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ANALYSIS OF TISSUE CREATINE PHOSPHOKINASE ISOENZYMES BY HISTOELECTROPHORESIS

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Tissue creatine phosphokinase isoenzymes in cattle and swine were analyzed by histoelectrophoresis. This method is modification of the polyacrylamide disc gel electrophoretic technique of DAVIS and has high practical value compared with previous tissue extracts electrophoretic methods.

The skeletal muscle predominantly had MM isoenzyme. The spinal cord had BB and MM isoenzymes. The cardiac muscle had MB and MM isoenzymes. The smooth muscle of cattle had BB and MM isoenzymes. The smooth muscle of swine had BB, MB and MM isoenzymes.

Key Words: creatine phosphokinase, CPK isoenzyme, tissue, cattle, swine, polyacrylamide disc gel electrophoresis, Veterinary medicine

INTRODUCTION

Creatine phosphokinase (CPK) is recognized as an important enzyme for muscular energy metabolism, and is found mostly in brain, skeletal, cardiac and smooth muscle.^{2,13)} The isoenzymes of CPK have been proved to be composed of MM (muscular type), BB (brain type) and MB (hybrid type).^{3,6)} Because of this tissue specificity, serum CPK isoenzyme analysis is of great diagnostic value.^{5,9)} Moreover, the method can be used to observe the variation of the tissue CPK isoenzyme pattern.

Serum CPK isoenzymes may be separated by means of electrophoresis, isoelectric focusing, the ionexchange method, the immunoinhibition method, the selective activation method and radioimmunoassay.⁸⁾ When tissue CPK isoenzymes are separated, the tissue extracts are commonly used as samples.^{1,7,10)} But this method is very complicated. Moreover as many as several grams of an organ are required to analyze tissue CPK isoenzymes.

The purpose of this study is to investigate the method by which a large number of samples of tissue CPK isoenzymes can be analyzed in a simple operation, and the method of micro-analysis, which allows the use of biopsy samples. The present

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investigation is concerned with modification of the polyacrylamide disc gel electrophoretic technique of DAVIS,⁴⁾ which allows separation and demonstration of CPK isoenzymes in tissue.

MATERIALS AND METHODS

The tissues used, obtained from cattle and swine within 30 minutes after slaughter, were as follows: diaphragm muscle, spinal cord, cardiac muscle, gastric smooth muscle, liver and kidney. Tissue samples were prepared by simply cutting them into the sizes of approximately 1 mg weight with ophthamo-scissors.

Modification of the technique of DAVIS was as follows: disc of 7.5% polyacrylamide gel in 0.19M tris-HCl buffer pH 8.9 was formed in a glass tube (inside diameter, 3.5mm; length, 10cm). After addition of the sample into each glass tube, electrophoresis was run at room temperature with 0.01M tris-0.08M glycine buffer at a constant current of 1mA/tube for 1 hour. Apart from this modification, the standard procedure for electrophoresis was carried out.

