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Bovine Myoglobin: Detection of Myoglobin in Sera and Urine of Calves with Nutritional Myopathy by Immunodiffusion Method

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ABSTRACT. Detection of myoglobin (Mb) in sera and urine of sixteen calves with nutritional myopathy was achieved by immunodiffusion method. Mb were detected in 12 serum samples out of 16 calves and in 9 urine samples out of 13 calves. Concentrations of Mb in serum and urine decreased markedly by treatment and were scarcely detected in any samples after one or two days. The minimal detectable concentration of Mb by immunodiffusion method was 5 μg per ml.—**KEY WORDS:** calf, myoglobin, nutritional myopathy.

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Calves with nutritional myopathy showed not only the marked elevations of serum enzyme activities, such as CPK, GOT and LDH, but also myoglobinuria in some of these calves [2, 3]. There have never been any reports on detection of bovine myoglobin (Mb) in blood and urine by immunodiffusion method. Myoglobin has been demonstrated by chemical testing [1], electrophoresis [7] and spectrophotometry [9]. However, these methods were insufficient to detect a small quantity of Mb and it was difficult to distinguish with certainty from hemoglobin. In our previous report [6], bovine Mb was purified, and specific anti-Mb serum was prepared. Then its antiserum was useful for detection and identification of Mb in serum and urine with myopathy. The present study describes the detection of Mb and the measurement of Mb levels in serum and urine of calves with nutritional myopathy by immunodiffusion method.

Mb was purified from bovine skeletal muscle by fractionation with ammonium sulfate and two cycles of crystallization in phosphate buffer. Then the purified Mb was

freeze-dried. Anti-Mb serum which was specific to Mb was prepared by immunizing rabbits against the purified Mb. Details of the method used were the same as described previously [6].

The potency of prepared anti-Mb serum by Ouchterlony method [8] was examined. Purified Mb was dissolved and diluted in 0.01 M phosphate buffer saline (PBS; pH 7.2). Ouchterlony plates were prepared with 1% agar in PBS. The plates containing Mb and antiserum were incubated at room temperature for 12 hrs and observed for precipitin lines. Consequently, the minimal concentration of Mb detectable with Ouchterlony method was 5 μg per ml as shown in Fig. 1. In comparison with other papers on the potency of anti-Mb serum, Watanabe *et al.* [10] reported that 6 μg per ml of horse Mb could be detected by the same method. The lowest detectable concentration of human Mb was reported as 0.5 μg per ml by Yakulis *et al.* [11] and 5 μg per ml by Miyoshi *et al.* [5].

Using prepared antiserum, detection of Mb in serum and urine of calves with

Fig. 1. Potency of anti-Mb serum by Ouchterlony method. (Ab) Anti-bovine Mb rabbit serum; (2)-(80) Concentration of purified bovine Mb as 2, 5, 10, 20, 40 and 80 μg per ml.

Fig. 2. Mb-positive finding of serum samples by Ouchterlony method. (Ab) Anti-bovine Mb rabbit serum; (Mb) Purified bovine Mb; (1)-(6) Serum samples of case No. 1, 3, 4 and 6.

Fig. 3. Mb-positive finding of urine samples by Ouchterlony method. (Ab) Anti-bovine Mb rabbit serum; (Mb) Purified bovine Mb; (4)-(9) Urine samples of case No. 4, 6, 7 and 9.

Table 1. Myoglobin concentration in serum and urine of calves with nutritional myopathy by single radial immunodiffusion method

Case No.	Serum ($\mu\text{g}/\text{ml}$)	Urine ($\mu\text{g}/\text{ml}$)
1	74	NT ^{a)}
2	—	—
3	6	—
4	6	20
5	—	—
6	18	137
7	6	14
8	—	NT
9	32	250
10	—	—
11	25	28
12	12	8
13	28	NT
14	14	31
15	12	10
16	6	6

a) Not tested.

nutritional myopathy was done by Ouchterlony method. During the period of January 1984 to June 1985, sixteen Japanese Black calves, which were 20 to 130 days of age, were examined. They were diagnosed by clinical symptoms of recumbency or stiffness and marked elevations of serum enzyme

activities (CPK; over 2,900 international units, GOT; over 1,360 Karmen units).

In detection of Mb in sera of the calves by Ouchterlony method, Mb-positive precipitin lines were observed in 12 samples out of 16 calves and the four positive cases are

Fig. 4. Changes of Mb concentration in serum and urine of calves with nutritional myopathy.

shown in Fig. 2. Then each of the precipitin lines fused completely with the line between anti-Mb serum and the purified Mb. In urine, Mb-positive precipitin lines were observed in 9 samples out of 13 calves and the four positive cases are shown in Fig. 3. Serum samples from those 9 calves which were Mb positive in urine were also positive for Mb.

Mb concentration in serum and urine were determined by single radial immunodiffusion method [4]. By this method all samples shown positive precipitin line by Ouchterlony method could be measured. The results of the measurement of Mb concentration in serum and urine are given in Table 1. Mb concentration in serum of case No.1 was 74 μg per ml and those of the other 11 cases ranged from 6 to 32 μg per ml. In urine of 9 cases, Mb levels ranged from 6 to 250 μg per ml. Particularly, Mb concentration of case No.9 was high (250 μg per ml) and the urine showed a reddish brown color.

Changes of Mb concentration in serum and urine of 8 cases are shown in Fig. 4. In

case No. 12 which was not treated with vitamin E, Mb in serum and urine were detected continuously until death. In the other calves, vitamin E or vitamin E-selenium were administered on the first day of onset. Mb concentration in serum and urine decreased progressively and it was not detected after one or two days.

In this study, Mb in serum and urine of the calves affected with nutritional myopathy were detected and identified by immunodiffusion method. But all of the cases were not always positive. The reason may be due to lower concentrations undetectable by immunodiffusion method. Further examining to detect a smaller quantity of Mb, technics on the detection of serum or urine Mb will appear to be specific and sensitive test for myopathy. Also the method may be utilized for evaluation on the status of diseases.

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要 約

免疫拡散法による仔牛の栄養性ミオパチーにおける血中と尿中ミオグロビンの検出について：納 敏・一条 茂（帯広畜産大学家畜内科学教室）——免疫拡散法によって栄養性ミオパチーの仔牛16例の血清および尿中ミオグロビン（Mb）の検出を試みた。Ouchterlony 法によって血中では16例中12例，尿中では13例中9例に Mb が検出され，かつこれらの陽性例は一元平板免疫拡散法によっても定量が可能であった。治療後の血中および尿中 Mb 濃度の減少は明瞭で，1～2 日後には検出されなかった。免疫拡散法による Mb の検出限界は 5 $\mu\text{g}/\text{ml}$ であった。