Eur. J. Clin. Chem. Clin. Biochem.
Vol. 29, 1991, pp. 775–782

Excretion of Urinary Enzymes in Female Sprague-Dawley Rats in Relation to Cellular Compartment, Creatinine Excretion and Diuresis

By *E. Bomhard*[1], *D. Maruhn*[2] and *H. Mager*[3]

[1] *Institute of Industrial Toxicology, Bayer AG, Wuppertal, Germany*
[2] *Clinical Research International, Bayer AG, Wuppertal, Germany*
[3] *Institute of Biometry, Bayer AG, Wuppertal, Germany*

(Received May 8/October 2, 1991)

Summary: One hundred and one young-adult female Sprague-Dawley rats were acclimatized to metabolic cages for 2 days. After that time 24-hour urine was collected at a constant cooling temperature of 0–4 °C. After gel filtration the enzyme activities were determined, and the resulting values were used to calculate 24-hour excretions. The following reference ranges (2.5 and 97.5 percentiles) were determined (in mU/24 h): lactate dehydrogenase 43–181; phosphohexoseisomerase 45–1445; glutathione-S-transferase 1–299; alkaline phosphatase 27–1239; leucine arylamidase 72–377; γ-glutamyltransferase 1334–9188; arylsulphatase A 59–309; β-galactosidase 76–305; β-glucuronidase 20–2756; β-N-acetyl-*D*-glucosaminidase 66–491; glutamate dehydrogenase 7–711. There was a significant (though not very high) correlation with diuresis for the lysosomal enzymes β-N-acetyl-*D*-glucosaminidase, arylsulphatase A and β-galactosidase, and for glutamate dehydrogenase, lactate dehydrogenase, phosphohexoseisomerase and alkaline phosphatase. The relation to creatinine excretion was markedly close for the lysosomal enzymes β-N-acetyl-*D*-glucosaminidase, arylsulphatase A and β-galactosidase ($r = 0.71–0.83$), as well as for alkaline phosphatase, leucine arylamidase and γ-glutamyltransferase. There was a relatively high correlation between the excretion of β-N-acetyl-*D*-glucosaminidase, arylsulphatase A and β-galactosidase among themselves ($r = 0.63–0.81$) as well as between leucine arylamidase and γ-glutamyltransferase ($r = 0.75$).

Introduction

Urinary enzymes are now well established indicators of pathological events in the nephron (1). In spite of their rapidly growing use very few data are available on normal variation in untreated rats. Such data seem to be necessary for the assessment and evaluation of effects. Available published data are restricted to a small number of enzymes (2–7), or were obtained with only small numbers of animals (8, 9). We therefore measured the excreted activity of a total of 11 enzymes in non-treated female Sprague-Dawley rats.

The relationship between the excretion of some enzymes and their relation to creatinine excretion and/or diuresis in human beings has been the subject of studies by several authors (10–14). But no relevant data are available for the rat. The objective of the following evaluation is therefore to determine associations between excreted enzymes, diuresis and creatinine excretion.

In addition, we investigated correlations between the excretion of those enzymes that stem from the same cellular structures (e. g. brush border, cytosol, lysosomes). Such data are known for humans (10, 15–17) but not for animals.

Materials and Methods

Animals

The study was carried out in young-adult female Sprague-Dawley rats bred by Lippische Versuchstierzucht (experimental animals breeding farm), Extertal, Germany. The animals had a body weight of approx. 250 to 350 g and were approx. 15 to 30 weeks of age. After their arrival the animals were acclima-

tized to the conditions in the animal room for at least one week. During that time they were kept individually in Makrolon Type-II cages. They were administered Altromin 1324 pellets as feed and tap water ad libitum. The room temperature was 22 ± 2 °C, humidity approx. 55 ± 10%. Continuous artificial lighting of the animal room took place from 7 a.m. to 7 p.m. After this adaptation period the animals were acclimatized to the metabolic cages for another 2 days with free access to feed and water. No other treatment was applied to the animals prior to urine collection.

