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Author(s)	YASUDA, Jun; TOO, Kimehiko
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## STUDIES ON SERUM CREATINE PHOSPHOKINASE ISOENZYME SEVEN CASES OF TETRAPLEGIA IN THE DOG

Jun YASUDA and Kimehiko TOO

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Canine serum creatine phosphokinase isoenzymes were determined in seven cases of Spondylosis deformans. The BB fraction of CPK isoenzymes in these cases was superior to that of other fractions except in one case which was accompanied by traumatic muscular damages because of decubitus. The isoenzyme distribution patterns changed into MM fraction superiority as in normal dogs as motor dysfunction improved.

Key words: creatine phosphokinase, CPK isoenzyme, dog, spondylosis deformans, veterinary medicine.

### INTRODUCTION

Plasma or serum creatine phosphokinase (CPK) is widely used as a diagnostic aid for detection of neuro-muscular and/or myocardial diseases such as progressive muscular dystrophy<sup>1,20,22)</sup> or cardiac infarction<sup>12,26)</sup> in human medicine. It is generally accepted that CPK occurs as a dimer of the subunits M and B, and three isoenzymes can be distinguished: MM, "skeletal muscles type"; hybrid MB, "myocardial type"; and BB, "brain-nerve type".<sup>2,3,6)</sup>

In domestic animals including the dog, plasma or serum CPK activity can be used as a diagnostic aid for detection of skeletal disease.<sup>3,10,11,14)</sup> However, in veterinary medicine, there have been few reports on clinical cases in which CPK isoenzyme was used.<sup>7,21)</sup> Therefore, the diagnostic values of CPK isoenzyme have been little known in this situation.

Peripheral neuropathies in dogs are frequently encountered in the daily clinic. The clinical manifestations of this disease are changes in sensations and varying degrees of motor dysfunction. Various causes of this disease have been reported,<sup>13)</sup> and many clinical case reports investigating the pathosis of the disease have been made by radiographic examination and/or neurological examination. On the other hand, few studies have been carried out from the clinico-enzymological viewpoint.

In the present study, CPK isoenzymes of two dogs in which tetraplegia was diagnosed were determined, and the change of isoenzyme pattern about CPK-BB was discussed.

TABLE 1 Summary of clinical data on the initial physical examination

CASE NO.	AGE(Y)	SEX	BREED	CLINICAL SIGN	RADIOGRAPHIC FINDINGS
1	4	M	Shitzu	Tetraplegia, Tenderness(Back)	N.S.I. (L 1-2)
2	7	M	Japanese spaniel	Tetraplegia, Tenderness(Back)	N.S.I. (T 11-12-13)
3	3	F	Dachshund	Limp & Tenderness(Right rear leg)	N.S.I. (T 10-11) S.B. (T 10-11) C.D.P. (L 2-3-4)
4	4	F	Maltese	Paralysis(Rear extremities)	N.S.I. (T 10-11-12-13) (L 2-3)
5	10	F	Dachshund	Staggering gait(Rear extremities)	N.S.I. (T 11-12)
6	6	F	Japanese spaniel	No tenderness & staggering gait Loss of muscular strength (Rear extremities)	C.D.P. (T 11-12) N.S.I. (T 13-L 1)
7	15	M	Collie	Tetraplegia, Tenderness(Back) Decubitus(Right side of the body)	N.S.I. (T 8-9-10-11-12-13) (L 1-2) S.B. (T 8-9-10, 11-12-13) C.D.P. (T 11-12-13)

T : Thoracic vertebrae      L : Lumbar vertebrae  
 N.S.I. : Narrowing of the spinal canal or intervertebral foramina  
 S.B. : Spondylotic Bridge      C.D.P. : Calcific Disk Protrusion

TABLE 2 Serum CPK isoenzyme in normal dogs

NO.	IU/l	MM %	MB %	BB %
1.	89.01	62	13	25
2.	83.50	51	14	35
3.	22.05	50.5	11.2	38.3
4.	55.02	53	19	28
5.	30.0	46.3	19.5	34.2
6.	11.1	52.6	14.5	32.9
7.	21.9	58.7	14.4	26.9
8.	15.8	49.4	22.1	28.5
9.	19.7	45.8	21.8	32.4
10.	15.2	51.0	11.1	37.9

