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The Diagnostic Value of Enzymuria, Cell Excretion, and Proteinuria in Experimental Renal Diseases

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Summary. Enzymuria, urinary cell excretion and proteinuria were simultaneously determined in renal diseases of female Wistar rats in order to investigate the diagnostic value of urinary enzymes. Investigations were carried out on rats with E. coli-pyelonephritis facilitated by oestradiolundecylate, aminonucleoside nephrosis, aminoglycoside induced renal lesions and pyelonephritic animals treated with therapeutic and toxic doses of tobramycin. - From the results of these studies it was concluded that the main diagnostic value of urinary enzymes is detection of drug induced tubular lesions in individuals with preexisting renal diseases.

Key words: Enzymuria, proteinuria, cell excretion, pyelonephritis, tobramycin, gentamicin.

Introduction

Opinions differ on the clinical significance of urinary enzymological investigations apart from the analysis of the alpha amylase. Some authors are of the opinion that the investigation of urinary enzymes gives important diagnostic and differential information about renal affections (7, 8). On the other hand, the lack of specificity of enzymuria has been pointed out (4, 6, 9) and its value therefore remains in doubt (5).

Therefore we tested Wistar rats with different renal diseases to determine whether enzymuria has any diagnostic value compared with proteinuria and cell excretion.

Material and Methods

1. Test animals. Test animals were female albino Wistar rats (strain AF/Han; s. p. f.), kept in macrolon cages in groups of 10 animals during the period between the tests, fed with dry pressed food altromin R 10, and given water through a feeding bottle. The average weight at the start of experiments was 200 g.

2. Urine collection. The animals were each given 6 ml tap water through a pharyngeal tube, and placed in metabolic cages before the nocturnal collection period which always lasted 12 hrs and took place at room temperature.

3. Treatment of urine. The analyses were started within 1 hr after the end of the collection period. The following analyses were performed:

3. 1 Volume measurement

3. 2 Determination of the cell excretion rate/min. Tubular cells (TC) and leucocytes were quantitatively determined using the Fuchs-Rosenthal chamber. Faecal and blood contaminated urine specimens were excluded from the analysis.

3. 3 Enzyme analysis. Before the analysis of urinary enzymes 2 ml of urinary specimen were centrifuged at 15,000 r. p. m. for 2 min and dialysis of the supernatant fluid was carried out for 90 min against flowing tap water using water vapour prepared cellophane. Then photometric determination of the following urinary enzymes was carried out using test combinations (Boehringer/Mannheim; Merck) at Eppendorf 5086 measuring place: Alkaline Phosphatase (AP), Malate Dehydrogenase (MDH), Lactate Dehydrogenase (LDH), Glutamic-Oxalacetic Transaminase (GOT). Table 1 gives information about the measuring conditions. Quality controls with Prezinorm (Boehringer/Mannheim) and Monitrol (Dade). Conversion of the measured enzyme activities/ml into the dimension mU/12 h was carried out taking into consideration the urinary volume.

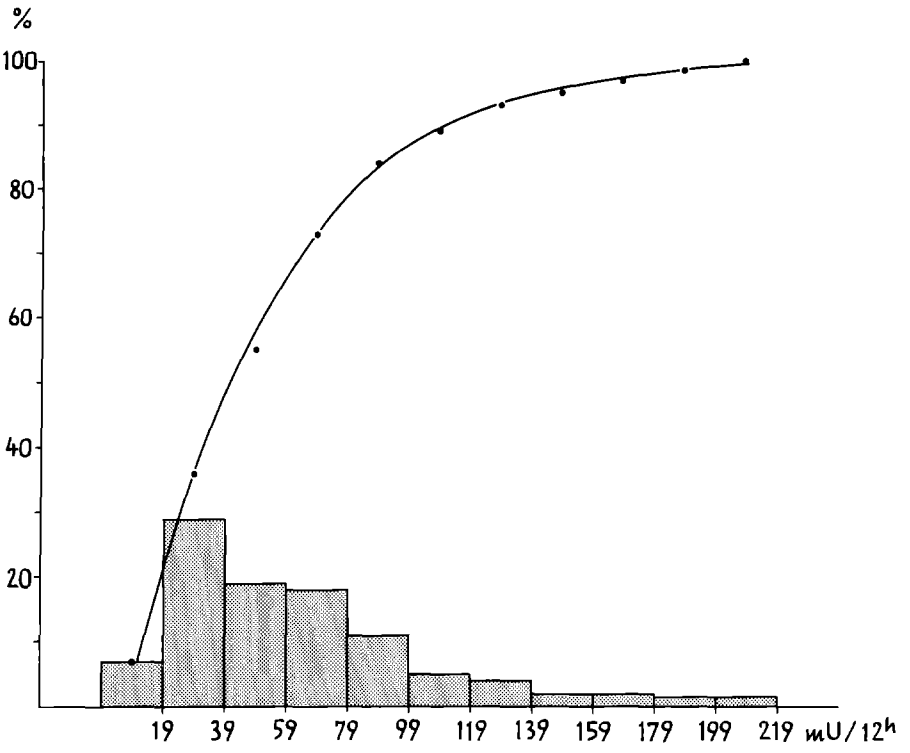

Fig. 1. Statistical distribution of MDH-activities in the nocturnal urine of female Wistar rats (n = 150) 12 hours before giving 6 ml tap water