Urine collection and preparation

After the acclimatization period, 24-hour urine was collected once from each animal. For this purpose polyethylene bottles were used, which were continuously cooled in order to maintain a temperature in the range of 0 to 4 °C. During that time the animals received tap water ad libitum. No feed was administered, to avoid contamination of urine. The metabolic cages consisted of stainless steel (Uno Inc., B.V. metalware factory, Zevenaar, Holland) and allowed separate collection of urine and faeces.

Urine samples were subjected to gel filtration as described previously (18).

Enzyme assays and measurement of creatinine

Microlitre methods were used for enzyme[1]) determinations. The activities of lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase and leucine arylamidase were measured using continuous assay methodology as described earlier (19).

Phosphohexoseisomerase was determined according to *Büding & McKinnon* (20) with slight modifications. Sample volume fraction was 0.166 and the continuous assay was at 334 nm and 25 °C using an Eppendorf photometer PCP 6121 (Netheler and Hinz, Hamburg, Germany).

Glutamate dehydrogenase was measured using a modification (sample volume fraction 0.287) of the method recommended by the German Society for Clinical Chemistry (21).

Arylsulphatase A, β-galactosidase, β-N-acetyl-*D*-glucosaminidase and β-glucuronidase were determined as described earlier (19, 22).

Glutathione-S-transferase was measured according to *Feinfeld* et al. (24) with the following modification: Sample volume fraction 0.48, continuous assay at 25 °C and 334 nm.

Quality control of the urinary enzyme assays was performed using a stable liquid control material as described (24).

The urine volumes were used to subsequently convert the measured activities into total excretion per 24 hour collection period.

[1]) Investigated urinary enzymes:

β-N-Acetyl-*D*-glucosaminidase	EC 3.2.1.30
Alkaline phosphatase	EC 3.1.3.1
Arylsulphatase A	EC 3.1.6.1
β-*D*-Galactosidase	EC 3.2.1.23
β-Glucuronidase	EC 3.2.1.31
γ-Glutamyltransferase	EC 2.3.2.2
Glutamate dehydrogenase	EC 1.4.1.3
Glutathione-S-transferase	EC 2.5.1.18
Lactate dehydrogenase	EC 1.1.1.27
Leucine arylamidase	
(cytosolic)	EC 3.4.11.1
(microsomal)	EC 3.4.11.2
Phosphohexoseisomerase	EC 5.3.1.9

If visual assessment of the urinary samples revealed bloody discoloration (a total of 4 animals), all results of the enzyme determination were excluded from biometric evaluation. In a few instances, no measurable enzyme activity was observed. In this case the value 0.00 mU/24 h was used in the statistical calculations.

In order to assess the associations between enzyme and creatinine excretions the latter were determined by unmodified AutoAnalyzer methodology N-11 B (Technicon Instruments Co., Tarrytown U.S.) according to *Chasson* et al. (25).

Biometric methods

The data were characterized per variable using appropriate measures of location and dispersion. In those instances where a logarithmic normal distribution showed a substantially improved fit when compared with the normal distribution, the geometric mean and standard deviation were also calculated.

The shape of the distributions was examined visually on the basis of the corresponding histograms, and the adequacy of the normal distribution assumption was judged using either the *Shapiro-Wilk* test (sample size lower than 51) or the *Kolmogorov-Smirnov* test (sample size exceeding 50). For all distributions fitted to the data a χ^2-goodness of fit test comparing expected and observed frequencies was run to obtain an overall indication of the fit of the models (nominal significance level $\alpha = 0{,}05$).

Linear associations between the various variables were assessed using the *Pearson* correlation coefficient (r), whereas general monotone trends were analysed by the *Spearman* rank counterpart (r_s). The critical values of the *Pearson* correlation coefficient for sample sizes approximately encountered in this study ($\alpha = 0.01$, $n = 100$ and 40, respectively) are 0.254 ($n = 100$) and 0.93 ($n = 40$). The former is valid for all correlations ($n = 99$ or 101) except for those with β-glucuronidase ($n = 39$). Note that due to the large number of available measurements a highly significant correlation coefficient does not necessarily imply a strong relationship.