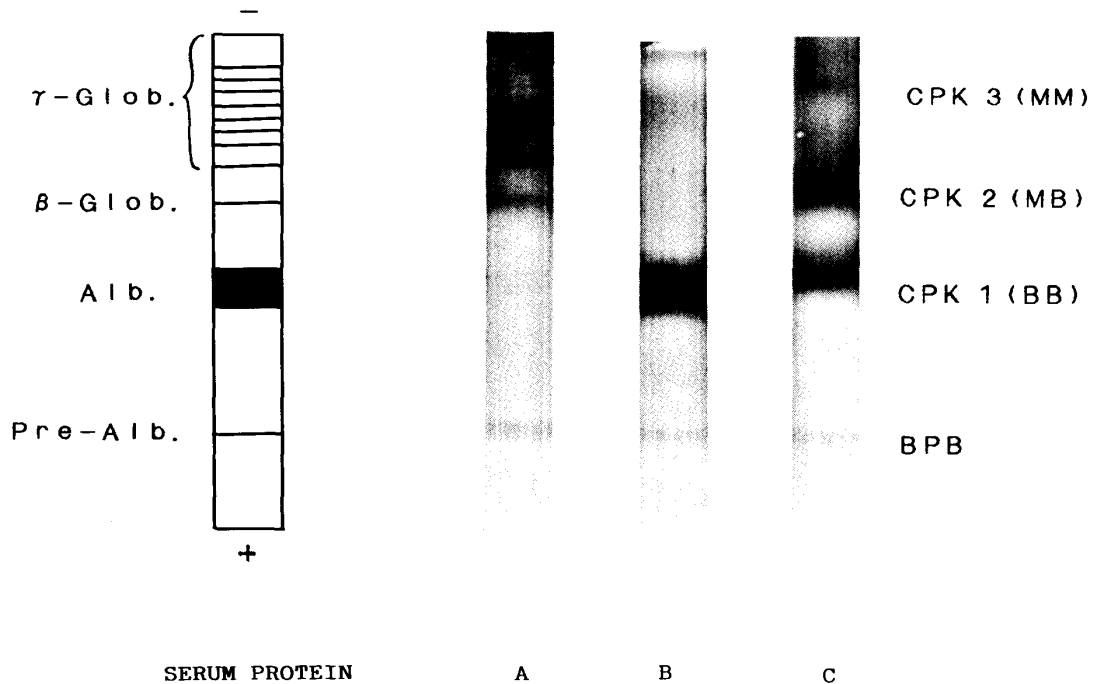
To visualize the isoenzymes in the gel, the following staining procedure was followed. A serum CPK staining kit (Iatrosset CPK-S: Iatron laboratories Inc.) was used after diluting it 4 times with 0.5% agarose. After the staining solution was distributed in a 700 μ l / 1 glass tube (inside diameter, 5mm; length, 10cm), the gel was soaked in the solution. To prevent the agarose from setting, this process should be done as quickly as possible. After the gel was incubated at 37°C for 1 hour, the stained gel was scanned with a densitometer (Cliniscan: Helena laboratories Inc.) at 595nm filter.

RESULTS

The CPK isoenzyme of the spinal cord of cattle and swine was anodic and had the same mobility as albumin. The CPK isoenzyme of the diaphragm muscle of cattle and swine, on the other hand, was cathodic and had the mobility of γ -globulin. The CPK isoenzyme of cardiac muscle of cattle and swine had the mobility of β -globulin, and was intermediate between the mobility of the spinal cord and that of the diaphragm muscle. Besides those bands, a slight band in the prealbumin region, overlapping the BPB band, which indicated the top of the migration, could be seen in all the samples (fig. 1). These band patterns agreed with those report by BENERJI et al, who applied polyacrylamide disc gel electrophoresis using human tissue extracts as samples.¹⁾

The skeletal muscle of cattle and swine predominantly had MM isoenzyme in addition to a small amount of MB isoenzyme. In swine skeletal muscle, very small amounts of BB isoenzyme were also demonstrated (fig. 2). The spinal cord of cattle and swine had BB isoenzyme as a sharp band (50%), and MM isoenzyme as a tailing

FIGURE 1 Swine creatine phosphokinase (CPK) isoenzyme pattern on polyacrylamide disk gel of
 gel of
 A : skeletal muscle ; B : spinal cord ; C : smooth muscle



band (50%) (fig. 3). The cardiac muscle of cattle had MB isoenzyme (15%) and MM isoenzyme (85%), and that of swine had MB isoenzyme (20%) and MM isoenzyme (80%) (fig. 4). The smooth muscle of cattle had BB isoenzyme as a sharp band (55%) and MM isoenzyme as a tailing band (45%), as well as the spinal cord of cattle. On the other hand, the smooth muscle of swine had BB (30%), MB (50%) and MM isoenzymes (20%) as a sharp band, respectively (fig.5). It was possible to demonstrate small amounts of BB isoenzyme in the liver and kidney of cattle and swine, but it was inseparable between the MB band and the MM band because of tailing (figs. 6 & 7).