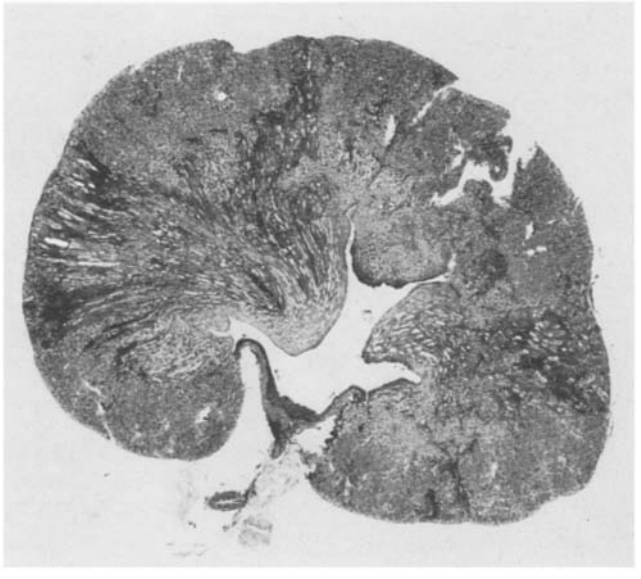

Fig. 2. Kidney: E. coli pyelonephritis (strain 0 25) facilitated by oestradiolundecylate(1 mg/kg/week) on the 21st.day of infection; PAS, 7 x. Sector like inflammatory infiltration of the renal parenchyma

3. 4 Quantitative determination of urinary protein. Determinations of protein in urine were performed with the biuret method (quality control with Monitrol/Dade).

4. Morphological investigations. The animals were sacrificed by depletive cardiac puncture under deep ether anaesthesia. After removing and bisecting, the kidneys were fixed in 5 % formalin. PAS-staining of 4-5 µ thick paraffin slices.

5. Statistical analysis. Since urinary enzymes and rates of cell excretion are distributed statistically almost log-normal (Fig. 1), methods free of distribution were used for the calculation of significance (U-test of Wilcoxon, Mann and Whitney; rank-variation-analysis of Wilcoxon and Wilcox; rank-correlation of Spearman), and the medians of the daily measuring series were determined. The limits of tolerance (TL; 5 % / 95 %) of the norm series were graphically ascertained in the net of probability, the confidence interval (C. I.) of the median determined tabularly (10). The normal values measured under these conditions are shown in Table 2.

6. Experimental renal diseases

6. 1 Pyelonephritis. Facilitation of infection in 20 animals by intramuscular injection of 1 mg oestradiolundecylate/kg/week. Transurethral instillation of 1 ml (= 10^8-10^9 bacteria) of a suspension of E. coli 025:19:12 on the 7th and 14th day after first application of hormone. Dissection of the animals was carried out 3 weeks after the last infection.

Table 1. Spectrophotometric measurements. Temperature: 25° C; Glass cuvette: 1 cm light path; TV: total volume; UV: urine volume added.

Enzyme	EC	Wavelength (nm)	TV (ml)	UV (ml)	Concentrations in the test volumes
AP	3.1.3.1	405	2,02	0,02	1,0 M diethanolamine-HCl-buffer (pH = 9,8); 0,5 mM $MgCl_2$; 10 mM p-nitrophenylphosphate
GOT	2.6.1.1	366	2.50	0,50	80 mM phosphate-buffer (pH = 7,4); 32,2 mM L-aspartate; 0,15 mM NADH; 6,4 mM alpha-oxoglutarate; 0,0078 mg MDH; 0,0078 mg LDH
LDH	1.1.1.27	366	3,15	0,10	50 mM phosphate-buffer (pH = 7,5); 0.18 mM NADH; 0,6 mM pyruvate
MDH	1.1.1.37	366	3,25	0,10	92 mM phosphate-buffer (pH = 7,4); 39 mM aspartate; 1,0 mM alpha-oxoglutarate; 0,18 mM NADH; 0,005 mg GOT

