Use of Micromethod for Phenylalanine in Management of Phenylketonuric Patients

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A micromethod for measurement of blood phenylalanine is described. The procedure is advantageous in management of phenylketonuric children under treatment.

Treatment of phenylketonuric infants with low phenylalanine diet requires frequent measurements of blood phenylalanine, since diet must be adjusted to changing phenylalanine requirements. The amount of blood required for repetitive determinations by the usual methods may be excessive. The need for frequent and rapid determinations led us to modify the method for serum phenylalanine (1) to permit use of blood obtained from a finger puncture—a modification suggested by methods for microchemical analysis for other blood constituents (2).

Blood is collected in capillary tubes* which are then sealed with a flame or in U-shaped tubes which require no sealing (3). The capillary tubes are centrifuged in a high speed centrifuge† or in an ordinary centrifuge in a cotton-packed cup. The portion containing cells is broken off and discarded. Serum from several capillary tubes is combined. In a small conical test tube, 0.20 ml. of 95% ethyl alcohol is added to 0.050 ml. serum (or plasma) to precipitate the protein. The precipitate is packed into the conical tip by centrifuging. Five spots are marked along a line 1 inch from the bottom of a sheet of Whatman No. 4 filter paper 7 in. long. The supernatant liquid is drawn into a 50-μl. pipet and carefully applied to a spot on the filter paper in aliquots of 5 μl. After each application, the alcoholic supernatant is dried.

*Obtainable from Scientific Products, Evanston, Illinois.
†International Equipment Co., Boston.
rapidly with a jet of warm air; the total amount of 50 μl. can be added to the paper in 2 or 3 min. On each of the other spots is placed a known amount of phenylalanine: 0.5, 1.0, 1.5, and 2.0 μg. The chromatogram is resolved in butanol-ethanol-water mixture (70-20-20) and developed with 0.2% ninhydrin reagent. About 3 hours are required for the solvent to rise when Whatman No. 4 paper is used. (Whatman No. 1 paper may also be used; the solvent rises in about 6 hours.) Quantitative estimation of the phenylalanine in serum can be made by comparing the density of color with that obtained with known amounts of phenylalanine.

For distinguishing between serum from normal and phenylketonuric individuals, visual comparison of phenylalanine spots is sufficient. The intensity of phenylalanine on a chromatogram prepared from phenylketonuric serum is usually greater than the 2.0 μg. standard (or more than 20 mg./100 ml.), while serum from normal individuals shows an intensity usually less than that of the 0.5 μg. standard (or less than 5 mg./100 ml. of serum).

This method is not proposed as a substitute for the more exact procedures for measuring phenylalanine concentrations in blood from nonphenylketonuric individuals (4, 5). However, it provides a means for distinguishing quickly between the phenylketonuric and non-phenylketonuric individual. It is most useful in following phenylketonuric children who are under treatment and in whom it is essential to determine the effectiveness of the restrictive dietary regimen.

References