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LACTATE DEHYDROGENASE AND CREATINE PHOSPHOKINASE ISOENZYMES IN TISSUES OF LABORATORY ANIMALS

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The lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) isoenzyme distributions in tissues of the ICR mouse, Wistar rat, guinea pig and golden hamster were analyzed by histoelectrophoresis. Tissues obtained were as follows: liver, pancreas, stomach, small intestine, heart, femoral muscle, uterus, kidney, spleen, lymph node, cerebrum, spinal cord and erythrocyte. Histoelectrophoresis was for the direct analysis of LDH and CPK isoenzymes in the tissues and had high practical value compared with previous tissue-extraction methods. In tissues of the mouse, guinea pig and golden hamster, LDH isoenzymes showed five bands. In the rat, LDH isoenzyme was separated into four fractions. CPK isoenzyme showed three bands; BB, MB and MM. In some tissues, the MM band was separated into two sub fractions.

Key Words: lactate dehydrogenase (LDH), creatine phosphokinase (CPK), isoenzyme, tissue, laboratory animal.

INTRODUCTION

Various biochemical markers concerning organic diseases have been applied by means of analyzing the activities of blood enzymes in both laboratory and domesticated animals[16,18]. We have developed a new method of histoelectrophoresis for the direct analysis of hepatic LDH isoenzyme patterns in bovines, instead of that with the serum[22]. Histoelectrophoresis is a modified technique of the method by Davis[5], and it has a high practical value compared with previous tissue-extract electrophoretic methods[21]. The subjected organs for this method in the common veterinary clinic are confined to liver or kidney, to which the biopsy techniques can easily be applied[9,11]. It is expected, however, that there would be many organs applicable to the direct electrophoretic method in the laboratory animals. In the present study using this method, we examined the distribution of lactate dehydrogenase (LDH) and creatine

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phosphokinase (CPK) isoenzymes in various tissues derived from four species of laboratory animals such as the mouse, rat, guinea pig and golden hamster and discussed the possibility of their value to analyze pathological conditions in experimental animals.

MATERIALS AND METHDS

Animals : The Jcl : ICR mice (21 males and 20 females at 5–23 weeks of age, closed colony), the Jcl : Wistar rats (20 females at 6–9 weeks of age, closed colony), the guinea pigs (6 females at 4 weeks of age) and the golden hamsters (one male and 6 females at 4–5 weeks of age) were used in this experiment. The mice and rats were housed in a SPF enviroment, and the guinea pigs and golden hamsters were in a conventional environment. The animals were distributed from the Animal Experimentation Division, Faculty of Medicine, Sapporo Medical College. All animals employed were in apparently healthy condition. The tissues obtained from these animals were as follows : liver, pancreas, the cardiac part of the stomach (gast A), the pyloric part of the stomach (gast B), small intestine, heart ventricle, femoral muscle, uterus, renal cortex, spleen, mandibular lymph node, cerebrum, spinal cord and erythrocyte (Tabs. 1–8). LDH and CPK isoenzymes in tissues were separated by polyacrylamide disc gel (PADG) electrophoresis. A 7–cm long rod of 6% PADG for LDH analysis and 7.5% for CPK were prepared in glass tubes (3.5–mm inside diameter and 100–mm length) as described previously[21,22]. A small piece (ca. 1 mg) of tissue for histoelectrophoresis was cut off with opthalmoscissors. After placing the samples on top of the gel, electrophoresis was run at a constant current of 1 mA/tube for 50 min with 1 mM Tris 77 mM glycine buffer at room temperature. To visualize the isoenzyme patterns, LDH–LQ and CPK–S kits (Iatron Laboratories, Inc.) were used. The enzyme reaction of LDH was stopped by immersing gels in 5% acetic acid solution. The resultant zymograms were scanned with a Cliniscan integrating densitometer (Helena Laboratories Co. Ltd.) at 570 nm. The zymograms of CPK isoenzymes were directly scanned, without stopping the enzyme reaction[21].