36.3±29    52.0±5.0    16.1±4.2    31.9±4.6

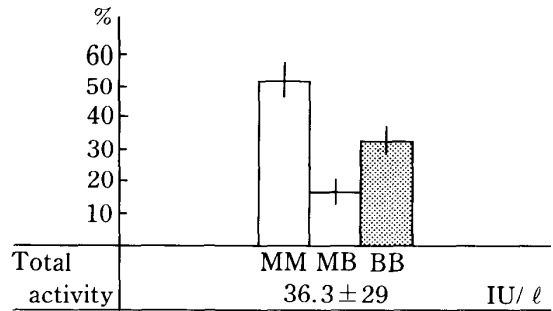


FIGURE 1 Serum CPK isoenzyme distribution pattern in normal dogs

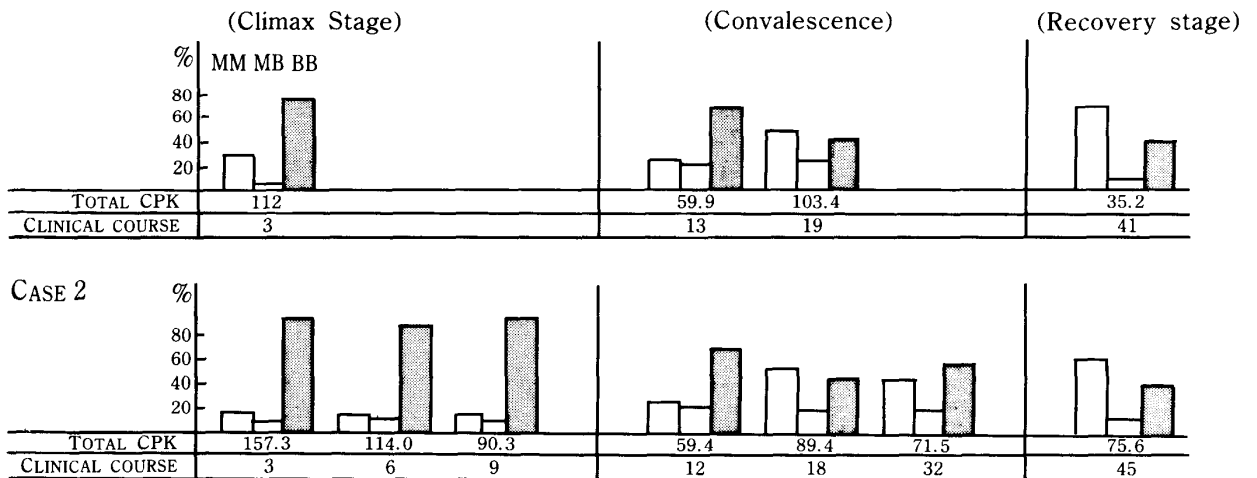


FIGURE 2 Serum CPK total activities and distribution patterns in Case 1 and 2

## MATERIALS AND METHODS

The dogs examined were admitted to the Veterinary Teaching Hospital at the University of Hokkaido because of tetraplegia and atrophy of paralyzed muscles in the rear legs. One dog was a 4 year-old, male Shitzu(case 1), and the other a 7 year-old, male Japanese spaniel(case 2), as shown in table 1. In addition to these two cases, 5 other dogs showing similar clinical findings were admitted to the hospital and determined CPK isoenzymes. The 5 cases were numbered 3, 4, 5, 6 and 7. The age, sex and breeds are shown in table 1. And as controls, 10 healthy dogs of various ages, sexes and breeds were used.

Peripheral blood samples were collected from the outpatients at different clinical stages. Simple lateral radiographs of the thoraco-lumber region were taken at the initial examination. Blood serums were separated as soon as possible and the CPK total activity was determined by Unikit-Rate reagents.\* CPK isoenzymes were separated chromatographically by a mini column of DEAE Sepharose CL-6B.\*\*<sup>16,18)</sup> The CPK activity of aliquots of column effluents were determined by the UV method\*\* and calculated the distribution ratio. Control serum samples from 10 healthy dogs were determined in the same manner.