FIGURE 2 – FIGURE 7 *Tissue CPK isoenzyme pattern of cattle (left side) and swine (right side) : Fig. 2, skeletal muscle ; Fig. 3, spinal cord ; Fig. 4, cardiac muscle ; Fig. 5, gastric smooth muscle ; Fig. 6, liver ; Fig. 7, kidney.*
The peak of the densitogram shows BB, MB, and MM band from left side to right side, respectively.

FIGURE 2 *Skeletal muscle*

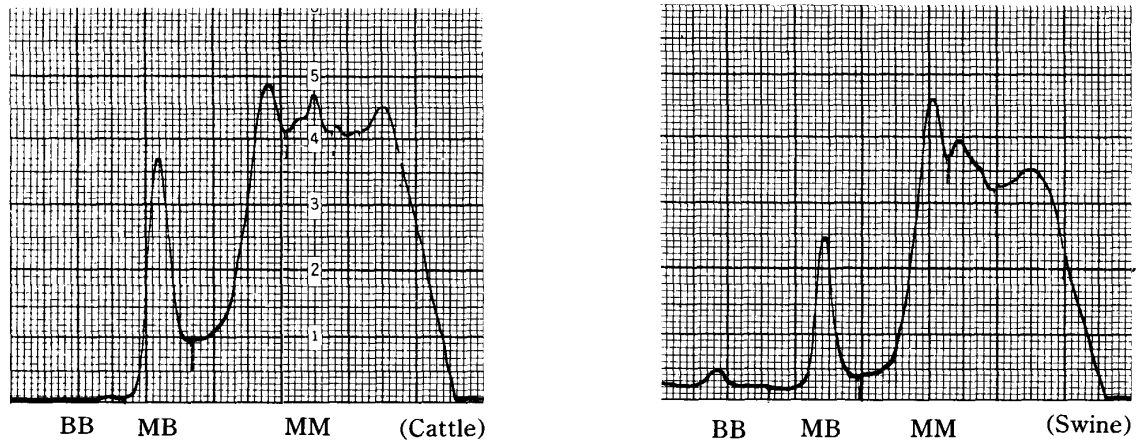


FIGURE 3 *Spinal cord*

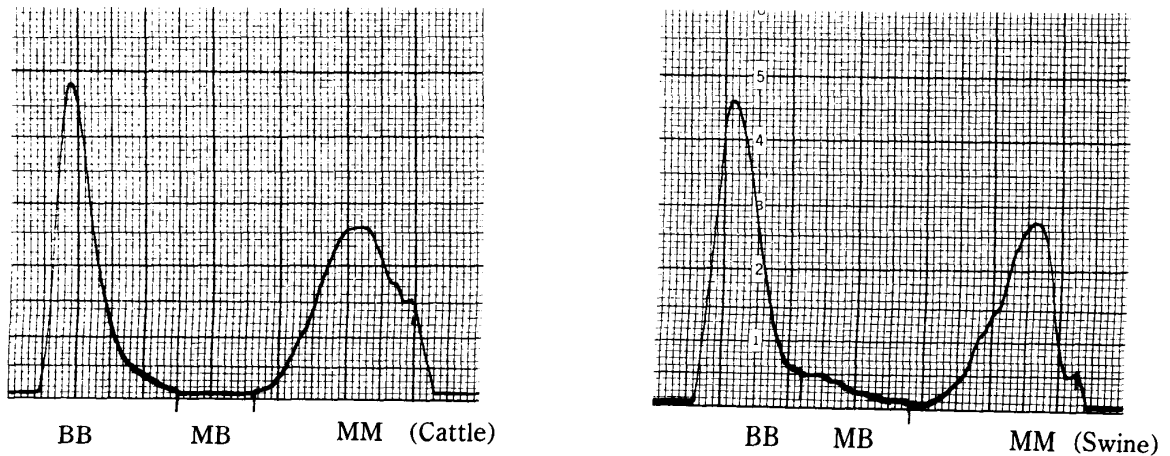


FIGURE 4 *Cardiac muscle*

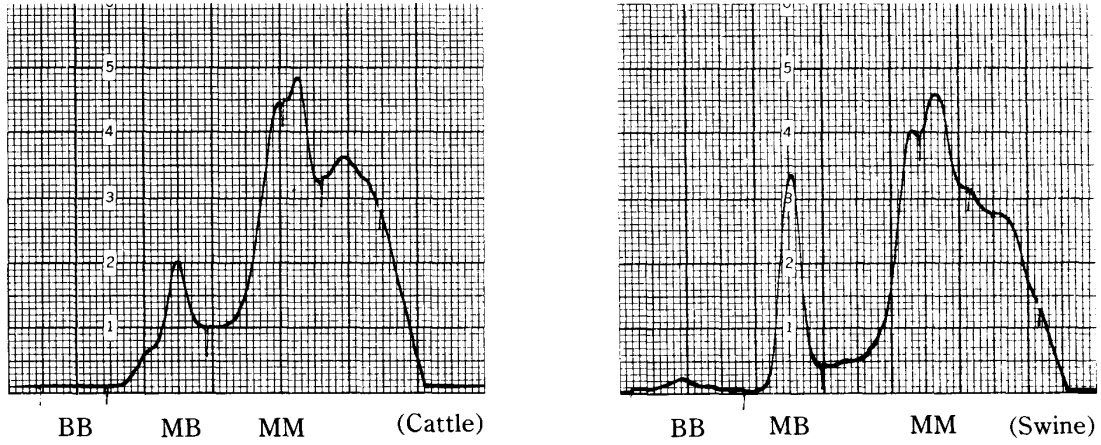


FIGURE 5 *Gastric smooth muscle*

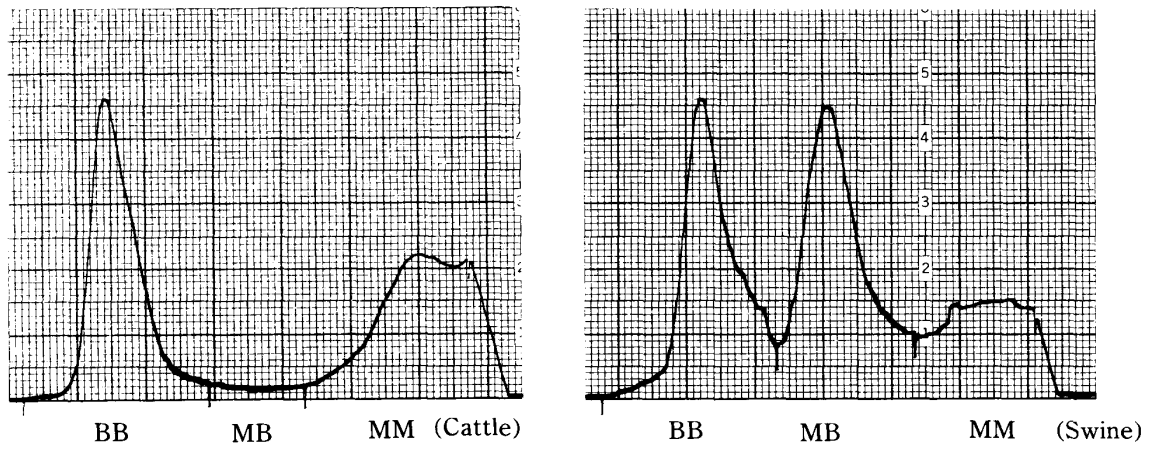


FIGURE 6 *Liver*

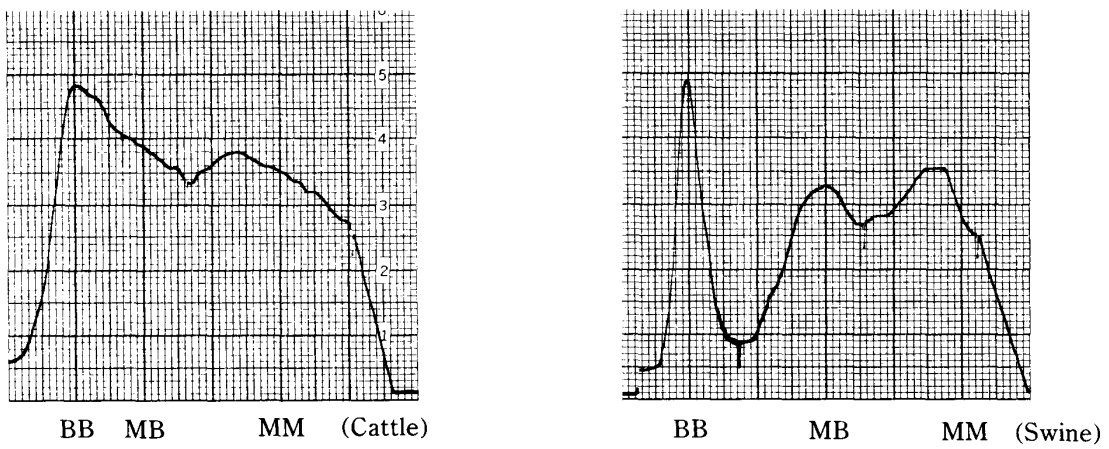
