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Modified Assay Method of Placental Alkaline Phosphatase in Human Blood

1. Electrophoresic Method

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1. **A method was developed for the separative assay of placental alkaline phosphatase in human blood from isozymes derived from other organs using the method of plate electrophoresis.**
2. **It was possible to measure correctly the placental alkaline phosphatase activity between 1 and 30 K-A U. using this new method.**

(Key Words: Placental Alkaline Phosphatase, Electrophoresis)

INTRODUCTION

To check the functions of human placenta, many kinds of methods have been developed in the clinical field, based on the principle of measuring the amounts of hormones or proteins such as estriol, human chorionic gonadotropin, human placental lactogen or placental alkaline phosphatase, secreted from the placenta.

Serum alkaline phosphatase was separated into six bands (Al-P_{1-6}) on agar gel plate by means of electrophoresis and diazo-staining in an extensive clinical survey (1, 2), but this method has several disadvantages. For example, it requires a long time for the separation of the isozymes and also the ratios of the recoveries of the isozymes using this method are not constant. In this paper, a method of isolating only placental alkaline phosphatase for clinical surveys of the placental functions, which has some advantages over the old methods, is described.

MATERIALS AND METHODS

Purification of placental alkaline phosphatase:

Purified enzyme was obtained from fresh human placenta by a modification of the method of Morton (3). It was eluted from the microsomal fraction by n-butanol treatment and passed through 1st DEAE cellulose column, a Sephadex G-150 column and 2nd DEAE cellulose column, successively. The enzyme is almost a single protein as judged from polyacrylamide gel disc electrophoresis.

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Determination of serum placental alkaline phosphatase:

The activity of alkaline phosphatase was measured by measuring phenol liberated from phenyl phosphate using alkaline phosphatase kit (Wako).

RESULTS AND COMMENTS

The electrophoretic method was as follows. From one to eight samples were applied, in proportions of 0.5 to 5 μl, to a Titan III XW Zip Zone Plate (6 × 7.7 cm, HELENA Lab.) in a HELENA electrophoretic apparatus. The electrophoresis buffer was 0.033 M Tris-Barbital buffer, pH 8.8 with 2×10^{-3} M $MgCl_2$ as a stabilizer of enzymatic activity. The electrophoresis was performed at 5° for 40 min, 33 V per cm. The staining technique was as follows. The reaction mixture was prepared by mixing thoroughly: 1.6 mg of α-naphtol phosphate ASMX and 5 mg of Fast Red Violet LB Salt in 2 ml of 0.05 M carbonate buffer, pH 10.5 with 2×10^{-3} M $MgCl_2$. After electrophoresis, the plate was placed carefully over another plate surface and equilibrated with the reaction mixture so that no air spaces were trapped between the two plates. The sandwiched plates were then incubated at 40° for 40 min in an air box. After incubation, the two plates were carefully stripped and washed with 5% formalin for 5 min for termination of the reaction. The two plates were carefully sandwiched again and activities of alkaline phosphatase isozymes were measured at 525 nm using a HELENA densitometer (Quick Scan Flur-Vis model).

Electrophoretic pattern of alkaline phosphatase isozyme in human

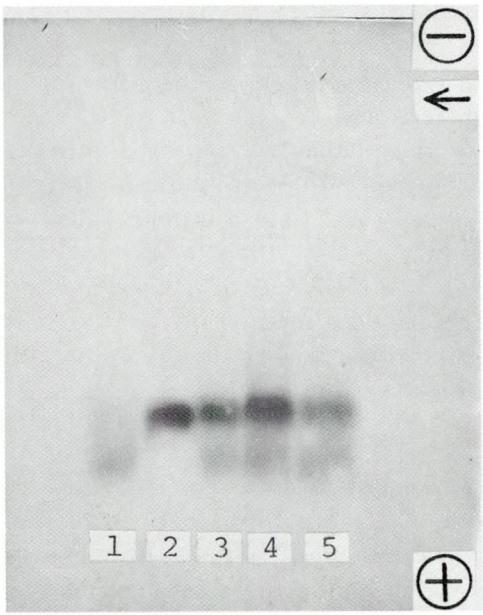

Photo 1. Electrophoretic patterns of human serum alkaline phosphatase and purified placental alkaline phosphatase.
1: Serum of Male
2: Purified placental alkaline phosphatase
3.: 1 + 2
4: Serum of pregnant woman (37 weeks)
5: Serum of pregnant woman (34 weeks)