Results

Reference values and distribution pattern

The results of the determinations are summarized in table 1 in the form of arithmetic mean values with standard deviation, medians, and the 2.5 and 97.5 percentiles (reference interval). The range between the 2.5 and 97.5 percentiles is to be regarded as the reference range.

The variability of individual values around the respective mean value was frequently very extreme. In some cases standard deviation even exceeded the mean value (phosphohexoseisomerase, glutamate dehydrogenase, β-glucuronidase, glutathione-S-transferase).

For the majority of enzymes[1]) (lactate dehydrogenase, alkaline phosphatase, leucine arylamidase, γ-glutamyltransferase, arylsulphatase A, β-galactosidase, β-N-acetyl-*D*-glucosaminidase) the upper limit of the reference range exceeded the median by not more than three times. For other enzymes, the upper limit

Tab. 1. Mean values, standard deviation (SD), median values and reference intervals (mU/d) for the 11 urinary enzymes investigated.

Enzyme	n	Mean (mU/d)	SD (mU/d)	Median (mU/d)	Reference interval (2.5–97.5 percentiles) (mU/d)
Lactate dehydrogenase	101	84	31	78	43– 181
Phosphohexoseisomerase	101	265	286	168	45–1445
Glutathione-S-transferase	101	69	84	42	1– 299
Alkaline phosphatase	101	415	284	399	27–1239
Leucine arylamidase	101	188	68	172	72– 377
γ-Glutamyltransferase	101	4253	1842	3930	1335–9188
Arylsulphatase A	101	181	64	183	59– 309
β-*D*-Galactosidase	101	193	57	191	76– 305
β-N-Acetyl-*D*-glucosaminidase	101	252	108	259	66– 491
β-Glucuronidase	39	748	746	523	20–2756
Glutamate dehydrogenase	101	110	143	74	7– 711

