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MULTIPLE FORMS OF HUMAN URINARY γ-GLUTAMYLTRANSPEPTIDASE

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OF several enzymes excreted in human urine, γ-glutamyltranspeptidase (GGTP) is considered as one of the important enzyme markers that can be used to assess the uro-renal system. Among different body fluids, urine was found to be a rich source of the transpeptidase. In cancer patients, though urinary GGTP activity was found to be increased, there was no significant change in serum transpeptidase activity. The work described here is part of a study of transpeptidase isozymes in urine of normal individuals and cancer patients.

Urine samples collected from normal and diseased individuals were dialysed extensively against 10 mM Tris–HCl buffer, pH 8.0, for about 24 h. The dialysed urine samples were centrifuged at 3,500 rpm for 15 min, and supernatants were freeze-dried for chromatographic and electrophoretic experiments.

A known aliquot of a solution of desalted concentrated urine samples in 10 mM Tris–HCl buffer, pH 8.0, was passed through a DEAE-cellulose column pre-equilibrated with the sample buffer. The column was developed with the sample loading buffer. The unbound protein fraction contained transpeptidase activity, and was called unbound isozyme. After washing the column with 2–3 bed volumes of the sample buffer, bound transpeptidase activity was desorbed into two peaks, bound-I and bound-II, by applying a linear salt gradient (0 to ,0.5 M NaCl) in the buffer. The enzymatically active fractions were dialysed and concentrated for polyacrylamide disc gel electrophoresis (PAGE)[1]. The concentrated samples were suspended in 1% Triton X-100 and electrophoresed in 7.5% acrylamide gels containing 0.01% Triton X-100. After the electrophoresis, transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2]. Each of the three enzymatically active fractions yielded one activity band.

Gel filtration chromatography of the desalted freeze-dried samples through Sephadex G-200 gave two enzymatically active peaks. Electrophoretic (PAGE) analysis of the first enzymatically active peak (high mol. wt form) showed one transpeptidase isozyme while that of the second (low mol. wt form(s)) peak gave two enzymatically active bands.

Direct electrophoresis of the desalted concentrated urine samples in 1% Triton X-100 gave three enzymatically active bands in the zymogram (figure 1). The pattern of transpeptidase isozyme distribution in the electrophoretogram was observed to be comparable to that of detergent-solubilized human kidney transpeptidase[3] preparation.

Rambabu *et al.*[4] showed the existence of the two transpeptidase isozymes and that the transpeptidase activity is increased in urine of leukaemia patients. But we have detected three transpeptidase isozymes in human urine. Grossly speaking, we also observed an increased level of the total transpeptidase activity in urine of leukaemia patients, but detailed investigations on several leukaemia patients have indicated that the activity of the unbound transpeptidase