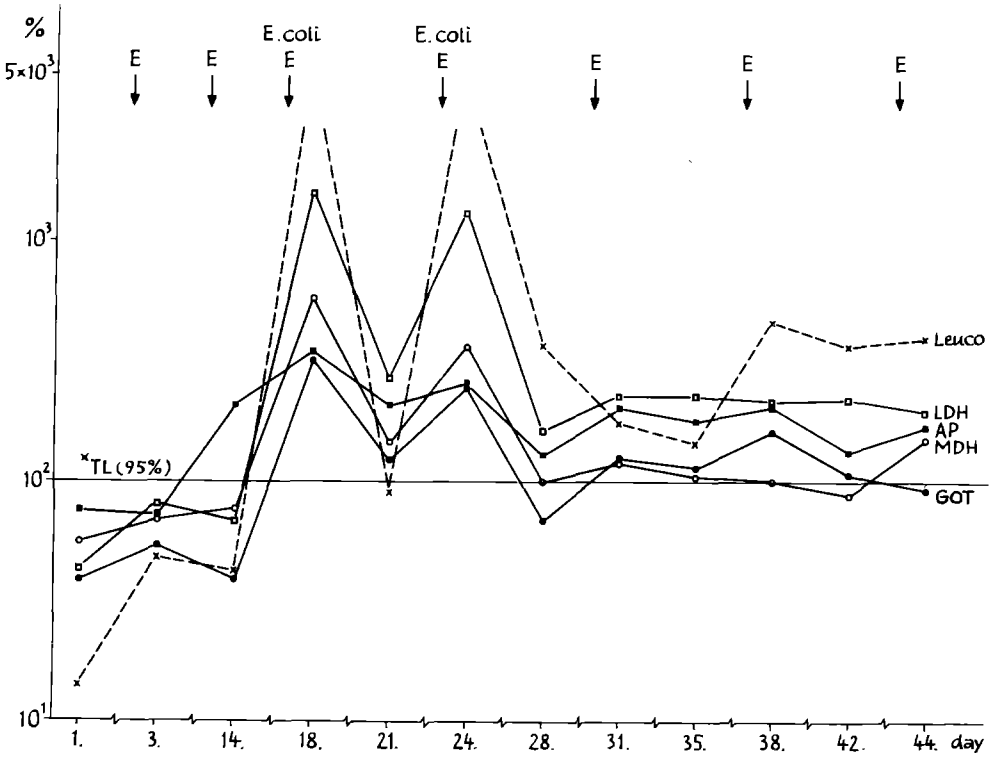

Fig. 3. Leucocyte excretion and enzymuria expressed as percentage during a 44 days course of oestrogen (E) facilitated pyelonephritis (E. coli strain: 0 25); n = 19 rats. TL (95 %) = upper limit of tolerance of the normal values

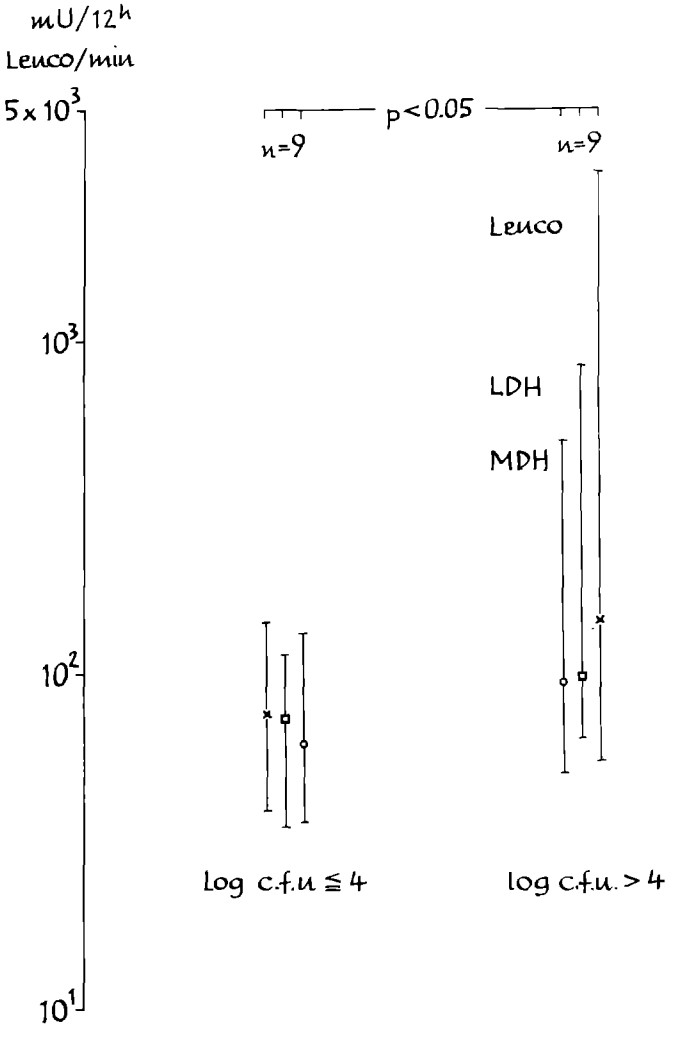

Fig. 4. Dependence of leucocyte excretion and enzymuria ($\bar{x}$, 95% - C.I.) on the renal quantity of colony forming units (c.f.u./g kidney) in oestrogen facilitated pyelonephritis; n = 9 rats/group

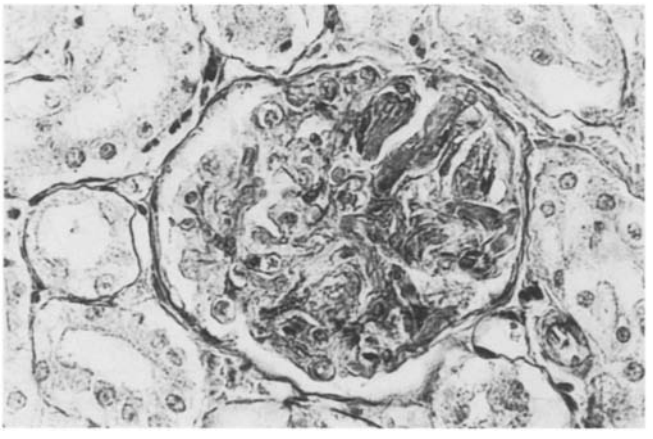

Fig. 5. Glomerulus: Thickening of the capillary basal membranes in aminonucleoside nephrosis (15 mg/kg/ day s.c.) 2 weeks after start of treatment. PAS, Obj. x 40

Quantitative bacteriological analysis of the renal homogenate was carried out by cultivation of the bacteria.

