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Ion-exchange thin-layer chromatographic screening test for phenylketonuria and other aminoacidaemias

By

JUDIT KOVÁCS

Section of Paediatrics, Péterfy Hospital, Budapest

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An ion-exchange thin-layer chromatographic method suitable for screening of aminoacidaemias in the newborn is described. The test is carried out from one drop of blood dried on filter paper. By means of the method, phenylketonuria was diagnosed in an 8-day-old infant. The diagnostic possibilities of the method in the identification of other aminoacidopathies is discussed.

In order to identify phenylketonuria as well as other disturbances of amino acid metabolism, screening tests are performed all over the world [9]. The possibilities of the application of thin-layer ion-exchange chromatography for screening of neonatal aminoacidopathies have recently been discussed [2, 3].

According to several authors [5, 8] thin-layer chromatography opens new perspectives in screening, while others [1, 7] emphasize the technical and economical disadvantages of the method. Between the well-known and generally used cellulose-acetate thin-layer chromatography and ion-exchange thin-layer chromatography, there are some basic theoretical and practical differences. The latter technique is much more simple, and its one-dimensional running in aqueous buffer solution ensures a simple, rapid and perfect separation of the amino acids in blood serum.

A further advantage of chromatography over the classical Guthrie test [4] consists in the fact that from a single blood sample almost the entire amino acid spectrum may be detected by simple observation, thus the demonstration of an increase in the individual amino acids does not require the application of microorganisms or culture media.

METHODS

In the ion-exchange thin-layer chromatographic procedure described by DÉVÉNYI [2] examination is carried out in three steps. During the practical trial, however, it has become clear that this form of the method would not be suitable for mass-screening purposes. Thus the following modifications have been made [3].

1) 20 to 50 μl capillary blood is dropped on filter paper and dried. The spot is 15 to 20 mm in diameter. This sample can easily be stored and mailed to the laboratory where the tests are performed.

2) The dried blood spot is punched into 6—7 small disks, which are put into small test tubes. To each tube 0.1 ml of a 10 : 1 mixture of 95% ethanol and concentrated hydrochloric acid is added.

3) The tubes are sealed and allowed to stand overnight at room temperature. This time is sufficient for the elution of amino acids.

4) The eluate is transferred during constant drying by means of a capillary upon the 10×10 cm chromatoplate (IONEX 25 SA, Na-cycle, of Macherey, Nagel & Co, Düren, GFR), which is coated with a strong cation exchange resin. Onto one plate, 7 blood samples and a control amino acid mixture can be transferred simultaneously.

5) The plates are put into a 15×25 cm glass chamber and developed in a pH 5.23 $Na^+ = 0.35$ N aqueous sodium citrate buffer solution at room temperature, for about 60 minutes.

6) The dried chromatogram is stained with ninhydrin spray [2, 3].

On the developed chromatogram the basic amino acids appear according to their increasing R_f values in the order arginine, histidine, lysine, phenylalanine, tyrosine, leucine. Since the concentration of these amino acids in blood is nearly identical, the spot of the pathologically increased amino acid will be conspicuous (Fig. 1).

A technical assistant may perform 10 examinations per hour. The method, the cost of which is about double of that of Guthrie's test is, however, suited for the simultaneous examination of the level of six amino acids.

Report of a case

B. K., a male infant, was born from the fourth pregnancy, weighing 2.800 g. The parents are healthy, there is no consanguinity. From the first pregnancy a healthy girl was born, which was followed by two spontaneous miscarriages.

Blood was drawn from the infant for the screening test on the 5th day of life. Evaluation of the blood sample was performed on the 8th day of life. The chromatogram demonstrated hyperphenylalaninaemia (Fig. 1). Owing to social reasons, the mother failed to present the child for control, therefore we repeated the blood sampling in the home of the infant at the age of 18 days. Some hours following the positive result of the repeated chromatogram, the infant was admitted to our ward. There a quantitative analysis (Beckman Unichrome Amino Acid Analyser) performed on the 21st day of life gave a serum phenylalanine value of 42 mg/100 ml. Dietary treatment was immediately introduced with Berlophen® (GDR). Quantitative control examinations performed monthly gave serum phenylalanine values at the beginning 2.0 to 2.5 mg/100 ml and later, 5 to 7 mg/100 ml. The infant accepts the preparation readily, he started to gain weight, his psychomotoric development is according to age (Fig. 2).

Discussion

By means of the method described above more than 3000 screening tests have been performed since August 1st, 1972. From mature newborns the blood sample is collected on the 5th, from premature ones on the 14th day of life. Until now, false positive results occurred in two cases. The parents were notified by mail and in both cases a control examination was performed within a week, which yielded negative results.

The method's sensitivity is similar to that of the Guthrie test [2, 3]. In addition, since the chromatography is independent of antibiotics, less false results are obtained than with the microbiological test. Where premature

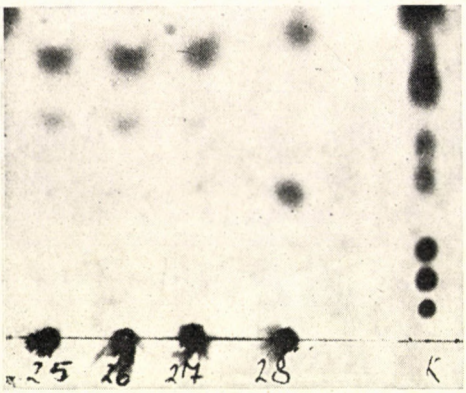

FIG. 1. Ion-exchange thin-layer chromatography of various sera. Nos. 25, 26, 27: normal. 28: phenylketonuric serum. K: control amino acid mixture