## RESULTS

Upon initial physical examination, two cases 1 and 2 showed clear consciousness, but extention and paralysis in the extremities had developed. Sensory examination revealed hypesthesia to touch and pain in the rear legs. Serious atrophy of the paralyzed muscles in the rear legs was also apparent along with tenderness in the lumber region.

Approximately 7-9 weeks after presentation, tenderness in the lumber region began to lessen. A slight extensor postural thrust reaction was revealed, while the muscle strength of the rear legs was still decreased. After approximately 10 weeks, the dogs were able to roll over on their sides and stand up for a short time. And after 12 weeks, they could walk lamely. At 41-45 weeks, their motor dysfunction had disappeared, and up to the present, no signs of recurrence have been noticed.

Clinical findings of the 5 similar cases(cases 3, 4, 5, 6 and 7) are shown in table 1. The degrees of tetraplegia or staggering gait were various. Lateral radiographs showed narrowing of the spinal canal or intervertebral foramina, spondylotic bridge and/or calcific disk protrusion. Based on these data, the above cases were diagnosed as Spondylosis deformans. Serious decubitus of the right side of the body was recognized in case 7. In some cases the follow-up survey could not be conducted as

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\* Chugai Pharm. Co., Tokyo, Japan

\*\* Fujisawa Pharm. Co., Tokyo, Japan

the dogs had to be returned to their owners.

The serum CPK total activities and isoenzyme distribution ratio of 10 control dogs are summarized in table 2. Total activity values ranged from 11.1–89.0 with a mean of  $36.3 \pm 29$  IU/l. The mean isoenzyme distribution ratio was  $52.0 \pm 5.0$ ,  $16.1 \pm 4.2$  and  $31.9 \pm 4.6\%$  for MM, MB and BB, respectively. The MM fraction dominated over the other fractions. (fig. 1)

Figure 2 gives the serum CPK total activities and isoenzyme distribution ratio of cases 1 and 2 in each clinical stage. The BB fraction dominated during the climax stage of motor dysfunction. Following the cure of motor dysfunction, the BB fraction showed a tendency to decrease. And in the recovery stage, CPK isoenzyme distribution patterns agreed with the control patterns.

The relationship between clinical findings and the CPK values of the 5 similar cases (cases 3, 4, 5, 6 and 7) is indicated in figure 3A–3E. Case 3: Serum CPK total and BB fraction activities were 37.3 and 16.7 IU/l, respectively, at the initial examination. When paralysis of the rear legs appeared after four days, a moderate increase of only the BB fraction was observed. Case 4: Staggering gait occurred repeatedly. When the gait was observed, CPK determinations were executed, at which time the isoenzyme distribution pattern was in agreement with the controls. Within a short time, the motor dysfunction disappeared, and after one month, the BB fraction increased moderately, and the staggering gait reappeared. Case 5: For all the tendency of BB fraction to decrease, the clinical signs of improvement could not be confirmed. Case 6: Agreement between the degrees of staggering gait and CPK-BB variations was observed. Case 7: A remarkable increase of CPK-MM originating from the damaged muscles occurred as a result of decubitus, while the activity of serum CPK-BB showed a slight increase. The dog died on the 15th hospital day without symptomatic improvement.

The results provide convincing evidence that canine serum CPK-BB variations correspond to the degree of clinical findings in peripheral neuropathies.

#### DISCUSSION

The terms peripheral nerve system in this study are defined on the basis of functional viewpoints and clinical findings; that is, the peripheral nerve systems are composed of all the lower motor neurons (LMN) such as motoneurons at the spinal cord, roots, root ganglia, spinal and peripheral nerves. The terms peripheral neuropathies refer to injury or disease of these components.