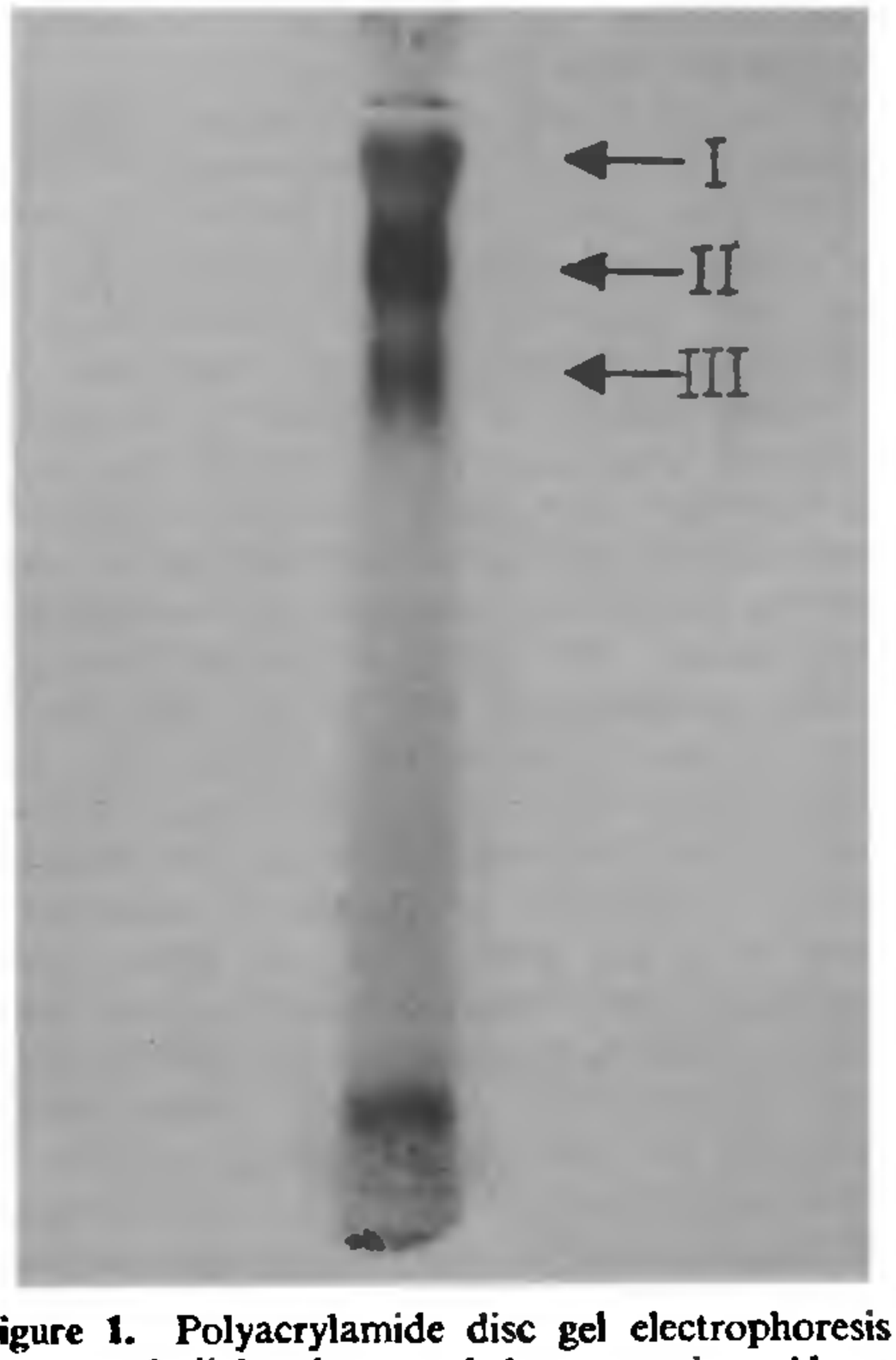

Figure 1. Polyacrylamide disc gel electrophoresis pattern of dialysed normal human urine. About 150 μg protein was electrophoresed and transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2].

isozyme is lower and that of the bound isozyme forms considerably higher to give an elevated total urinary GGTP activity in leukaemia patients[5]. However, other cancer patients (except those under treatment) showed no change in urinary transpeptidase activity.

Tsuji *et al.*[6] have reported that liver GGTP presumably leaks into serum, because many properties of liver and serum GGTP are comparable. Hence the level of GGTP in the serum can be used as an index of hepatic function.

GGTP in the human kidney is located on the extrinsic surface of the brush border membrane and, to some extent, elsewhere in the kidney[7]. Although the molecular weights of GGTP from urine and kidney were found to be different[8], Scherberich *et al.*[9] have shown immunological identity between urinary GGTP and GGTP of proximal tubule cells. Based on these similarities (immunological and electrophoretic properties), it can be presumed that urinary GGTP is leaked from the kidney membranes into urine, and that any change in the proportions of the urinary transpeptidase isozymes can be used to assess the dynamic status of the brush border membrane. Further, this type of study might be of diagnostic importance in differentiating between non-renal and renal conditions. However, more studies are required to establish the actual clinical use of estimating the isozymes of urinary enzymes like GGTP, alanine aminopeptidase, alkaline phosphatase, lysozyme, etc.

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INDUCTION OF LYSOSOMAL ENZYMES IN EHRLICH ASCITES TUMOUR CELLS BY BROMOPHOS

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IN mammals xenobiotics are detoxified mainly in liver cells by enzymes located predominantly in the endoplasmic reticulum. These enzymes are known as