Quantitative Immunological Determination of
Brush-Border Protein in Urine
Their Role in the Progression of Inflammatory and Toxic Renal Damage¹

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Brush-border membranes of human kidney cortex were separated by differential centrifugation and further enriched by using a continuous and discontinuous sucrose density gradient (4, 6, 8). This brush-border fraction was bound to marker enzymes. In addition to alkaline phosphatase (AP), known to be specific for brush-border membranes, an alanine-aminopeptidase (AAP) and a -glutamyltranspeptidase (-GTP) are part of this membrane fraction. Proteolytic treatment of this brush-border fraction with papain leads to a specific release of AAP and -GTP localized on the membrane surface, while AP is integrated more deeply into the membrane structure (8). Rabbits were immunized with human brush-border membranes producing anti-brush-border antisera (4).

Concentrated urine from patients undergoing rejection episode and acute tubular necrosis was used as antigen and resulted in precipitates in electroimmunodiffusion technique as described in our previous papers (5, 7). These anti-brush-border sera turned out to be very useful also in the study of the output of brush-border membrane proteins in urine in various kidney diseases.

By applying a one-dimensional immune electrophoresis Lurell (2) a very clear presentation of the excretion of brush-border proteins is possible in urine usually 100-fold concentrated. In one- and two-dimensional electro-immunodiffusion (EID) we were able to identify the precipitin lines of AAP and AP by enzyme specific staining.

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Table I. Enzymatic determination of alanine-aminopeptidase (AAP), alkaline phosphatase (AP) and
-glutamyltranspeptidase (-GTP) in ultrafiltrated urine from patients with acute glomerulonephritis (AGN), chronic glomerulonephritis (CGN) and lupus erythematosus (LE).
AAP was also measured immunologically by radial immunodiffusion technique (AAPimmun.). Further clinical data (creatinine clearance,
proteinuria, edema and blood pressure) are shown in this table
(+)=Proteminurine under 1%0,Esbach; + = 1- 4%0; ++=4-9%0 and +++= more than 9%0.

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The quantitative immunological determination of AAP and AP was conducted according to Mncini (3). The plates were stained specifically for AP and AAP activity and subsequently with amidoblock 10B. A purified AAP from human kidney served as a standard for quantitative determination of AAP in the Mncini technique².

In all concentrated urines we also determined AAP, AP and -GTP with enzymatic methods. Alkaline phosphatase (AP) alanine-amino-peptidase (AAP) and -glutamyl-transpeptidase (-GTP) were assayed according to Biochemica Informations, Boehringer (1).

Urine was collected over 24-hour periods. Urine samples were concentrated with AMICON ultrafiltration system DC2, using hollow fiber HIDP10.

Urine concentrates of one patient with acute glomerulonephritis (AGN), 22 with chronic glomerulonephritis (CGN), 21 of them verified histopathologically, as well as five patients under cytostatic treatment with a high dose, ten patients with a low dose over a longer period and 13 patients under the 'De-Vita scheme' were studied.

In 14 of 25 cases with glomerulonephritis, remarkable amounts of membrane-bound AAP could be recognized (table I). Using the Mncini (3) technique with following specific staining of the AAP, the excretion ranged from 1,819 to 17,669 mU/24 h. In these cases the correlation between AAP measured enzymatically and immunologically was r = 0.822 and two < 0.001. In 100-fold concentrated urine of 44 normal persons the output of AAP measured enzymatically was very low (table II) and in Lurell (2) electrophoresis no brush-border proteins were detectable. From 22 patients with CGN, those with nephrotic syndrome had the highest output of AAP. But there was no correlation between the intensity of proteinuria and AAP elimination in urine. Proteinuria was in one case of AGN and two cases of lupus erythematosus very low, the immunologically measured AAP was high.

Table II. Enzymatic determination of alanine-aminopeptidase (AAP), alkaline peptidase (AP) and -glutamyltranspeptidase (-GTP) in concentrated urine of 44 normal persons. Control group. Age: 3-66 years, male and female

2 We thank Prof. Pfleiderer from the Ruhr University in Bochum for the use of his purified AAP preparation.
Fig. 1. Two-dimensional tandem-crossed electro-immunodiffusion of papain-digested brush-border fraction (M) and a urine sample of a patient suffering from lupus erythematosus (U). The plate was stained AAP specific and subsequently with amidoblack 10B. The AAP-precipitin line is identical. The solubilized membrane fraction shows two additional proteins. Anti-brush-border antiserum 10 %, agarose 0.8 %, sodium barbital buffer pH 8.6.

Creatinine clearance was decreased in 12 of 14 cases showing kidney membrane proteins in urine and only three of 11 cases without tissue proteinuria. Patients with recognizable membrane proteins in urine showed tendencies toward progression of their kidney diseases. Contrary to these findings most patients without membrane proteins in urine had clinically no definite signs of progression of their disease. Two patients with lupus erythematosus had a continuous high excretion of membrane proteins (fig. 1). The constant elimination of AAP was accompanied by an immunologically measurable content of AP which seems to us a sign of progression of the kidney disease. Within a few weeks both patients had reached terminal renal insufficiency.

In glomerulonephritis the excretion of -GTP was always higher than of AAP. With specific anti--GTP sera the -GTP precipitate could be verified. Different from these findings was the excretion pattern of AAP and -GTP in concentrated urine of patients under cytostatic treatment. The elimination of membrane proteins with AAP activity was generally equal or higher than those of -GTP. In the first group in which patients were treated with a high dose of cytostatic drugs, membrane-bound AAP activity up to 40,000 mU/24 h were measured. Treatment consisted of cyclophosphamide, vincristine sulfate, vinblastine sulfate, cytosine arabinoside, chlorambucil and cytarabin. One may conclude that in cases of high elimination of AAP, surface structures of the brush border were altered. In cases treated with a high dose of cytostatic drugs, membrane proteins appeared very soon (fig. 2). Patients treated with a low dose over a longer period of time clearly showed membrane protein elimination which started considerably later (fig. 3). The more often such cytostatic treatment was applied, the earlier and more pronounced was the membrane protein excretion in urine. This study seems to us to be a first step towards determining a parameter for the testing of nephrotoxicity of pharmaceuticals.
Fig. 3. 62-year-old female with acute lymphoblastic leukemia under treatment with cytosine arabinosid. The lines and broken lines represent the alanine-aminopeptidase (AAP), alkaline phosphatase (AP) and -glutamyltranspeptidase (-GTP) enzymatically determined. The columns show the immunologically measured AAP.

Fig. 4. Alanine-aminopeptidase (AAP), alkaline phosphatase (AP) and -glutamyltranspeptidase (-GTP) measured in 24-hour collections of urine from a patient with Hodgkin's disease. The empty columns represent the appearance of AAP which could be determined with anti-brush-border antisera in radial immunodiffusion technique.

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Summary

In urine concentrates from patients with acute and chronic glomerulonephritis and patients under cytostatic treatment, remarkable amounts of membrane-bound alanine-aminopeptidase, alkaline-phosphatase and -glutamyltranspeptidase could be recognized. With an anti-brush-border antisera membrane-bound enzymes could be differentiated from soluble fractions of other origins. Patients with recognizable membrane proteins in urine showed tendencies toward progression of their kidney diseases. Patients under cytostatic treatment with high dose showed a very early output of these enzymes while others under low dose started after a longer treatment with the excretion of membrane-bound enzymes.

References

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