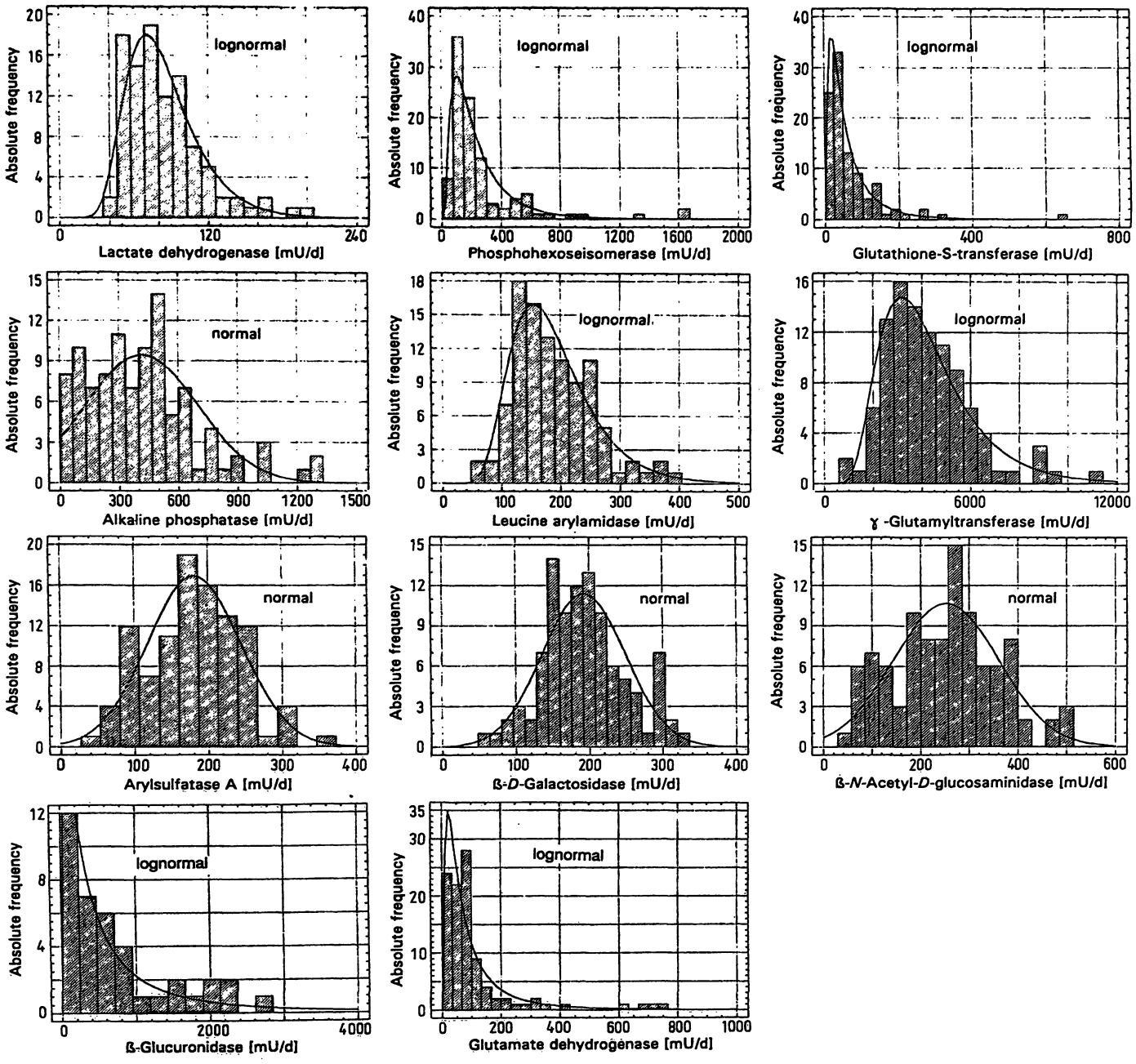

Fig. 1. Absolute frequency distribution of individual excretion rates (in mU/d) for the 11 urinary enzymes investigated

of the reference range is conspicuously greater than the median: β-glucuronidase (5.5 times), glutathione-S-transferase (8 times), phosphohexoseisomerase (18 times) and glutamate dehydrogenase (23 times).

The individual values for each investigated enzyme were arranged according to magnitude. The percentage frequency of the individual activity ranges is given in figure 1.

The normal distribution hypothesis was not rejected for alkaline phosphatase, γ-glutamyltransferase, arylsulphatase A, β-galactosidase and β-N-acetyl-*D*-glucosaminidase, nor was the hypothesis of logarithmic normal distribution (left-hand steep) for lactate dehydrogenase, leucine arylamidase, γ-glutamyltransferase, β-galactosidase, β-glucuronidase and glutathione-S-transferase. Consequently, both null hypotheses could not be rejected for γ-glutamyltransferase and β-galactosidase. However, in agreement with the results of the χ^2-goodness of fit test, the form of frequency distribution indicates that β-galactosidase is rather normally and γ-glutamyltransferase is rather lognormally distributed. For alkaline phosphatase a two-peak distribution cannot be excluded. After a square-root transformation, however, the data follow approximately a normal distribution.

Although phosphohexoseisomerase and glutamate dehydrogenase obviously show neither normal distribution nor logarithmic normal distribution, the description of data should be given on the basis of the parameter estimates of a logarithmic normal distribution. Otherwise one would have to use more complicated transformations.

By far the highest excretion on average was measured for γ-glutamyltransferase, amounting to 3000 mU/24 h. The second highest excretions were those of β-glucuronidase and alkaline phosphatase. The excretions of glutathione-S-transferase were relatively low, followed by glutamate dehydrogenase and lactate dehydrogenase.