RESULTS

The mean values of the percentages of tissue LDH and CPK isoenzyme fractions in the animals employed are shown in Tables 1–8 and Figures 1–4, respectively.

In tissues of the mouse, LDH1, LDH2 and LDH5 were the main isoenzymes only in the spinal cord. LDH4 and LDH5 were predominant isoenzymes in the pancreas, gast B, small intestine, femoral muscle, uterus, spleen and mandibular lymph node. Liver and erythrocyte predominantly had only fraction LDH5. Gast A, heart ventricle, renal cortex and cerebrum possessed five LDH fractions *i,e,* LDH1 through LDH5 (Tab. 1 & Fig. 1). Pancreas, gast A and B, small intestine, uterus, spleen, mandibular lymph node, cerebrum and spinal cord mainly had CPK–BB and MB isoenzymes.

TABLE 1. LDH isoenzyme distribution in main tissues of the mouse

		LDH ISOENZYME (% : MEAN±S.E.)				
	N	LDH 1	LDH 2	LDH 3	LDH 4	LDH 5
Liver	6	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	99.9±0.1
Pancreas	7	1.1±0.7	1.0±0.6	0.0±0.5	23.0±1.7	74.5±2.1
Gast. A	8	16.8±1.1	22.0±0.5	15.9±1.1	21.2±1.2	24.2±1.8
Gast. B	6	2.4±0.6	2.5±0.6	5.8±2.1	26.7±6.2	62.6±7.6
Small intestine	7	1.6±0.6	3.6±1.0	3.5±1.1	13.2±4.3	78.1±3.5
Heart ventricle	7	19.7±1.5	29.5±1.1	29.2±0.7	16.6±1.7	4.9±0.7
Femoral muscle	6	0.7±0.2	1.5±0.4	3.1±0.5	13.3±1.4	81.4±2.1
Uterus	6	5.1±0.7	10.1±0.9	13.1±1.4	30.8±0.9	40.9±3.2
Renal cortex	9	19.6±1.5	27.5±1.7	21.6±2.0	20.7±2.0	10.5±2.1
Spleen	7	0.1±0.0	0.0±0.0	0.1±0.1	5.4±1.9	94.3±2.0
Mandibular Lym.	7	3.4±0.7	5.5±1.1	4.0±0.9	24.7±3.0	62.5±2.9
Cerebrum	7	24.6±3.6	23.5±2.0	19.9±1.6	20.5±2.8	11.4±1.7
Spinal cord	6	39.2±3.9	25.3±2.8	7.1±1.2	7.6±1.8	20.7±4.5
Erythrocyte	6	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2	99.8±0.2

TABLE 2. CPK isoenzyme distribution in main tissues of the mouse

		CPK ISOENZYME (% : MEAN±S.E.)		
	N	BB	MB	MM
Liver	13	10.2±2.4	89.8±2.3 *	
Pancreas	7	37.1±7.5	59.1±7.6	3.8±1.8
Gast. A	7	55.7±2.5	41.5±1.7	2.8±1.2
Gast. B	7	60.3±4.7	36.6±4.0	3.1±1.0
Small intestine	7	63.7±2.9	24.8±4.0	11.6±3.0
Heart ventricle	8	2.6±1.0	55.6±3.9	41.8±4.3
Femoral muscle	8	1.7±0.7	40.2±3.3	58.0±3.8
Uterus	6	71.9±5.1	26.7±4.7	1.3±0.7
Renal cortex	8	8.3±1.6	40.3±5.4	51.4±4.8
Spleen	9	44.7±3.8	49.7±2.5	4.5±1.6
Mandibular Lym.	7	39.1±5.8	56.5±5.3	4.4±1.8
Cerebrum	6	63.1±2.9	34.6±2.8	2.3±0.7
Spinal cord	12	53.3±4.1	34.2±2.1	12.6±3.1