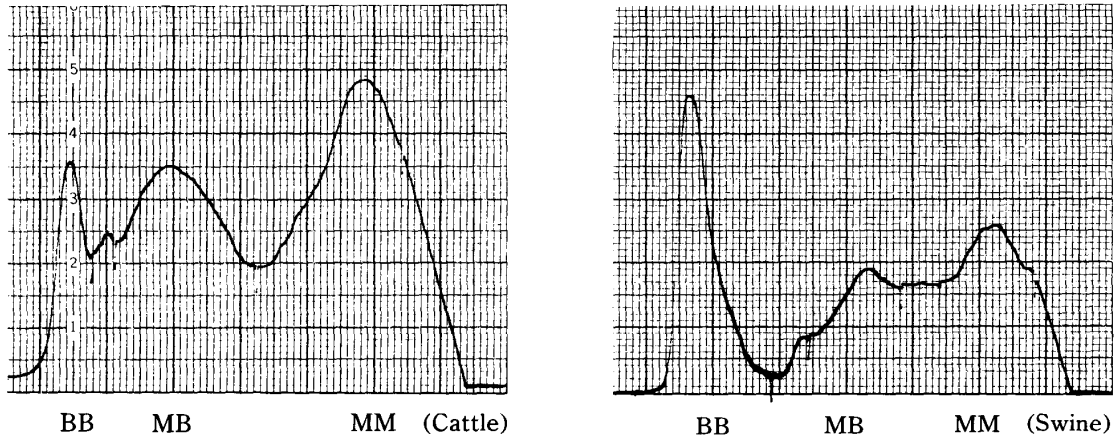


FIGURE 7 *Kidney*

DISCUSSION

The technique of CPK isoenzymes described in this study has two advantages over other methods. The main one is the high resolution that is attainable by the use of polyacrylamide gel as the separation medium.^{11,12)} This technique eliminates the need of an extracting operation to separate tissue CPK isoenzymes due to modification of the gel concentration and the buffer concentration of DAVIS's technique. The staining of CPK requires a coupling enzyme such as hexokinase and glucose-6-phosphate. But the small pore size of polyacrylamide gel prevents easy absorption of the coupling enzyme into the gel. This problem leads to contamination with the product of the reaction in the staining solution.¹²⁾ To remedy this, 0.5% agarose should be added to the staining solution to prevent diffusion of the product of the reaction.

A second advantage is that this technique enables to separate tissue CPK isoenzymes using very small amounts of samples. Those advantages make this technique a useful diagnostic tool in clinical examinations.

In the present investigation, the modified method of electrophoresis and staining was used to demonstrate only CPK isoenzymes. However, the technique may also be applied to other isoenzymes.

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