serum are shown in Photo 1. The rates of the migrates of isozymes are compared to hepatic and placental alkaline phosphatase, which are known previously (4). There were two separate active fractions, one of which migrated toward the anode faster than the other. The faster one migrated to the position of liver type alkaline phosphatase in normal serum (Photo 1-1) and the position of the latter was fixed with purified placental type alkaline phosphatase (Photo 1-2). Both activities were completely separated from each other by using sera of pregnant women (Photo 1-4, 5) or normal serum with purfied placental alkaline phosphatase added (Photo 1-3).

As shown in Fig. 1., the activity of placental alkaline phosphatase was completely recovered after electrophoresis treatment for 40 min by the addition of 2×10^{-3} M $MgCl_2$, an activator and stabilizator of the enzyme, in the serum and the buffer used.

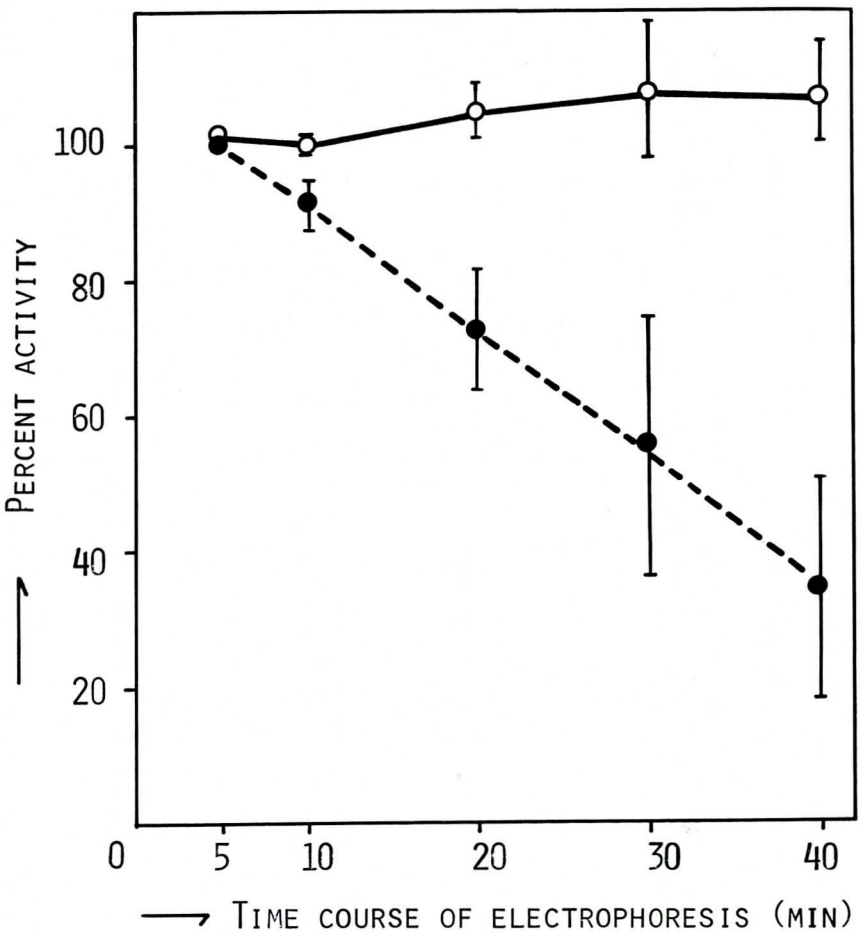

Fig. 1 Stability of placental alkaline phosphatase during electrophoretic treatment.
○—○ Placental alkaline phosphatase activity using our method.
●---● Placental alkaline phosphatase activity using the HELENA kit.
Each value represents the mean for three samples.