* MB and MM were not distinguished

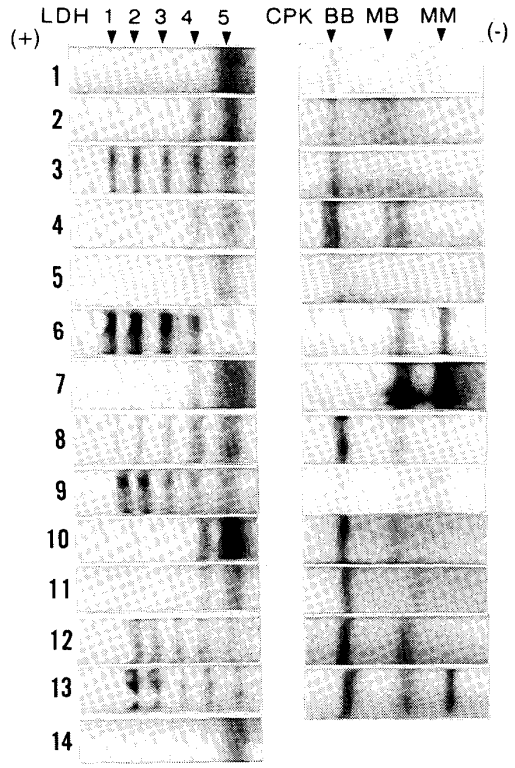

Fig. 1 Zymograms of LDH and CPK isoenzyme in the mouse. 1: liver, 2: pancreas, 3: gast A., 4: gast B., 5: small intestine, 6: heart ventricle, 7: femoral muscle, 8: uterus, 9: renal cortex, 10: spleen, 11: mandibular lymph node, 12: cerebrum, 13: spinal cord, 14: erythrocyte.

TABLE 3. LDH isoenzyme distribution in main tissues of the rat

	N	LDH ISOENZYME (% : MEAN±S.E.)			
		LDH 1	LDH 2	LDH 3	LDH 4
Liver	6	1.2±0.7	1.0±0.5	2.1±1.6	95.7±1.8
Pancreas	6	0.0±0.0	0.2±0.1	3.1±1.4	96.6±1.3
Gast. A	7	4.4±1.5	12.4±4.9	27.9±2.5	55.3±8.4
Gast. B	7	4.4±1.6	20.7±4.7	30.9±3.1	45.2±7.0
Small intestine	6	0.6±0.6	6.1±1.7	24.1±2.4	69.2±3.5
Heart ventricle	8	21.1±1.4	34.7±1.4	29.2±1.1	15.0±1.3
Femoral muscle	6	6.8±1.6	14.7±3.8	19.7±3.1	58.9±8.2
Uterus	6	5.0±1.6	19.3±2.8	31.8±3.6	42.9±6.0
Renal cortex	8	30.4±2.1	31.8±2.0	20.2±1.6	17.6±2.7
Spleen	7	0.9±0.4	5.3±1.8	25.9±2.9	67.9±4.9
Mandibular Lym.	6	2.3±1.0	8.4±2.2	25.1±3.0	64.4±2.4
Cerebrum	8	15.9±2.0	21.0±2.0	34.7±2.0	28.4±2.5
Spinal cord	8	38.8±4.3	25.9±1.9	18.2±2.0	16.7±3.5
Erythrocyte	6	0.2±0.2	0.0±0.0	0.0±0.0	99.8±0.1

Fraction CPK–MB was separated into two sub fractions in the gast B. Heart ventricle, femoral muscle and renal cortex predominantly had CPK–MB and MM fractions. It was possible to demonstrate a small amount of CPK–BB isoenzyme in the liver, but the MB band and the MM band were inseparable because of tailng (Tab. 2 & Fig. 1).