6.2 Aminonucleoside nephrosis. Induction of this disease by daily subcutaneous application of 15 mg aminonucleoside of puromycin/kg/day (Nutritional Biochemicals, Cleveland) for 10 days in 20 animals. Section 14 days after beginning the test.

6.3 Aminoglycoside-induced renal lesions. I.m. injection of 2.5, 5, 10 and 100 mg tobramycin/kg/day or gentamicin for 5 days in 9 single doses at 10 animals each. Section always on the 12th day after the first application of the aminoglycoside.

6.4 Therapy of pyelonephritis. Induction of pyelonephritis in 40 animals as in 6.1. Start of the 1-week-therapy with tobramycin (2.5 mg or 100 mg/kg/day) 10 days after the second infection.

Results

1. Pyelonephritis. The oestrogen induced pyelonephritis of the rat was chosen for urinary enzymological analysis, because this experimental disease with its focal interstitial infiltrates in the histological picture (Fig. 2) and its chronically progressive course corresponds largely to the human non-obstructive pyelonephritis, and therefore investigations using this model could have clinical application.

In Fig. 3 the behaviour of urinary enzyme activities and of the leucocyte excretion rates in this type of pyelonephritis during a 7 week test period is demonstrated. Before the experimentally induced infection only the activity of the AP is significantly elevated in fact because of an oestrogen implied enzyme induction (2).

The day after endovesical coli infection there appeared a massive leucocyturia as expected. Of

Table 2. Normal values in the nocturnal urine volume of n female Wistar rats after giving 6 ml tap water 12 hrs before. Limits of tolerance: 5%/95%.

	n	$\bar{x}$	Limits of tolerance
AP	150	432mU/12h	120/660
GOT	150	9mU/12h	3/ 29
LDH	150	31mU/12h	11/ 90
MDH	150	52mU/12h	15/155
Tubular Cells	150	14/min	6/ 37
Protein	40	7 mg/12h	4/ 11

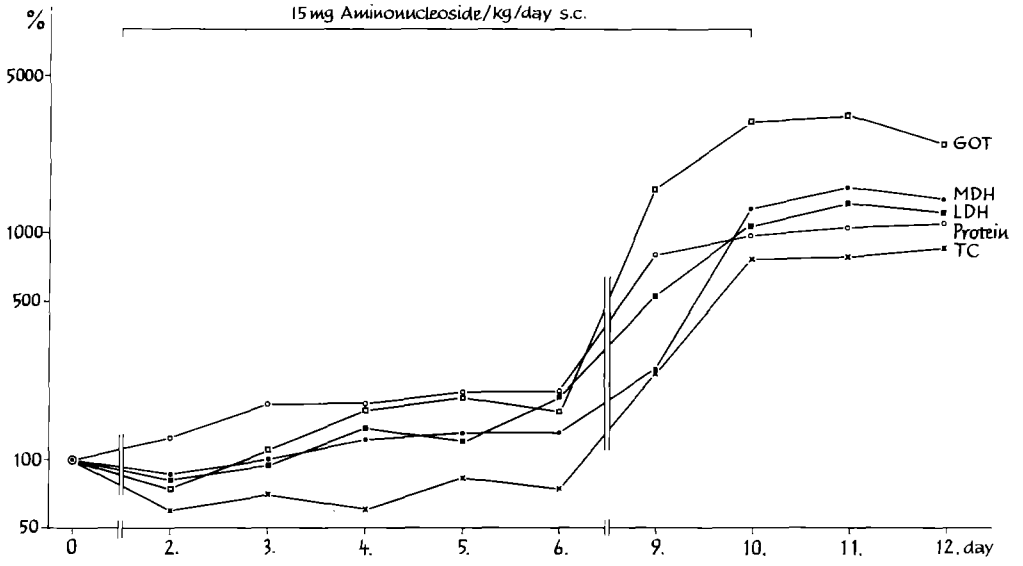

Fig. 6. Proteinuria, enzymuria and excretion of tubular cells (TC) expressed as percentage during the development of aminonucleoside nephrosis of the rat (n = 20); norm-$\bar{x}$ = 100 %

