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Rapid thin-layer chromatographic microassay of ε-aminocaproic acid in urine

A simple method for the estimation of ε-amino-*n*-caproic acid (EACA) became necessary to obtain information about the absorption of this potent inhibitor of plasminogen activation which might be of great interest in dentistry.

Few techniques[1–5] are known to determine the presence of EACA in urine, blood and saliva by means of paper chromatography, column chromatography, high-voltage paper electrophoresis. Moreover they are not easy to deal with and rather time consuming for our purpose. We developed a convenient and simple procedure for the determination of microamounts of EACA using thin-layer chromatography (TLC).

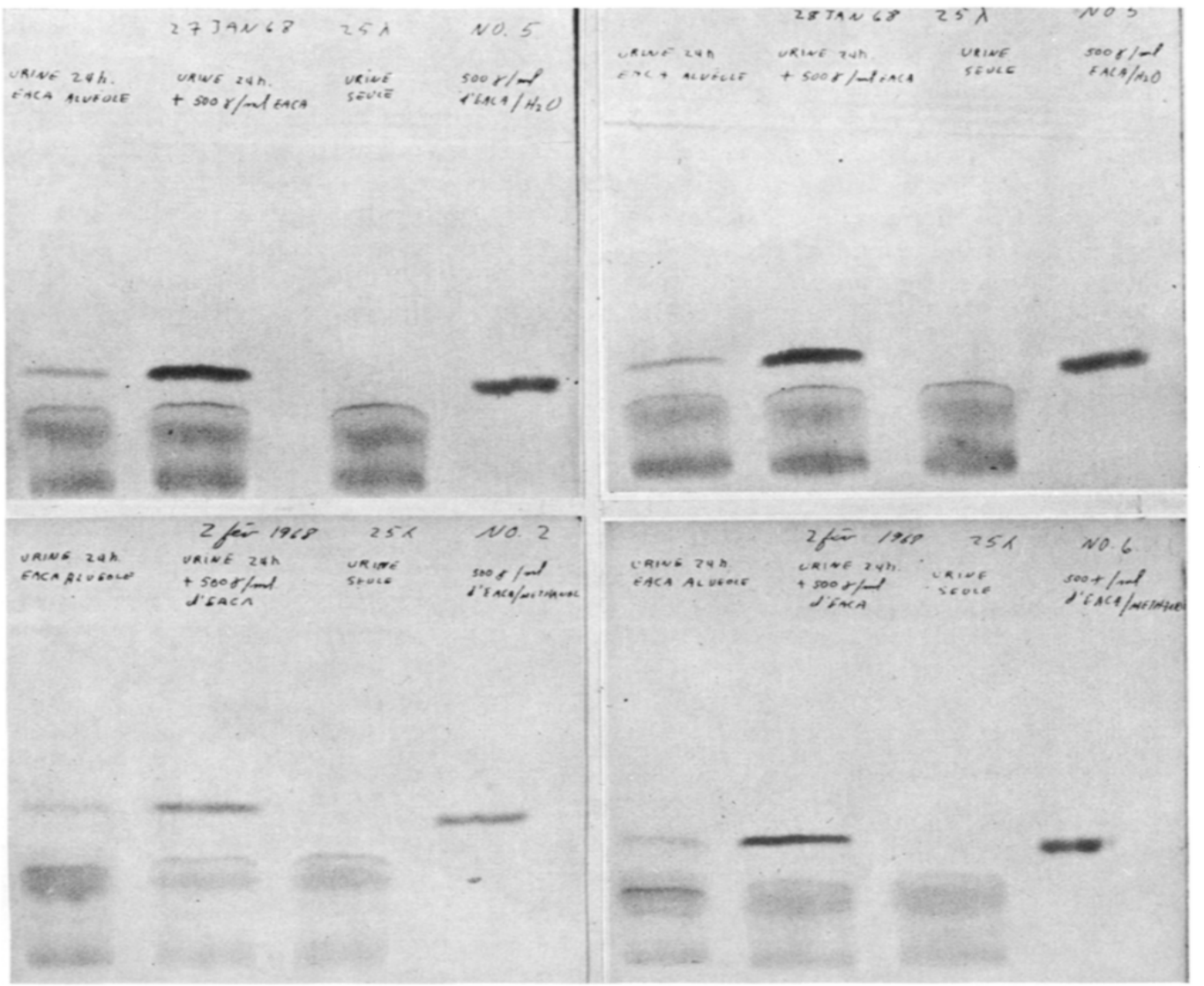

Fig. 1. Chromatographic pictures showing the migration and separation of EACA from the other constituents of urine. From right to left: the control solution (EACA dissolved in methanol or water); urine without EACA; urine in which we added EACA; urine collected from rabbits who received EACA.

Method

EACA is chromatographed on Silica Gel G layers which give better results than Alumina or Kieselgur G. Fisher micropipets were used for spotting. The EACA was supplied by Lederle of Canada. The solvent is a mixture of the following: *n*-butanol–glacial acetic acid–distilled water (8:2:2). Thin-layers, 250 μ thick, are prepared with a Shandon applicator, air-dried for 30 min, heated in an oven at 100° for at least 1 h and allowed to cool before use. The detection reagent applied with chromatosprayer is ninhydrin 0.5% in methanol or water. The chromatograms are photographed with a 35-mm Nikon.

Rabbits (17) weighing from to 2 to 3 kg, are used. After general anesthesia with pentobarbital (Nembutal, Abbott Laboratories, Canada), the lower left central incisor is removed and the socket is packed with an absorbable gelatine sponge (Gelfoam, Upjohn Co., Canada) approx. 20 mm × 20 mm × 7 mm. The Gelfoam has been filled before with EACA at a concentration of 10 mg/kg. The socket is sutured with catgut No. 4. The rabbit is then placed in a metabolism cage and the 24-h urine is collected and filtered. 24-h urine was also collected from rabbits (10) not operated on. For a third group (10) we added in the 24-h urine, 500 μg/ml of EACA. A standard solution was prepared by dissolving 500 μg of EACA per ml of methanol or distilled water. The sample spots are applied, using a volume of 25 λ for each solution, in a straight line parallel to the margin of the layer in order to obtain the cleanest separation. The chromaplates are placed in the chamber for 60 min, removed and dried 10 min with a hot-air blower. Using a Shandon spraygun, the ninhydrin solution is sprayed on in a fume hood. The plate is then heated in an oven at a temperature of 80° during 20 min.

Results

The solvent system butanol–acetic acid–water permits excellent migration and separation. It is the solvent of choice for TLC of small amounts of EACA, the lower detection limit being from 5 to 10 μg. The colour shown by EACA in urine, EACA dissolved in methanol or water is a deep rose (magenta).

The method suggested gives an excellent separation of EACA from normally occuring amino acids in urine as can be seen by the different chromatograms (Fig. 1). These results from 17 rabbits provided with small amounts of EACA in the socket of a lower incisive tooth prove that the compound has been absorbed.

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