In the rat, LDH isoenzymes were separated into four fractions. LDH1, 2 and 3 bands were migrated like to the mouse, but LDH4 band in the rat were migrated between LDH4 and LDH5 bands in the mouse. Heart ventricle, renal cortex and spinal cord mainly had LDH1, LDH2 and LDH3. Liver, pancreas and erythrocyte only had LDH4. In the other tissues, LDH4 was the predominant fraction (Tab. 3 & Fig. 2). Gast A, small intestine, uterus, spleen, mandibular lymph node, cerebrum and spinal cord mainly had CPK–BB isoenzyme and had a small amount MM isoenzyme as well. While, liver, pancreas, heart ventricle, femoral muscle and renal cortex chiefly had CPK–MM isoenzyme in addition to a small amount of fraction BB (Tab. 4 & Fig. 2).

In the guinea pig, LDH1, LDH2 and LDH3 were predominant isoenzymes in the pancreas, gast A, heart ventricle, renal cortex, cerebrum and spinal cord. Erythrocyte mostly had fraction LDH1. LDH5 was the main band only in the femoral muscle. LDH3 and LDH4 were the main isoenzymes in the other tissues (Tab. 5 & Fig. 3). CPK–BB and MM were recognized in the uterus, mandibular lymph node, cerebrum and spinal cord. Heart ventricle and femoral muscle mainly had CPK–MM and had a small amount of fraction MB. Fraction CPK–MM was separated into two sub fractions. Liver, gast A and B, small intestine, renal cortex and spleen predominantly had three isoenzymes: CPK–BB, MB and MM. The MB and MM bands were inseparable in the pancreas because of tailing (Tab. 6 & Fig. 3).

In the golden hamster, LDH1 and LDH2 isoenzymes were the main fractions in the gast A and B, heart ventricle, renal cortex, cerebrum and spinal cord. On the other hand, LDH4 and LDH5 were the predominant fractions in the liver, pancreas, small intestine, femoral muscle, spleen and mandibular lymph node. LDH2, LDH3 and LDH4 were the main bands in the uterus and erythrocyte (Tab. 7 & Fig. 4). The other tissues mostly had CPK–BB and had a small amount of CPK–MM. Liver, pancreas, heart ventricle, femoral muscle and renal cortex predominantly had CPK–MM isoenzyme and had a small amount of CPK–BB. Fraction CPK–MM was separated into two sub fractions. CPK–MB bands were recognized in the gast A, small intestine, heart ventricle and uterus (Tab. 8 & Fig. 4).

Discussion

The isoenzyme patterns of each tissue vary in each species of animal[3,10,14,15], but the patterns of the same tissue of the closely related species are similar to each other[17]. Although the mouse (*Mus musculus*), rat (*Rattus norvegicus*), guinea pig

TABLE 4. CPK isoenzyme distribution in main tissues of the rat

	N	CPK ISOENZYME (% : MEAN±S.E.) BB	MB	MM
Liver	10	24.0±2.6	0.1±0.1	75.9±2.7
Pancreas	7	26.2±7.4	0.9±0.6	72.8±7.7
Gast. A	6	63.6±2.3	7.9±1.5	28.5±2.5
Gast. B	6	45.6±4.5	6.1±2.3	48.2±5.6
Small intestine	8	59.9±4.6	5.4±1.1	34.6±5.0
Heart ventricle	7	6.6±1.3	22.5±2.6	70.9±3.2
Femoral muscle	6	2.2±1.0	2.7±0.7	95.1±1.6
Uterus	6	62.5±2.6	6.0±2.5	31.5±4.5
Renal cortex	6	33.0±6.2	2.8±1.7	21.7±5.1 42.5±6.0 *
Spleen	8	67.6±4.6	2.4±1.2	29.9±4.8
Mandibular Lym.	6	55.5±6.0	0.9±0.6	43.6±6.6
Cerebrum	7	65.7±3.2	0.3±0.2	34.0±3.3
Spinal cord	8	57.1±4.8	0.8±0.3	42.7±4.2

