	24.0	0.86	Sunstroke
425	7.2	48.8	20.8	5.6	15.2	8.8	21.6	0.95	Chronic endometritis

Discussion

The fractionation of the serum proteins in cattle by means of electrophoresis with cellulose acetate membrane has been reported by Ek [3], Herz and Hod [5], Shimada [15] and Osbaldiston [14]. In the serum proteins of cattle, it has hardly been observed that α-gl was divided into two fractions, α_1- and α_2-gl, and that β-gl and γ-gl were separated clearly from each other. In dogs [4], horses [1, 10] and man [13], the serum proteins have been divided distinctly into five or six fractions, such as albumin and α_1-, α_2-, β_1-, or β_2-, and γ-gl. It is generally assumed that the low separativity of the serum proteins in cattle may be attributed to the high viscosity and the small difference in isoelectric point among the protein fractions of serum. By vertical electrophoresis with polyacrylamide gel, however, the α-gl fractions of cattle are divided into two parts of more [16].

Ek [3] reported that by use of a modified buffer solution containing 0.38 g of calcium acetate in 1.0 *l* of barbital buffer solution, it was clearly possible to separate β-gl from γ-gl, but not to divide α-gl into two fractions. From the results of the present investigation, it is presumed that even α-gl of bovine serum may be separated into α_1- and α_2-gl by modifying the composition of the buffer solution to be used.

The distance of migration is influenced by the pH and ionic strength of the buffer used, current volume and voltage [6]. In the present investigation, the addition of calcium, which is a bivalent cationic element, to the buffer solution was examined for effect, so that the ionic strength of the buffer solution might be increased enough to control the occurrence of Joul's heat. As a result, it was indicated that a modified buffer solution (pH 8.6) containing 2.0 g of calcium lactate in 1.0 *l* of 0.06 M barbital buffer solution was able to divide α-gl into α_1- and α_2-gl in 83% of the serum samples used and separate β-gl distinctly from γ-gl.

The percentages of the serum protein fractions in healthy dairy cattle were measured and analyzed statistically on mean value, standard deviation, confidence limit and rejection limit. The authors considered that the confidence limit and the rejection limit were within a physiological range and on the borderline of normal values, respectively. They obtained results which nearly agreed with those reported by Shimada [15], Ek [3] and Osbaldiston [14]. In their investigation, however, albumin and β-gl showed a little low percentages and α-gl a somewhat high percentage. These results are interpreted that by using the barbital calcium buffer solution, some protein of the globulin migrated from albumin and β-gl to α-gl.

The diagnostic significance of the fractionation of α_1- and α_2-gl has previously been reported for various diseases in human medicine. Namely, α_1-gl increases in compensation in patients who show a decline of albumin level in incipient liver disturbances caused by acute hepatitis [11], Hodgkin's disease [17], carcinomatosis [2] and the like. On the other hand, α_2-gl increases in the nephrotic syndrome [17], acute and chronic infections [18], sarcoidosis [10] and metastatic carcinomatosis [17], and decreases in cirrhosis of the liver [8].

In the present study, an increase in α_1-gl was recognized during the clinical course in the cases of acute endometritis, secretion of milk of normal acidity and positive for the alcohol test, acute hepatitis and downer syndrome after parturition. An increase in α_2-gl was observed in the cases of incipient theileriosis, sunstroke, glomerular nephritis, ketosis, bracken poisoning and chronic

endometritis. From these results, it is assumed that the fractionation of α_1- and α_2-gl may be useful for the clinical diagnosis of some diseases in cattle.

Hereafter, the authors will endeavor to establish the diagnostic significance of the changes in percentage of α_1- and α_2-gl.

Acknowledgements: The authors wish to acknowledge the advice and encouragement of Dr. Yasuhisa Yasuda, of the Faculty of Agriculture, Iwate University, and the technical assistance of Mr. Masayasu Tomabechi and Mr. Yasuhiro Kinoshita, of the Department of Veterinary Medicine, School of Animal Sciences, Kitasato University.