There were relatively large differences in variability. For example, approx. 80% of the values of lactate dehydrogenase were within the very narrow range between 50 and 100 mU/24 h. Further enzymes with the majority of values in a relatively narrow activity range included arylsulphatase A (86% of the values between 100 and 250 mU/24 h), β-galactosidase (72% of the values between 120 and 240 mU/24 h), leucine arylamidase (82% of the values between 120 and 280 mU/24 h), glutathione-S-transferase (approx. 81% of the values between 0 and 100 mU/24 h). Variability is relatively wide in the case of γ-glutamyltransferase (here, for example, only 65.4% of the values are located between 2000 and 5000 mU/24 h), in the case of alkaline phosphatase (which is conspicuous by a frequency peak between 7.5 and 100 mU/24 h, another in the range from 300 to 500 mU/24 h, and additionally by a major proportion of values > 500 mU/24 h) and especially in the case of β-glucuronidase. Concerning the latter only 61.5% of the values are located within the wide range between 20 and 600 mU/24 h. This is the reason why further determination of this enzyme did not seem sensible. With respect to their variability the other enzymes lie between these two groups.

Relations between the excretion of enzymes, urine and creatinine

The following correlation coefficients (r) were calculated between the enzyme activities excreted within 24 hours and the urine volumes and creatinine excretion respectively:

Enzyme	Urine volume	Creatinine
Lactate dehydrogenase	0.48	0.44
Phosphohexoseisomerase	0.32	−0.03
Glutathione-S-transferase	0.06	−0.02
Alkaline phosphatase	0.29	0.62
Leucine arylamidase	0.16	0.32
γ-Glutamyltransferase	0.14	0.33
Arylsulphatase A	0.43	0.83
β-Galactosidase	0.39	0.71
β-N-Acetyl-*D*-glucosaminidase	0.49	0.77
β-Glucuronidase	0.14	0.25
Glutamate dehydrogenase	0.48	0.24

According to these results the extent of the correlation between enzyme excretion and the excretion of urine is generally rather low. The coefficients of correlation vary from r = 0.06 (glutathione-S-transferase) to r = 0.49 (β-N-acetyl-*D*-glucosaminidase).

Non-significant (nominal α-level = 0.01) correlation coefficients were obtained for glutathione-S-transferase, β-glucuronidase, leucine arylamidase and γ-glutamyltransferase. On the whole, r and r_S correspond sufficiently well. Consequently, one can proceed on the assumption that the excretions of lactate dehydrogenase, phosphohexoseisomerase, glutamate dehydrogenase, alkaline phosphatase, arylsulphatase A, β-galactosidase, and β-N-acetyl-*D*-glucosaminidase in urine show a tendency to rise as the urine volume increases.

Except for phosphohexoseisomerase and glutathione-S-transferase the release of all other enzymes into the urine exhibits a positive relationship to creatinine excretion. The correlations are relatively high for the lysosomal enzymes, arylsulphatase A, β-galactosidase, and β-N-acetyl-*D*-glucosaminidase as well as for alkaline phosphatase, the latter originating from the brush border (r > 0.60).