It was possible to measure correctly the placental alkaline phosphatase activity between 1 and 30 K-A U. using this new method, as shown in Fig. 2.

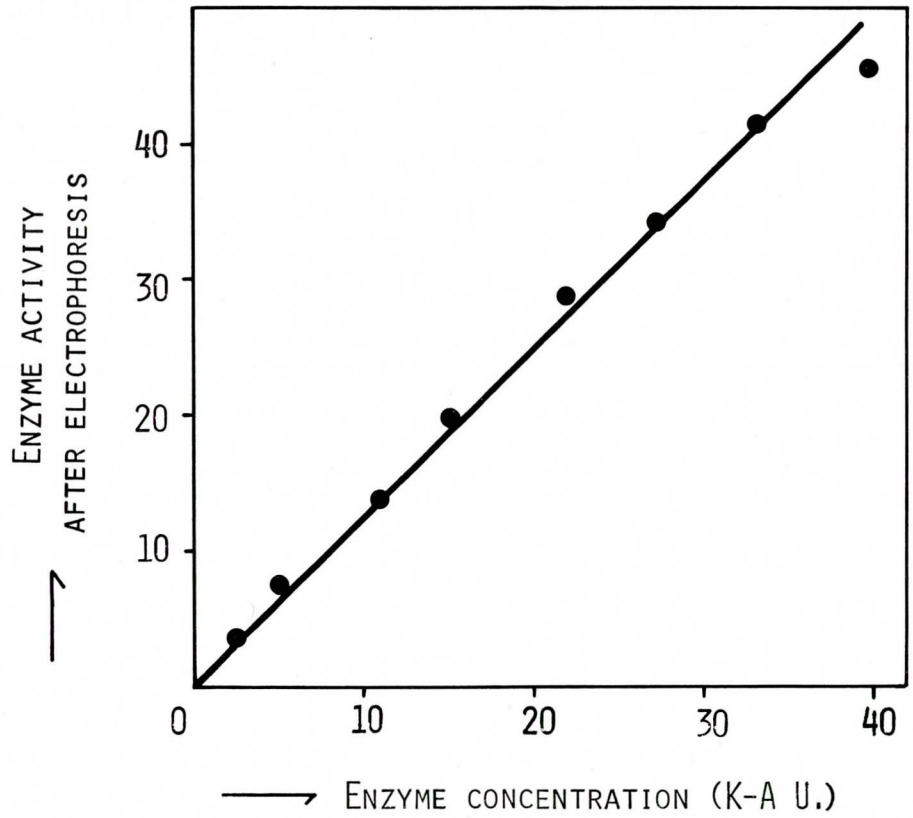

Fig. 2 Relationship between the activity using plate electrophoresis and enzyme concentration in the placental alkaline phosphatase assay. (K-A U. = King Armstrong Unit).

Conversely, when $MgCl_2$ was omitted, the activity of the enzyme disappeared during electrophoresis and the recoveries of enzyme activity were not constant.

The old methods (4, 5) require a long time for the separation of the isozymes. Furthermore, the ratios of the recoveries of the isozymes using these methods are not constant. However, our new method is considered to have some advantages over the old methods, because it is possible to isolate the isozymes and measure their activities within the period of only 90 min accurately.

REFERENCES

1) Boyer S. H.: Science 134: 1002, 1961
2) Oda Z.: Metabolism and Disease 2: 331, 1965
3) Morton R.K.: The Purification of Alkaline Phosphatase of Animal Tissues: Biochem J 57: 595–603, 1954
4) Okudaira Y., Miura S., Hayakawa K. et al.: Separative assay method for placental alkaline phosphatase isozyme using the method of electrophoresis: The World of Obstetrics and Gynecology 6(28): 568, 1976
5) Suzuki H., Yamanaka M. and Oda T.: Ann N Y Acad Sci 160: 811, 1969