The serum CPK total and BB activities of healthy dogs obtained in this study were in agreement with other reports as shown in table 3.<sup>5,9,17,24)</sup>

As in man, only the BB fraction activity is separated from canine nervous tissues.<sup>19,24)</sup> On the other hand, CPK-BB activity in canine serum is originally higher than in man,<sup>15,23)</sup> cattle<sup>25)</sup> and the horse<sup>7,21)</sup> as shown in table 3. Therefore, in the

FIGURE 3 Relationship between the change of CPK isoenzyme patterns and the clinical signs of cases 3-7 are illustrated in A-E, respectively

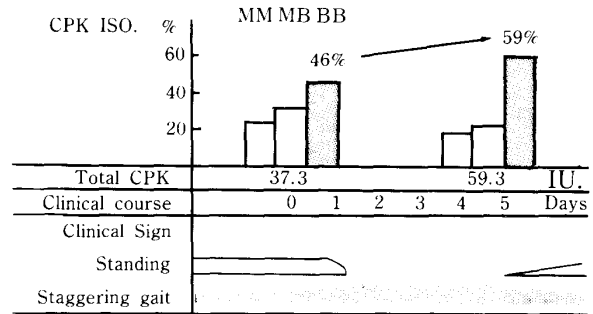


Fig3-A (case 3)

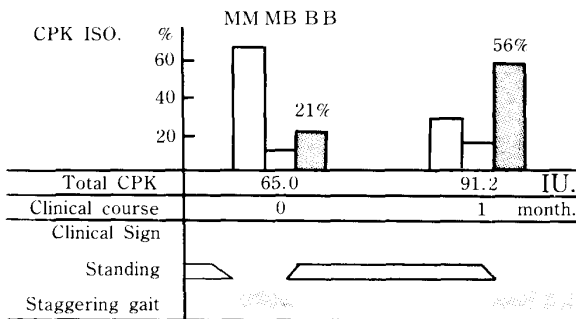


Fig3-B (case 4)

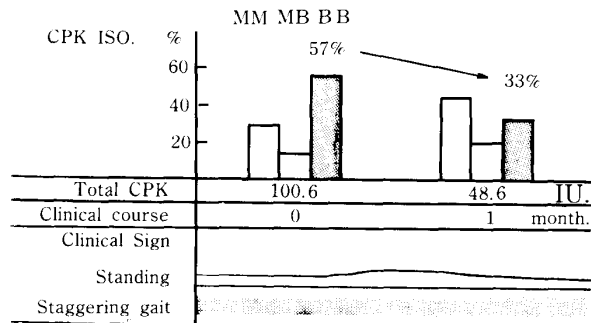


Fig3-C (case 5)

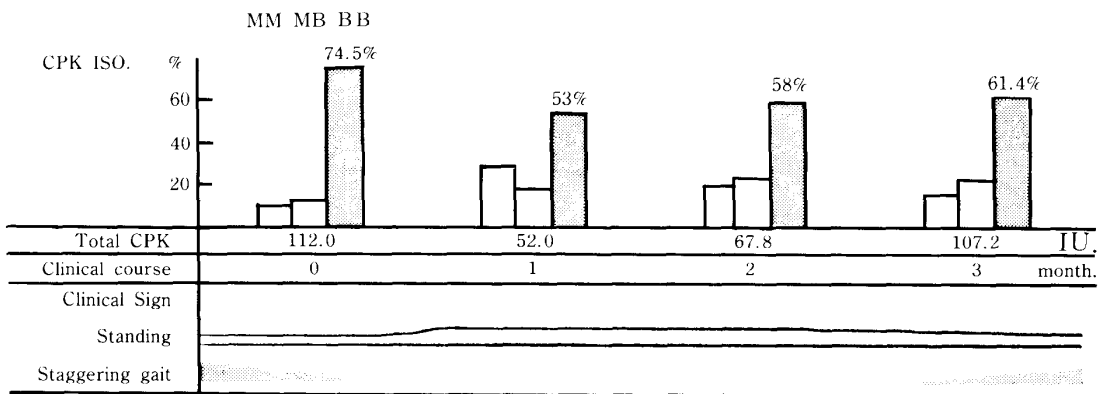


Fig3-D (case 6)