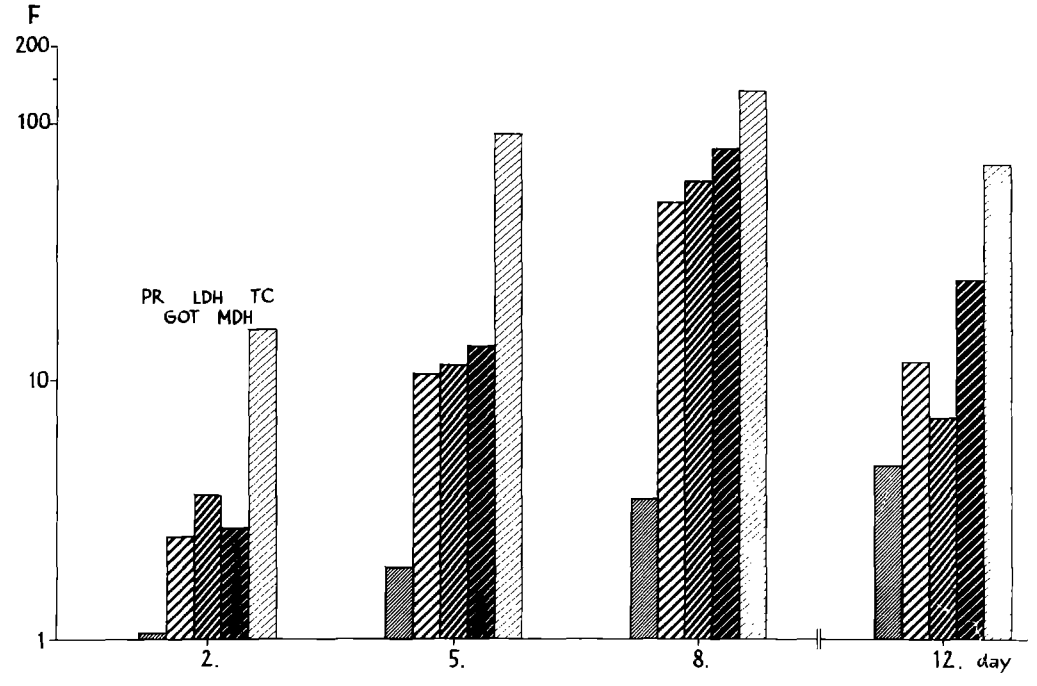

Fig. 7. Comparison of the relative increases of proteinuria (PR), enzymuria (GOT, LDH, MDH) and excretion of tubular cells (TC) during and after a 5-days treatment with 100 mg gentamicin/kg/day i. m. Norm-$\bar{x}$ = 1; F = factor of parameters increase; n = 10 rats

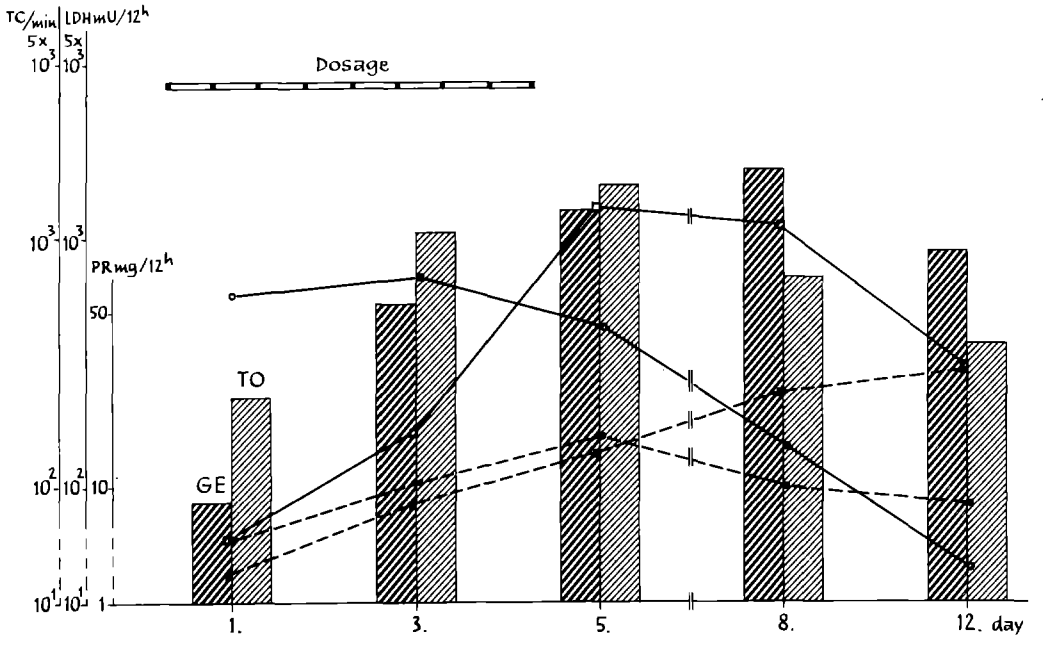

Fig. 8. Excretion of tubular cells (TC, histogram), enzymuria (LDH, ———) and proteinuria (PR, ----) during treatment with gentamicin (□ ■) or tobramycin (○ ●) in comparison ($\tilde{x}$). Dosage: 100 mg/kg/day i.m.; n = 10 rats/series