References

[1] Berrier, B. W. (1969). Electrophoretic analysis of blood serum and plasma proteins of normal horses. *Amer. J. vet. Res.*, **30**, 2237–2240.

[2] Betsuyaku, T. (1962). Immunoelectrophoretic studies of serum protein subfractions—on α-globulin fraction. *Sapporo med. J.*, **22**, 269–287.

[3] Ek, N. (1969). Studies on electrophoresis on cellulose acetate membrane of bovine serum proteins in healthy animals. *Acta vet. Scand.*, **10**, 118–126.

[4] Fukuda, Y., and Shibanai, D. (1971). Cellulose acetate membrane electrophoretic studies on serum plasma protein in dogs. *Bull. Fac. Agric., Yamaguti Univ.*, No. 22, 393–412 (in Japanese).

[5] Herz, A., and Hod, I. (1969). The albumin/alpha globulin ratio in various physiological states in cattle. *Brit. vet. J.*, **125**, 326–329.

[6] Hirai, H. (1967). Introduction of Electrophoresis. *In* Method of Electrophoretic Experiment, Hirai, H., Abe, M., and Shimao, K., editors, Bunkodo, Tokyo, 1–23 (in Japanese).

[7] Hirayama, C., Fukuda, T., Yoshikawa, T., and Koga, S. (1964). Metabolism of plasma proteins. *Metabolism and Disease*, **1**, 572–581 (in Japanese).

[8] Hirayama, C., Fukuda, T., and Murai, N. (1968). Liver diseases and plasma proteins. *Saishin-Igaku*, **23**, 1598–1606 (in Japanese).

[9] Kozma, C. K., Pelas, A., and Richard, A. S. (1967). Electrophoretic determination of serum proteins of laboratory animals. *J. Amer. vet. med. Ass.*, **151**, 865–869.

[10] Mattheeuws, D. R. G., Kaneko, J. J., Loy, R. G., Cornelius, C. E., and Wheat, J. D. (1966). Compartmentalization and turnover of I^{131}-labeled albumin and gamma globulin in horses. *Amer. J. vet. Res.*, **27**, 699–705.

[11] Nagel, R., and Katz, R. (1963). Serum proteins in acute hepatitis: Their prognostic significance. *Amer. J. med. Sci.*, **240**, 198–205.

[12] Ogawa, Y., Abe, M., Kitamura, M., Kosakai, N., Shimao, K., Tomita, J., Hirai, H., and Kadoma, K. (1966). Standard technique of fractionation of serum proteins by electrophoresis with cellulose acetate membrane. *Physico-Chemical Biology*, **11**, 351–356 (in Japanese).

[13] Ogawa, Y. (1968). Standard method of cellulose acetate membrane electrophoresis. *Jap. J. clin. Path.*, Suppl. No. 11, 46–67 (in Japanese).

[14] Osbaldiston, G. W. (1972). Serum protein fractions in domestic animals. *Brit. vet. J.*, **128**, 386–393.

[15] Shimada, Y. (1969). Applications of clinical biochemistry for diagnosis in cattle. I. Fractionations of serum, and plasma proteins by cellulose acetate membrane electrophoresis. *J. vet. Med. (Tokyo)*, No. 500, 869–875 (in Japanese).

[16] Schmidt, J. (1968). Differentiation of animal protein by vertical electrophoresis in polyacrylamide gel. I. Technique, and bovine serum proteins. *Deut. tierärztl. Wschr.*, **75**, 87–91.

[17] Sunderman, F. W. (1964). Studies of the serum proteins. VI. Recent advances in clinical interpretation of electrophoretic fractionations. *Amer. J. clin. Path.*, **42**, 1–21.

[18] Sunderman, F. W., Jr., and Sunderman, F. W. (1957). Clinical applications of the fractionation of serum proteins by paper electrophoresis. *Amer. J. clin. Path.*, **27**, 125–158.

[19] Torii, T., Takahashi, K., and Doi, I. (1965). *In* Stochastics for Medicine and Biology. University of Tokyo Press, Tokyo, 2–53 (in Japanese).