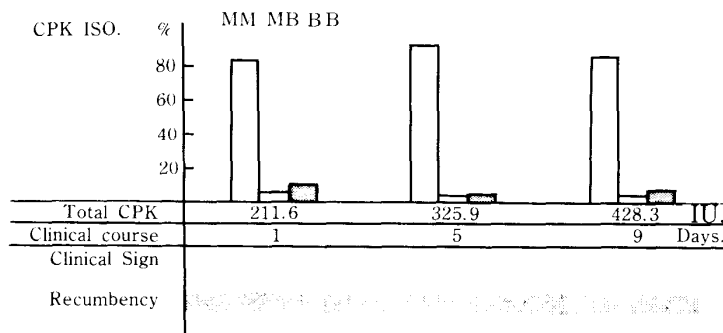


Fig3-E (case 7)

TABLE 3 *Serum CPK values in domestic animals and man*

	T-CPK IU/L	ISOENZYME %			
		MM	MB	BB	
Dog	36.3±29	52.0±5.0	16.1±4.2	31.9±4.6	YASUDA et al.
	115.6±91.5	53.0±5.0	1.4±2.4	45.5±20.4	WATANABE et al.(1982)
	22.0				HEFFRON et al.(1976)
Horse	23.4±6.3	77.4	2.1±1.2	18.5±6.8	FUJII et al.(1980)
	24.3	90.6	7.0	2.3	OHISHI et al.(1978)
Cattle	18.4±15.4	84.6±8.3	11.7±7.0	3.3±2.2	YASUDA et al.(1982)
Man	13-64	97-98	2-3	0	SASAKI et al.(1976)
	72	92	6	2	KAKEI et al.(1977)

case of motor dysfunction, it can be considered as the increase of serum CPK-BB activity in canines is observed to be more remarkable than in man, cattle and the horse.

According to the conception of leakage enzymes, it is conceivable that the leakage values of the enzyme originating from the nervous tissues increase in the case of motor dysfunction.<sup>3,8)</sup> However, there are some reports that serum CPK-BB activity in man does not always increase due to the neuropathies.<sup>12,17,27)</sup> Considering the fact of the remarkable elevation of CPK-BB activity in this study, it may be conceivable that the enzymes originating from the nervous tissues have leaked into the peripheral blood in the canine. Whether peripheral neuropathies can be cured or not depends on the qualities and quantities of organic damages in the peripheral nervous system. In other words, even in cases where organic damages in the nervous system have spread widely, there is some possibility that they can be cured clinically as far as their stages are reversible or non-progressive. It is probable that the leakage of CPK-BB decreases with the cure of the affected parts.

Generally, motor dysfunction is accompanied by decubitus because of oppressive hindrance of circulation in the muscles. It appears that a remarkable increase in serum CPK total activity occurs in decubitus.<sup>5)</sup> In such cases it is difficult to distinguish nervous damage from muscular damage by means of serum CPK total activity. It was reported that serum CPK-MM shows increase in muscular damage.<sup>21)</sup> However, in the present study, decubitus in the muscles was not observed in small-sized dogs, and there was almost no leakage of CPK-MM originating from the muscles into the serum. Accordingly, the change of serum CPK-BB was singularly observed. However, in large-sized dogs such as case 7, or in large animals such as cattle and horses, tetraplegia is always accompanied by decubitus. In such cases, as the MM fraction occupies the major part of the isoenzyme, the change of BB is not observed alone. It may be that the leakage value of CPK-MM originating from the damaged muscles is significantly higher than that of CPK-BB originating from damaged nervous systems. Thus in cases of canine tetraplegia with traumatic muscular damages, it is necessary to determine the level of serum CPK-BB not only by relative values but also by absolute values. In the present study, only one case of tetraplegia accompanied by traumatic muscular damage was examined (case 7), and its clinical course was very long with a chronic and nonprogressive stage of neuropathies. Even in such a case, the absolute values of serum CPK-BB showed a slight increase. Moreover, the clinical course of neuropathies is generally longer than that of traumatic muscular damages. Our experience demonstrated that the recovery course of traumatism should be taken into consideration in evaluating canine serum CPK isoenzymes.

On the basis of our clinical observations, we concluded that determination of serum CPK-BB fraction in canine peripheral neuropathies is a useful method for



judging the prognosis and selecting the most appropriate treatment of nonsurgical or surgical intervention.

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