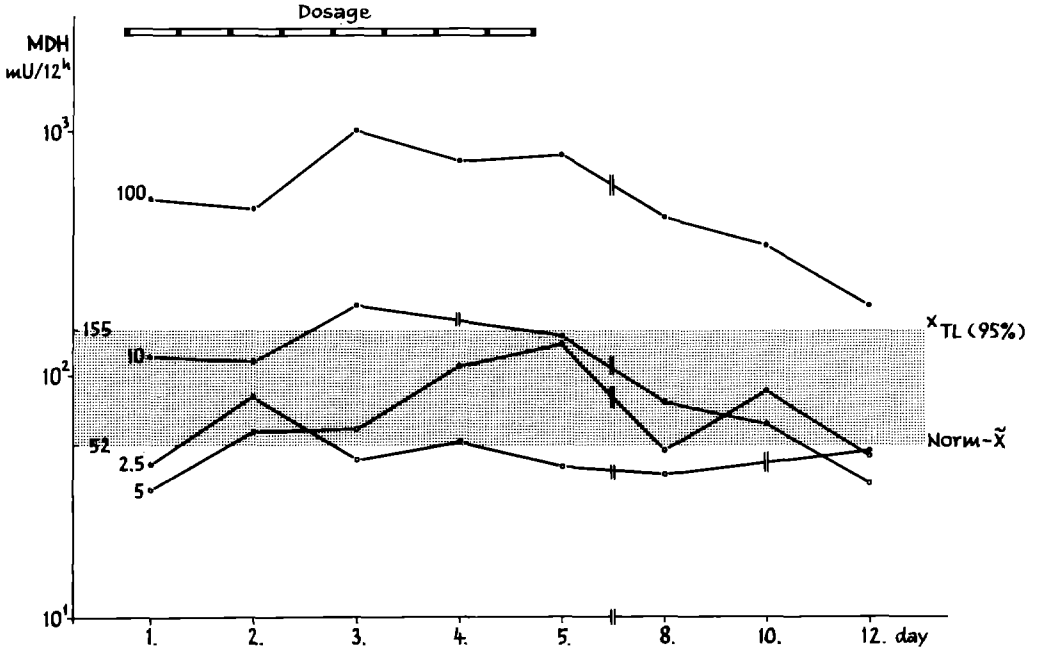

Fig. 9. MDH ($\tilde{x}$) in 12^h urine volume of Wistar rats during treatment with different doses of tobramycin (2,5, 5, 10, 100 mg/kg/day i.m.); n = 10 rats/dose. TL (95%) = upper limit of tolerance of the normal values

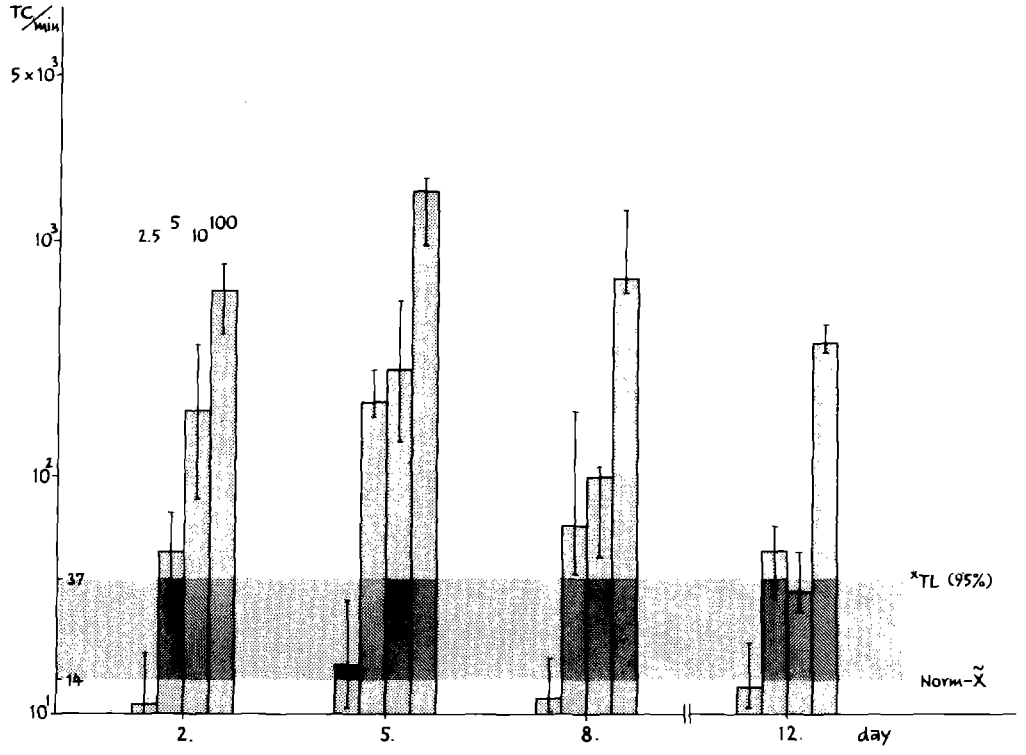

Fig. 10. Excretion of tubular cells (TC; $\tilde{x}$) during 5-days treatment with different doses of tobramycin (2.5, 5, 10, 100 mg/kg/day i.m.); n = 10 rats/dose. TL (95%) = upper limit of tolerance of the normal values