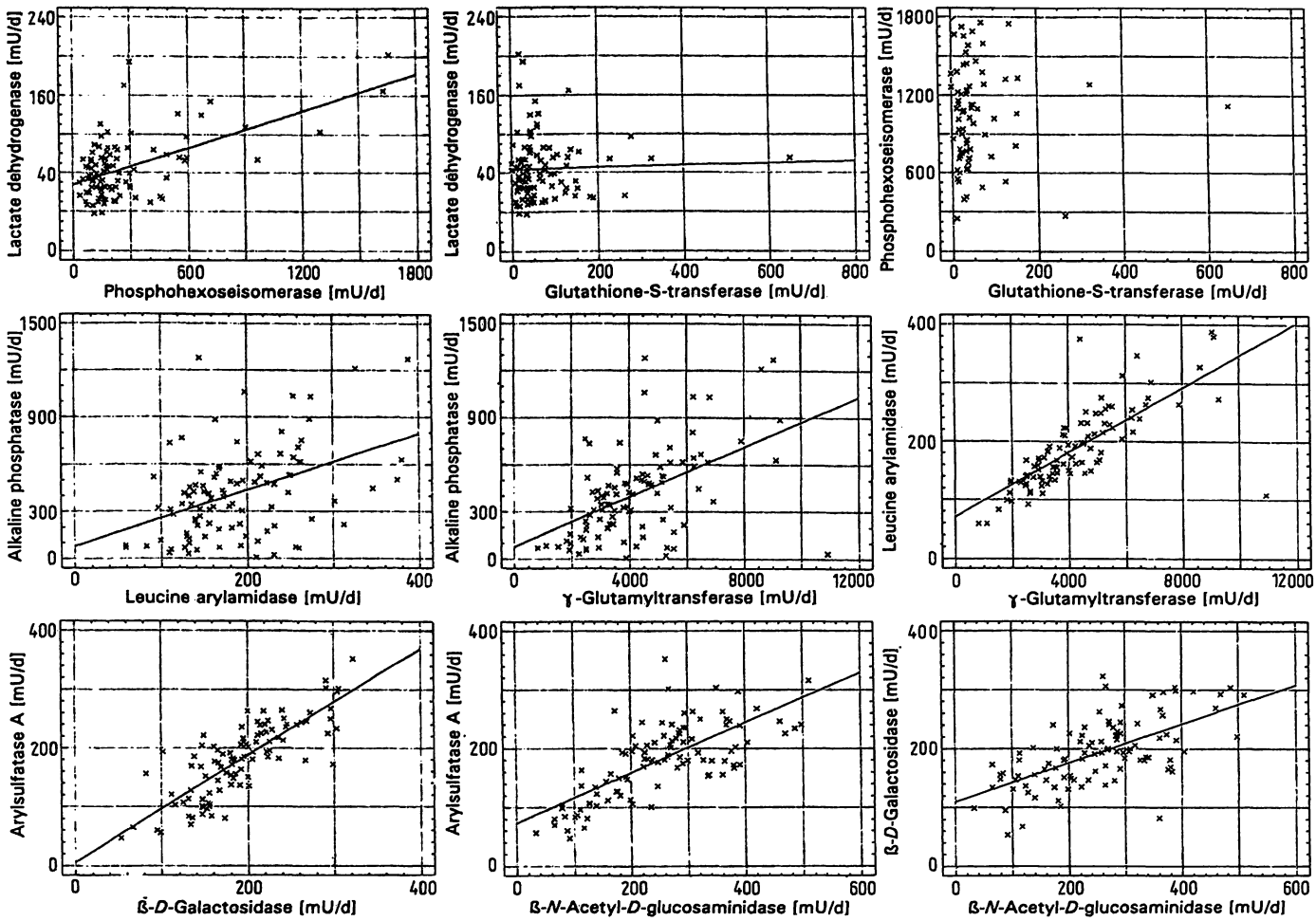

Fig. 2. Correlation between urinary enzymes of cytosolic, brush border or lysosomal origin.

Coordinacy of excretion of enzymes from the same cellular compartment

The following correlation coefficients were calculated for the enzymes originating from cytosol.

	Phosphohexose-isomerase	Glutathione-S-transferase
Lactate dehydrogenase	0.58	0.03
Phosphohexoseisomerase		0.13

According to these results no linear relationship exists between lactate dehydrogenase on the one hand and phosphohexoseisomerase and glutathione-S-transferase on the other hand. Furthermore, as can be seen from figure 2, the relatively high correlation ($r = 0.58$) between lactate dehydrogenase and phosphohexoseisomerase is essentially determined by 4 to 5 points well separated from the rest of the data; logically the coefficient of rank correlation showed a value of only 0.37. Thus, a real parallelism between the excreted activity of the two enzymes does not seem to exist.

In contrast, brush border enzymes display closer associations:

	Leucine arylamidase	γ-Glutamyl-transferase
Alkaline phosphatase	0.43	0.51
Leucine arylamidase		0.75

In this case the correlations between enzymes, especially between leucine arylamidase and γ-glutamyltransferase are reflecting a real general tendency; however, only the relation between leucine arylamidase and γ-glutamyltransferase seems to be sufficiently close. The coefficients of rank correlation are well in accordance with the above mentioned values.