* MM was separated in two sub fractions

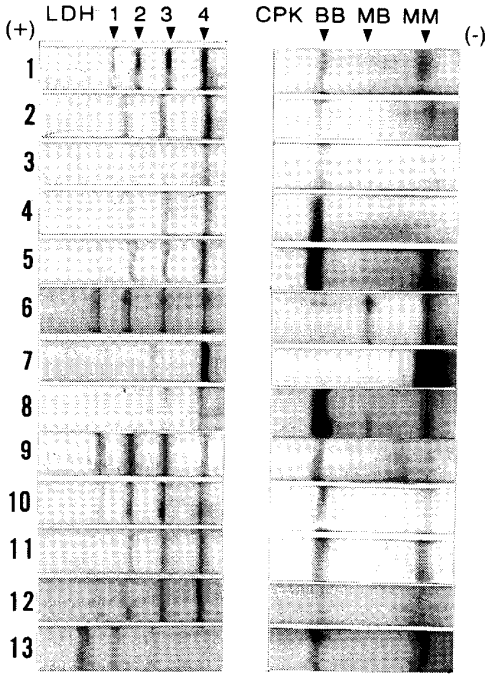

Fig. 2 Zymograms of LDH and CPK isoenzyme in the rat. 1–13: See Fig. 1.

TABLE 5. LDH isoenzyme distribution in main tissues of the guinea pig

	N	LDH ISOENZYME (% : MEAN±S.E.)				
		LDH 1	LDH 2	LDH 3	LDH 4	LDH 5
Liver	6	12.6±2.4	24.8±3.5	34.4±2.1	21.3±4.1	6.8±3.0
Pancreas	6	22.1±1.0	35.0±0.9	34.1±0.9	8.6±1.1	0.3±0.2
Gast. A	6	52.3±4.3	17.3±0.8	19.7±2.1	12.2±1.7	2.6±0.9
Gast. B	5	28.1±9.0	14.1±1.4	31.2±5.0	20.7±3.6	4.9±1.9
Small intestine	6	5.5±0.9	13.3±0.6	33.1±2.6	32.4±2.0	15.6±4.0
Heart ventricle	6	29.1±1.8	33.9±0.7	28.1±1.3	8.4±0.8	0.5±0.2
Femoral muscle	6	3.7±1.0	4.0±0.5	8.8±1.1	17.6±1.7	65.9±3.5
Uterus	6	9.2±1.9	22.9±2.1	38.3±1.8	24.3±3.4	5.3±3.4
Renal cortex	6	53.5±3.8	25.2±1.5	15.8±2.4	4.4±0.9	1.1±0.3
Spleen	6	8.6±2.4	14.9±3.7	33.4±3.2	30.0±5.1	13.1±5.5
Mandibular Lym.	3	12.2±6.0	14.7±3.5	28.6±2.7	32.2±5.2	12.4±3.5
Cerebrum	6	23.6±1.8	28.6±1.1	33.2±1.6	14.2±2.3	0.2±0.2
Spinal cord	6	44.4±2.5	27.7±1.5	21.2±1.5	7.3±2.2	0.6±0.3
Erythrocyte	6	85.4±3.3	8.9±2.1	4.5±1.8	0.9±0.5	0.3±0.1

TABLE 6. CPK isoenzyme distribution in main tissues of the guinea pig

	N	CPK ISOENZYME (% : MEAN±S.E.)		
		BB	MB	MM
Liver	6	24.2±1.4	11.0±2.8	64.8±2.8
Pancreas	5	11.3±2.9	88.9±3.0 *	
Gast. A	6	46.1±6.7	14.2±3.9	39.7±9.6
Gast. B	6	41.2±6.2	14.2±2.1	44.6±7.3
Small intestine	6	54.0±4.6	18.8±5.3	27.2±7.7
Heart ventricle	6	1.4±0.5	7.9±1.5	90.8±1.9
Femoral muscle	6	0.3±0.2	2.2±1.0	97.5±1.1
Uterus	6	51.2±3.6	4.8±2.8	40.9±2.9
Renal cortex	6	22.4±3.6	9.9±2.5	67.4±3.4
Spleen	6	39.4±8.2	7.0±1.8	53.4±7.4
Mandibular Lym.	6	62.4±3.5	2.0±0.9	35.7±4.0
Cerebrum	6	52.5±3.3	1.6±0.6	45.9±3.1
Spinal cord	6	53.2±6.2	2.3±0.6	44.5±5.8

