Communications to the Editor

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Separation of L-Asparaginase-like Substance from Male Human Urine

An attempt was made to detect L-asparaginase activity in adult male human urine and to separate the active principle therefrom. With condensation by salting-out with ammonium sulphate, it was found that original urine contains 0.1—2.1 enzyme units of active principle per 100 litres. Its optimal pH is 8.0 with a stable range of pH 9.0—7.0.

The elution pattern as observed, using Sephadex G-100, reveals a mono-peak in enzyme activity, whose molecular weight is about 56000 as against 130000—140000 for *E. coli*-deriving L-asparaginase. Electrophoretically, its migrative pattern is also different from that of *E. coli*-deriving L-asparaginase.

Keywords——human urine; L-asparaginase; L-asparaginase-like substance; molecular weight; stability; optimum pH; electrophoresis

L-Asparaginase is now clinically used in Japan as an antitumor agent. Its resources, however, are entirely dependent on microorganisms so that there are still problems, such as anaphylaxis, that are yet to be solved.

An attempt has been made by the authors to confirm the presence of L-asparaginase in adult male human urine as well as to separate the substance. The urine was collected under chilled conditions, with 0.01% bovine serum albumin as the stabilizer and 0.005% NaN_3 as the antiseptic.

The L-asparaginase activity was measured by Cooney's enzymatic method[1] while protein was determined by Hartree's modified Copper–Folin method[2] using bovine serum albumin as the standard protein. The protein concentration was estimated by optical absorbance at 280 nm.

Slowly ammonium sulphate was added with stirring to the collected urine until obtaining an 80% saturation, after which it was allowed to stay overnight at 4°. The resultant precipitate was subjected to centrifugation and then a 1/20 part of 0.1 M boric acid buffer solution (pH 8.0) against the original urine volume was added and stirring done for 2 hr at room temperature. Centrifugation was further performed and the supernatant resulted was condensed through a Diaflow-membranefilter (MP-30, Amicon) and washed repeatedly with same buffer solution, obtaining thereby a crude enzyme fraction. The amount of an L-asparaginase-active enzyme obtained by this procedure was 0.1—2.1 enzyme units (μmol/min) per 100 litres of original urine. Also, to find out the recovery rate of L-asparaginase from urine, 1.5 enzyme units of L-asparaginase (Boehringer) originating from *E. coli* were added to original urine per litre. The recovery rate obtained was 74.6%.

This crude enzyme fraction exhibited its highest activity at pH 8.0, decreasing as the pH moved toward the acid side and reaching below 20% of the highest at pH 4.0. The activity at pH 10.0 was 80%.

Its stability was also examined against pH (37°, 2 hr), with results that the activity was 100% at pH 9.0, decreasing gradually as the pH moved toward the acid side. It stood at 76% at pH 6.0. At pH 10.0 the activity suddenly lowered to 36%. Stability against heating was checked at 50° (pH 8.0) for 30 min but the activity was not lowered at all.

The elution pattern was observed, using Sephadex G-100, with respect to an active fraction obtained by salting-out with ammonium sulphate. The molecular weight of this

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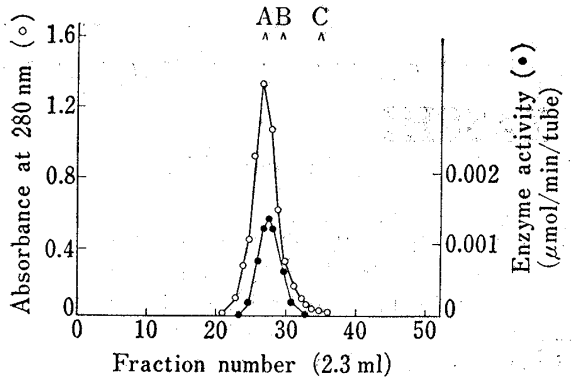

Fig. 1. Elution Pattern by Sephadex G-100 of Crude Enzyme Solution from Male Human Urine

Column size 1.6φ × 90 cm, 0.2M borate buffer solution pH 8.0, A: bovine serum albumin (MW 67000), B: ovoalbumin (MW 45000), C: soybean trypsin inhibitor (MW 21400).

enzyme, as estimated from the calibration curve made of a protein of known molecular weight in the same column, was about 56000 (Fig. 1).

Further the active fraction was compared with an *E. coli*-deriving L-asparaginase by means of starch-block-electrophoresis. It was found that when subjected to phoresis concurrently with bromphenol blue used as marker under conditions of Veronal buffer solution pH 8.6 (μ=0.05), 200 V, 30 mA and 18 hr, the active fraction migrated to the anodic side ahead of the marker and the *E. coli*-deriving enzyme behind.

The foregoing results testify to the presence in adult male human urine of an L-asapraginase-like substance which is predicted to be a new substance, being distinctly different from a microorganism-deriving L-asparaginase in molecular weight (56000 as against 130000—140000)[3,5,6] and in electrophoretic pattern, if similar in stability and other respects.[3,4]

It is not yet known whether this enzyme is always found in normal human urine since checks were not made by us with respect to individual person's urine. Judging, however, from the fact that the yield varied considerably among the batches of urine collected on different days from several persons, it can be well anticipated that there exist individual differences in content. The distribution of asparaginase in human organs has been reported by Tursky[7] to be 4.4 (μmol/hr/g) in the brain, 1.2 in the liver, 3.4 in the kidneys and 0 in the serum, and it is of interest to know whether this asparaginase-like substance found in urine is excreted *via* the basilar membrane of the kidney.

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