The generally closest relations between excreted activities were found for the lysosomal enzymes (excluding β-glucuronidase) (see also fig. 2). The differences with respect to the coefficients of rank correlation are maximally 0.05.

	β-Galactosidase	β-N-acetyl-*D*-glucosaminidase	β-Glucuronidase
Arylsulphatase A	0.81	0.72	0.17
β-Galactosidase		0.61	0.09
β-N-acetyl-*D*-glucosaminidase			0.14

Discussion

A prerequisite for the differentiation of pathological conditions, e. g. in the kidney, with toxic nephropathy being of particular interest here, is a knowledge of the normal range. Such information was found (often mentioned only incidentally) in the literature for the following enzymes: alkaline phosphatase, leucine arylamidase, γ-glutamyltransferase, β-N-acetyl-*D*-glucosaminidase, lactate dehydrogenase, and glutamate dehydrogenase. The comparison of such values can be problematic, not least because of different urine collection conditions (with or without cooling, and at which temperature), the period over which urine was collected, methods of measurements etc. The results are also rather substantially influenced by the animal strain employed and by age and sex of the animals. Aspects of this problem together with appropriate examples have been discussed inter alia by *Plummer* (4) and by *Plummer* et al. (26). Different treatment of urinary samples prior to enzyme determination itself can also have an important effect on the results. We used gel filtration of urines (18). This method removes interfering and inhibitory factors from the urine as well as reduces preparation times. These are essential advantages over dialyses and thus generally result in higher enzyme activity values.

Despite these qualifications a comparison with the values of other authors seems to be sensible, since it is not to be expected that they will shift e. g. in their order of magnitude and variation.

A relatively great number of normal values is available for the brush border enzymes γ-glutamyltransferase, alkaline phosphatase and leucine arylamidase. Median glutamyltransferase excretion was 2400 mU/24 h with 95% percentiles at 8400 mU/24 h in male Wistar rats (7). *Stoykova* et al. (5) examined adult male Wistar rats with a total of seven 24-hour urine collection periods within 140 days. They found glutamyltransferase excretions of about 7000 mU/24 h (range: 6180 – 8300 mU/24 h), which were highly constant. *Grötsch* et al. (8) observed the excretion of γ-glutamyltransferase over a period of 65 days in male and female Wistar rats each. They found a pronounced age-related increase, which ranged from an average of 905 (day 1) to 8867 (day 60) mU/24 h for males and from 784 (day 1) to maximum 2187 mU/24 h (day 40) for females. This apparent contradiction is probably due to the fact that the animals used by *Grötsch* et al. (8) were essentially younger at the start of study than those used by *Stoykova* et al. (5). From about day 30 of the experiment, the excretion rates of the rats employed by *Grötsch* et al. (8) are highly constant. *Zbinden* et al. (6) found a mean excretion of approx. 9900 ± 170 mU/24 h in female rats of the strain Iva: SIV50. These results reveal that the urines of adult animals, independent of the rat strain employed, show a very high γ-glutamyltransferase activity, which lies on average between 2000 and 7000 mU/24 h.

As far as alkaline phosphatase is concerned *Ngaha & Plummer* (3) and/or *Plummer* (4) established a value of 420 ± 374 mU/24 h in male Wistar rats. *Planas-Bone* (27) determined a value of 3885 ± 221 mU/24 h in male Heiligenberg rats. Both the mean value and the standard deviation of our own data correlate very well with those of *Plummer*'s team.

Median leucine arylamidase excretion was 84 mU/24 h with 95% percentile of 252 mU/24 h in male Wistar rats (7). These values seem to be negligibly lower than those of the study in hand.

With regard to the enzymes located in the cytosol, most information about normal values was found for lactate dehydrogenase. In female Sprague-Dawley rats 24-hour excretions of 117 ± 23 mU and 390 ± 86 mU/24 h have been published (28, 29). *Bogatzki* (30) reports a value of 103 mU/24 h per 100 g body weight in albino rats. In male Wistar rats excretion was 132 ± 112 mU/24 h (3, 4). In the same order of magnitude is the median of 168 mU/24 h (with 95% percentiles at 504 mU/24 h) reported by *Zekert & Mautner-Markhof* (7) for male Wistar rats.