* MB and MM were not distinguished

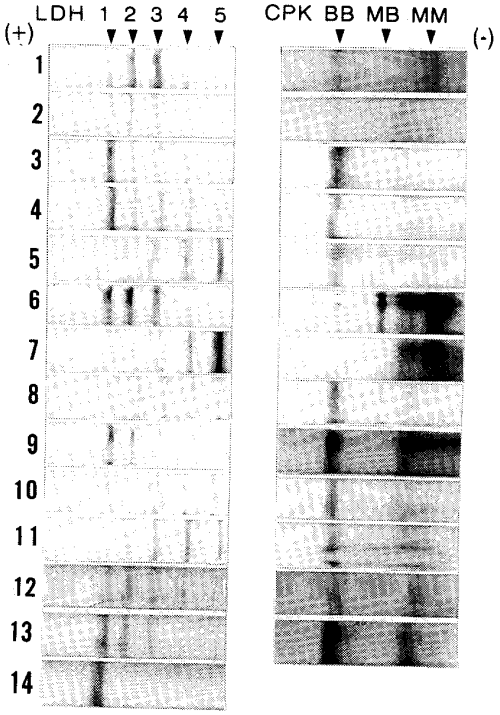

Fig. 3 Zymograms of LDH and CPK isoenzyme in the guinea pig. 1–14: see Fig. 1.

TABLE 7. LDH isoenzyme distribution in main tissues of the hamster

	N	LDH ISOENZYME (% : MEAN±S.E.)				
		LDH 1	LDH 2	LDH 3	LDH 4	LDH 5
Liver	7	1.2±0.5	1.1±0.4	0.8±0.6	3.5±1.2	93.4±2.0
Pancreas	6	0.9±0.3	2.1±0.6	11.7±3.2	14.5±1.5	70.7±4.8
Gast. A	6	22.8±3.5	33.9±2.6	26.2±2.6	9.1±0.9	7.0±1.1
Gast. B	6	32.3±4.9	35.1±1.7	18.5±2.6	7.4±2.6	6.7±2.1
Small intestine	6	9.7±2.3	8.7±1.5	3.6±1.4	2.0±0.7	76.0±5.4
Heart ventricle	7	46.2±2.4	36.7±0.9	11.4±0.6	3.2±0.9	2.5±0.9
Femoral muscle	7	4.2±1.1	9.8±2.8	9.9±1.8	11.7±1.8	64.3±5.7
Uterus	7	2.9±0.8	15.4±3.1	23.4±4.0	17.1±3.3	29.6±3.5
Renal cortex	7	31.6±2.0	39.6±3.0	20.6±1.3	5.1±1.2	3.1±0.9
Spleen	6	2.2±0.8	5.1±1.4	13.2±1.7	17.9±2.6	61.7±3.4
Mandibular Lym.	6	0.8±0.4	2.5±0.5	10.9±1.7	15.1±0.5	70.7±2.1
Cerebrum	7	24.8±2.1	38.5±1.4	22.2±1.3	9.1±1.6	4.6±1.4
Spinal cord	6	34.4±2.2	35.3±2.3	20.3±2.1	7.2±1.2	2.8±0.7
Erythrocyte	6	0.1±0.1	17.2±2.3	30.7±1.4	25.0±1.9	27.0±2.0