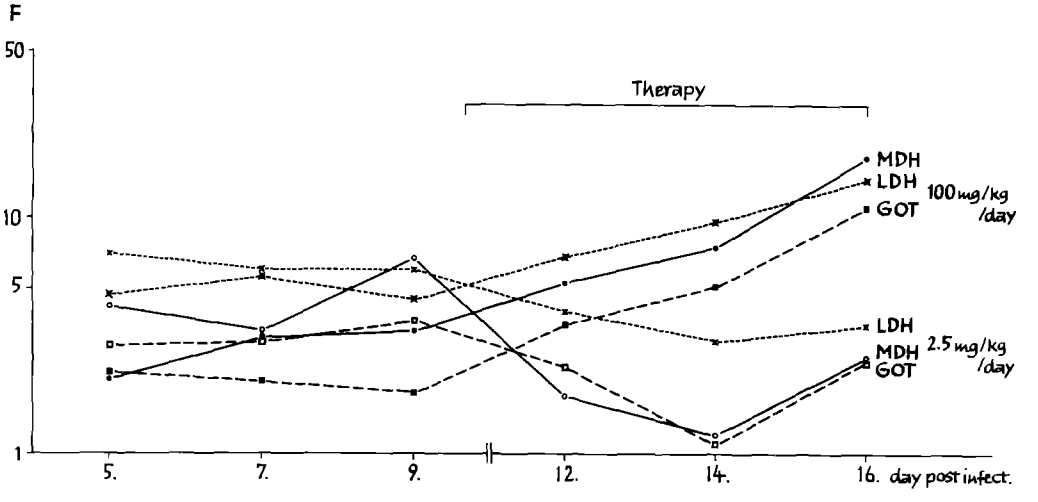

Fig. 11. Enzymuria before and during therapy of pyelonephritic Wistar rats with different doses of tobramycin (2, 5 or 100 mg/kg/day); start of therapy: 10. day after the 2. infection; n = 20 rats/dose. F = factor of activity increase, (norm-$\tilde{x}$ = 1)

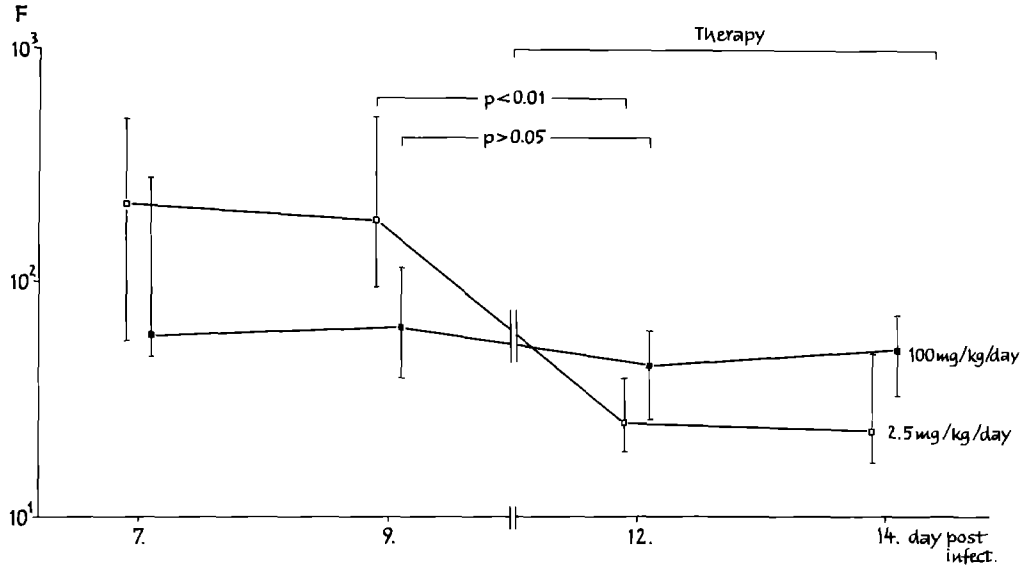

Fig. 12. Cell excretion before and during therapy of pyelonephritic Wistar rats with different doses of tobramycin (2,5 or 100 mg/kg/day); start of therapy: 10. day after the 2. infection; n = 20 rats/dose. F = factor of activity increase, (norm-$\bar{x}$ = 1)

the almost parallely increasing urinary enzymes the LDH is dominating, which is probably of granulocyte origin. Also during the further 3 weeks test period leucocyturia and enzymuria remain on a nearly comparable level. The shifting of proportion between LDH and MDH compared with the normal, which is already known in the obstructive model (7), could be confirmed. A proteinuria appeared only inconstantly.

The excretion of leucocytes shows, as expected, a correlation with the degree of renal inflammation, measured by the number of colony forming units/g kidney. As demonstrated in Fig. 4 there exists an analogous relation between the activity of pyelonephritis and enzyme efflux (LDH and MDH) on the same level of significance (5%).

2. Aminonucleoside nephrosis. After 10 days of treatment with aminonucleoside a typical glomerular nephrosis develops with thickening of the capillary basement membranes (Fig. 5) and protein cylinders in the renal tubules throughout. Further there are to be bound focal necroses of the proximal renal tubular epithelium.

The behaviour of enzymuria, proteinuria and cell excretion during the 12 days test period is demonstrated in Fig. 6. As one can see, a largely parallel and highly significant increase of the urinary diagnostic parameters appears between the 6th and 9th day of the investigation. After the end of the treatment the activities of urinary enzymes (MDH and LDH), the cell excretion, and the protein concentration remain in a pathological range on a comparably high level until the end of the test.

3. Aminoglycoside induced renal lesions. If 100 mg gentamicin/kg/day are given to rats over a course of 5 days, a complex renal lesion appears, which is histologically characterized by extensive tubular necroses and a nonspecific glomerular nephrosis with droplike storage of PAS-positive material in the capillary covering epithelium (11).

In Fig. 7 the behaviour of parameters during the 12-days observation is compared, expressed as percentage. In all phases of the test a pronounced increase of cell excretion, and proteinuria besides a significant enzymuria were detected, which persisted longer than the 5 day period of treatment. Enzyme efflux and rates of cell excretion were statistically closely correlated. Only the analysis of AP yielded non-characteristic results, because its activity in urine is inhibited by aminoglycosides, as was shown by our own additional investigations.