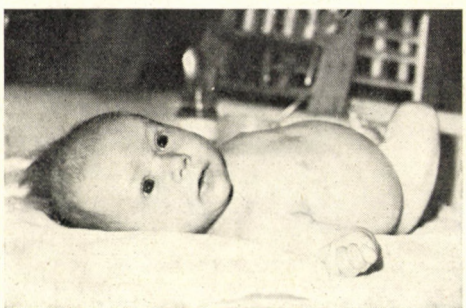

FIG. 2. B. K. 9-week-old phenylketonuric infant, treated with Berlophen®

newborns are concerned, the screening test is not disturbed by previously administered plasma or amino acid infusions, which may induce a transitory increase in all amino acids in the serum. Thus, the chromatogram would demonstrate an identically increased intensity of the spots of each amino acid. Spots of outstanding intensity only may be evaluated as pathologic, or as suspect of being pathologic. Obviously, the method is suitable also for the regular biochemical control of children on a phenylalanine-poor diet.

The aminoacidopathies which may be detected by this type of screening method, are represented in Table I, demonstrating also their biochemical and clinical characteristics.

A differentiation between the various types of hyperphenylalaninaemia seems of importance, taking into consideration the genetic heterogeneity of the phenylalanine-hydroxylase enzymatic defect [6]. In infants, the differentiation of phenylketonuria is

TABLE I

Amino acid metabolic disorders detectable by Ionex 25 SA-chromatoplate method

Diagnosis	Amino acids or organic acids increased in plasma	Amino acids or organic acids increased in urine	Biochemistry	Clinical features and treatment
Phenylketonuria	Phenylalanine	Phenylalanine o-OH-phenylacetic acid, phenylpyruvic acid	Phenylalanine hydroxylase deficiency	Usually (but not always) mental retardation. Convulsions, eczema, fair hair and complexion. Treatment: low phenylalanine diet
Hyperphenylalaninaemia	Phenylalanine		Not precisely known; phenylalanine transaminase in some cases	May be normal, depends on type. Treatment if serum phenylalanine exceeds 20 mg/100 ml
Tyrosinosis (several clinical types)	Phenylalanine, Tyrosine		Transient p-OH-phenylpyruvic acid oxidase deficiency	General failure to thrive, convulsions. Temporarily responds to low-phenylalanine diet
Hyperlysinaemia	Lysine; also ammonia	Lysine, N-acetyllysine, Homoarginine	Lysine-acylase, Lysine-dehydrogenase, or lysine ketoglutarate-reductase	Convulsions and coma related to protein feeding
Histidinaemia	Histidine	Histidine, Alanine, Threonine	Histidine ammonialyase	Slurred, inarticulate speech. Variable incidence of mental retardation. Low histidine diet has been attempted; results questionable
Oasthouse (Smith—Strang) syndrome	Not reported	Phenylalanine, Methionine, Tyrosine, Leucine and Isoleucine, alpha-OH-butyric-acid	Unknown	Infantile spasms with interim flaccidity and unresponsiveness, sparse hair, retardation, general unawareness
Branched chain ketoaciduria	Isoleucine, Valine, Leucine, Allo-isoleucine	Leucine Isoleucine Valine	Branched chain keto acid decarboxylase. Partial and intermittent forms	Neonatal difficulty in feeding, anorexia, convulsions, other CNS signs. Mild, intermittent and thiamine dependent forms

absolutely indicated from therapeutic aspects. This may be carried out easily by appropriate loading tests as well as by regular quantitative control of the serum phenylalanine level [6].

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References

1. Coradello, H.: Dünnschichtchromatographische Untersuchung von Kapillarblut Neugeborener. Klin. Pädiat. **184,** 358 (1972).
2. Dévényi, T.: Thin-layer ion-exchange chromatography on resin-coated chromatoplates. I. Separation of aromatic and basic amino acids (phenylketonuria-test). Acta biochim. biophys. Acad. Sci. hung. **5,** 435 (1970).
3. Dévényi, T., Báti, J., Kovács, J., Kiss, P.: Thin-layer ion-exchange chromatographic screening test for aminoacidemias in blood samples dried on filter paper. Acta biochim. biophys. Acad. Sci. hung. **7,** 237 (1972).
4. Guthrie, R., Susi, A.: A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics **32,** 338 (1968).
5. Hübschmann, K.: Aktuelle Probleme der Erfassung angeborener Stoffwechselstörungen durch Siebteste bei Neugeborenen. Pädiat. u. Grenzgeb. **10,** 115 (1971).
6. Kempe, H. C., Silver, H. K., O'Brien, D.: Current Paediatric Diagnosis and Treatment. 2nd ed., Blackwell, Oxford 1972, p. 875.
7. Schön, R., Thalhammer, O.: 25.000 routinmäßige Dünnschichtchromatographien bei Neugeborenen. Ergebnisse und Vergleich mit Guthrie-Testen. Z. Kinderheilk. **111,** 223 (1971).
8. Urner, U., Ahlbehrendt, I., Friedmann, H., Wagenknecht, C.: Multiphasische Testung zur Erkennen von genetischen Störungen des Aminosäure- und Kohlenhydratstoffwechsels, eine Pilotstudie. Dtsch. Gesundh.-Wes. **28,** 49 (1973).
9. WHO scientific group. Screening for inborn errors of metabolism. WHO techn. Rep. Ser. 401 (1968).

Dr. J. Kovács
Péterfy Kórház
H-1076 Budapest, Hungary