TABLE 8. CPK isoenzyme distribution in main tissues of the hamster

		CPK ISOENZYME (% : MEAN±S.E.)			
	N	BB	MB	MM 1	MM 2 *
Liver	6	22.5±2.6	0.7±0.4	21.7±4.3	55.1±5.4
Pancreas	6	17.2±1.8	1.0±0.4	76.1±2.3	5.7±2.9
Gast. A	6	55.0±4.6	17.3±2.0	27.1±3.5	0.7±0.4
Gast. B	6	59.9±4.7	4.0±1.2	28.7±5.7	7.4±3.0
Small intestine	6	63.7±2.7	10.5±2.2	24.8±3.0	1.0±0.5
Heart ventricle	6	2.9±0.9	14.4±1.7	44.0±2.7	38.7±2.5
Femoral muscle	6	1.6±0.6	2.9±1.0	33.1±1.2	62.1±1.8
Uterus	6	52.8±2.9	12.4±0.8	31.8±1.5	3.1±1.5
Renal cortex	6	31.2±5.3	1.0±0.6	64.7±6.0	3.9±2.5
Spleen	6	61.6±5.9	2.2±0.8	34.8±5.3	1.5±0.4
Mandibular Lym.	7	55.2±1.8	3.5±1.0	39.9±1.5	1.4±0.5
Cerebrum	6	51.6±5.5	1.6±0.6	34.8±4.5	12.0±5.0
Spinal cord	6	56.0±5.0	3.6±0.7	34.4±3.8	6.0±2.1

* MM was separated in two sub fractions, MM 1 and MM 2

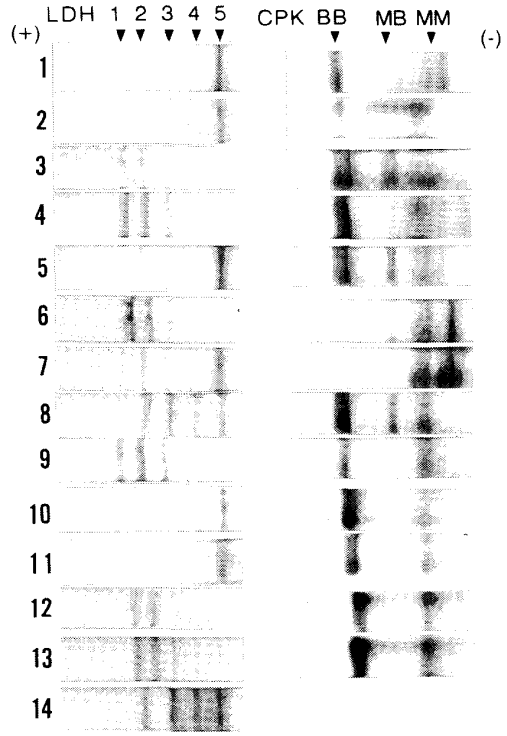

Fig. 4 Zymograms of LDH and CPK isoenzyme in the hamster. 1-14: see Fig. 1.

(*Cavia porcellus*) and golden hamster (*Mesocricetus auratus*) are all belonging to Rodentia, the isoenzyme patterns of LDH and CPK in some tissues varied from each other in this experiment. Therefore, it is necessary to understand the distribution of tissue isoenzymes of each animal. In laboratory animals such as the mouse and rat, there have been many reports about parts of tissuee isoenzymes[1,4,7,8,15,17], but there are few reports that have systematically analyzed isoenzymes of various tissues at the same time[13,19]. Liver and erythrocyte LDH isoenzyme patterns of laboratory animals obtained by the direct electrophoretic method were similar to those obtained by the extracting method[1,17,20]. CPK isoenzyme patterns in main tissues of laboratory animals were similar to those of humans and other mammals[2,6,12]. Analytical values obtained by the tissue-extracting method, however, varied more than those obtained by this direct method[14]. There is also evidence that enzyme activity can be inactivated during extraction. Therefore, the histoelectrophoretic method is thought to be appropriate for the detailed analysis of tissue isoenzyme patterns in laboratory animals. This method has different significance from serum isoenzyme analysis and may be the markers concerning organic damages in laboratory animals. As enzyme proteins are direct products of gene expression, variation at the genetic level is reflected in variations of the tissue isoenzyme compositions. This technique may also be applied to elucidate ontogenetic and phylogenetic classification of inbred laboratory animals.

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