The urinary diagnostic parameters, too, showed differences in the type of toxic influence of the aminoglycosides also with extensive parallelism (Fig. 8). 100 mg tobramycin/kg/day produced a significantly higher increase of enzyme efflux, cell excretion and proteinuria during the period of treatment than the equal dose of gentamicin. On the other hand the statistical relation of all values was reversed after the end of the treatment. The investigation of enzyme activities under different aminoglycoside doses showed, that the enzymes MDH, LDH and GOT have a dose dependent reaction, and therefore allow the determination of the dose of toxic threshold.

Fig. 9 shows the relations between dose and effect regarding the behaviour of MDH after application of different tobramycin doses; furthermore, that 10 mg/kg/day significantly increased enzyme activities.

The rates of cell excretion (Fig. 10) show a similar dose dependent behaviour, which indicates the beginning of renal pathology at a dose of 5 mg/kg/day.

4. Therapy of pyelonephritis. Fig. 11 shows the behaviour of urinary enzymes in groups of 20 pyelonephritic animals, which were treated with different doses of tobramycin. As one can see, the initially - as the result of pyelonephritic process - raised activity of GOT, LDH and MDH tend to normalise under the therapeutic dose of 2.5 mg/kg/day. Compared with this a further significant increase of enzyme activities appears under the toxic tobramycin dose of 100 mg/kg/day.

Cell excretion under therapeutic doses declines correlated with the motion of the urinary enzymes (Fig. 12). But the excretion rates remain on the same level, whereas the enzymes increase, if toxic doses of tobramycin are given.

Discussion

To clarify the diagnostic value of enzymuria various urinary enzymes, cell excretion and proteinuria were investigated in Wistar rats with pyelonephritic disease, nephrosis, and aminoglycoside induced renal lesions.

As can be seen from the results of the pyelonephritic series the activity curves of LDH, MDH and GOT characterise the course of disease in a similar way as the excretion rates of leucocytes. Also the cell excretion informs about the bacterial activity of the renal inflammation as well as the enzyme efflux, in an equivalent way. Since however a raised leucocyte excretion in contrast to the increased activity of urinary enzymes is always a sign of urinary tract infection - and furthermore the quantitative investigation of urinary cells is methodically less time consuming compared with the analysis of urinary enzymes, the estimation of urinary enzymes is of no additive diagnostic importance in pyelonephritis. Only the shifting of the ratio of LDH:MDH in favour of the LDH compared with the normal activity seems to be specific for bacterial inflammatory renal processes. Within the range of urinary enzymological studies of nephrotoxicity the evidence allows the recognition and elimination of pyelonephritic animals which could not be done using the quantitative sediment, since the tubular epithelial cells and leucocytes cannot clearly be differentiated in the native preparation.

As in pyelonephritis the parameter enzymuria can be diagnostically renounced in aminonucleoside nephrosis too, because morphologically informative inferences can be made about a tubular lesion with the quantitative determination of the tubular cells; furthermore the finding of a large proteinuria proves a disorder of glomerular filtration. Thus, using these two parameters it is possible to draw conclusions about the kind of morphological disturbance, which under clinical conditions can then be classified by renal biopsy.

Also the complex aminoglycoside induced renal lesion, as well as differences in the type of toxic action regarding tobramycin and gentamicin can be described by the urinary diagnostic parameters proteinuria, enzymuria, and cell excretion. Regarding the correlation between enzyme efflux and cell excretion one first may be tempted to direct attention towards the studies of nephrotoxicity using only the analysis of cellular excretion rates and to renounce urinary enzymological investigations. The demonstrated results concerning the examination of dose effect relations also seem to support this simplified test arrangement: Indeed the urinary enzymes showed dose dependent renal reactions; but the quantitative sediment produced at least equivalent diagnostic information.

From this statement it can be said that when seeking the limits of renal tolerance for an antibiotic substance, the registration of the cellular excretion rates alone can be considered as sufficient criterion. Therefore in the first testing of chemotherapeutics in patients with healthy kidneys the measurement of cell excretion before, during and after application is in our opinion sufficient for detection of tubular toxic reactions.

In clinical tests newly developed antibiotics are often given to patients with renal diseases in order to observe their antibacterial effectiveness upon the pyelonephritic process and to detect simultaneously undesired side effects of the tested substances. Therefore it was necessary to investigate whether or not the cell excretion indicates tubular toxic influences sufficiently even in the case of preexisting renal disease. These results showed that pyelonephritic rats treated with a toxic tobramycin dose react with a further urinary enzyme increase under therapy, while the cell excretion remains on the pathologic level provoked by the renal infection. If one considers that leucocytes in the natively investigated urine cannot be differentiated from tubular cells with certainty, the missing increase of cells in urine must be derived from the two drug induced counter-current developments. On the one side the antibacterial substance produces a suppression of inflammatory activity and thus a reduction of excretion of leucocytes. On the other hand the toxic dose leads to tubular necroses with increased desquamation of cells. So under these test conditions the analysis of urinary enzymes dominates clearly over the selective registration of the cell excretion and should therefore always be used in clinical investigations of nephrotoxicity in pyelonephritic patients.

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