ELECTROPHORETIC STUDIES OF PHOSPHATASES

III. PAPER ELECTROPHORESIS OF ALKALINE PHOSPHATASE OF BOVINE SERUM

KAZUSHIGE TANAKA

Institute of Medical Chemistry, Yamaguchi Medical School, Ube

Director : Prof. S. Nakamura

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The method of paper electrophoresis, as one of zone electrophoresis proposed by *Tiselius* (1), proved especially usefull in the clinical serum analysis. Its application to the enzyme chemistry is now increasing, even though certain limitations are recognized (2).

Recently *Nakamura* and *Tanaka* (3) have studied the distribution of the alkaine phosphatase in serum by the method of electrophoretic frontal analysis and showed that the alkaline phosphatase migrates between α- and β-globulins. In the present experiment, this finding has also been confirmed by the paper electrophoresis.

EXPERIMENTAL AND RESULTS

Material: Bovine serum was obtained by centrifuging the clotted blood at 2,000 r. p. m. for 20 minutes.

Methods: Electrophoresis was carried out by a *Grassmann* type apparatus (4). A sheet of No. 50 Toyo filter paper of 30×10 cm. in size was used, applying about 0.05 ml. of serum on the narrow side of it. A veronal acetate buffer of pH 8.6 and ionic strength 0.1 was used.

After the electrophoresis of about 20 hours, the paper was dried in the air without heating. Then a strip of 2 cm. width was cut off from the paper along its length. The strip was then stained according to *Grassmann* with amido black 10 B and the extinction was measured at every 2mm. with a photoelectric colorimeter using red filter.

The rest of the paper was used for the estimation of the alkalne phosphatase. First it was cut at intervals of 5mm. along its length, producing fragments of 5×80mm. Each fragment was further cut into several pieces and thrown into a test tube.

The activity of alkaline phosphatase was measured by the method of *Bessey et*

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al (5) as follows: to each test tube, 0.2 ml. of 0.4 per cent sodium *p*-nitrophenyl phosphate and 0.8 ml. carbonate buffer of 0.1M and pH 9.8 were added. After incubation for 3 hours at 38°C, 4 ml. of 0.02 N NaOH were added and with the aliquote, extinction coefficients at 410 mμ. were masured with a Beckman DU type spectrophotometer.

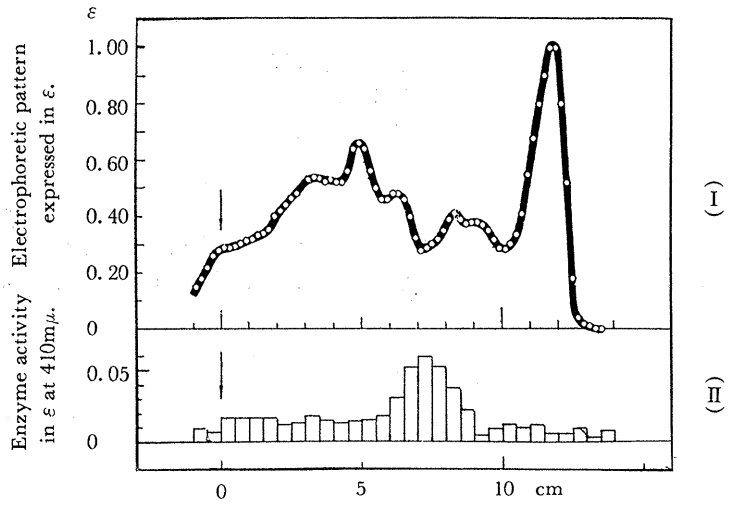

Fig. 1. Electrophoretic pattern of bovine serum and the distribution of alkaline phosphatase by paper electrophoresis.

One of the results obtained is shown in Fig. 1. Curve I represents the electrophoretic pattern of bovine serum and Curve II, the pattern of alkaline phosphatase. From the results obtained it is obvious that the alkaline phosphatase migrates between theα- and β-globulins. Thus the results obtained by *Nakamura* and *Tanaka* (3) and *Tanaka* (6) were confirmed also by the method of paper electrophoresis.

LITERATURE

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