No glutathione-S-transferase activity could be identified in urine (and in serum) by means of an enzymatic assay (31). The fact that in our test series we failed to detect activity in only two animals is probably due to the use of a 2-fold sample volume, as well as the application of gel filtration.

Surprisingly little information about normal values was found for the lysosomal β-N-acetyl-*D*-glucosaminidase which is very frequently investigated in nephrotoxicity experiments. *Nakamura* et al. (9) determined a mean excretion of 185 ± 12 mU/24 h in male as well as 125 ± 9 mU/24 h in female SCL-SD rats. In Wistar rats (n = 5) mean excretion rates were between 77 ± 39 and 237 ± 80 for males and 90 ± 39 to 204 ± 70 mU/24 h for females (8). The low values

were determined in relatively young animals in each case. The differences with respect to our own data do not seem to be important.

Mean excretion of mitochondrial glutamate dehydrogenase was 25 ± 33 mU/24 h for male Wistar rats (4). From the data of *Bogatzki* (30), a mean excretion of 74 mU/24 h can be calculated for male albino rats.

With regard to diuresis-dependency of the excretion of urinary enzymes, *Jösch & Dubach* (11) found that the release of lactate dehydrogenase, alkaline phosphatase and aryl-amidase increases in diuresis and decreases in antidiuresis. This was valid both for healthy humans and for rats. A hyperbolic relation between diuresis and alanine aminopeptidase excretion was established as a result of investigations with 10 test subjects (10). Corresponding results were obtained by *Thiele* (13) who measured γ-glutamyltranspeptidase excretion in humans. Measuring β-N-acetyl-*D*-glucosaminidase, alanine aminopeptidase, alkaline phosphatase and γ-glutamyltransferase in 6 healthy male humans, *Jung* et al. (12) found that all 4 enzymes showed increased excretion with rising urinary flow. The excretion of the brush border enzymes was more strongly affected than that of lysosomal β-N-acetyl-*D*-glucosaminidase. Our own investigations revealed a linear correlation, although not very close, for the lysosomal enzymes β-N-acetyl-*D*-glucosaminidase, arylsulphatase A and β-galactosidase, as well as the mitochondrial glutamate dehydrogenase and the cytosolic lactate dehydrogenase; the correlation was less close for phosphohexoseisomerase and alkaline phosphatase. No significant correlation existed for glutathione-S-transferase, β-glucuronidase, leucine arylamidase, and γ-glutamyltransferase.

Creatinine excretion, which is often taken as a reference value, also significantly correlated with volume. There was an especially close relationship between creatinine and the lysosomal enzymes β-N-acetyl-*D*-glucosaminidase, arylsulphatase A, and β-galactosidase (correlation coefficient between 0.71 and 0.83), as well as alkaline phosphatase ($r = 0.62$).

The correlation of leucine arylamidase and γ-glutamyltransferase was higher with respect to creatinine excretion than with respect to volume. Interestingly, the converse is true in the case of the cytosolic enzymes.

Correlation of the lysosomal enzymes arylsulphatase A, β-galactosidase, and β-N-acetyl-*D*-glucosaminidase among themselves was very high. This suggests that these enzymes originate from the same nephron sections and are released via similar pathways from the cell. However, there was virtually no correlation between these three enzymes and β-glucuronidase. No data relevant to this observation are known from literature on animal experiments. *Paigen & Peterson* (15) investigated a population of 125 healthy adult persons; they also observed a very high correlation with respect to the excretion of the lysosomal enzymes β-glucuronidase, α-galactosidase, β-galactosidase and β-hexoseaminidase among themselves, but not with cytosolic lactate dehydrogenase. The correlation coefficients ($r = 0.753-0.849$) were very close to the range reported here.

Their investigations, however, showed a β-glucuronidase response similar to that of the other lysosomal enzymes. *Burchardt* et al. (32) on the other hand, did not find a significant correlation between arylsulphatase A and β-glucuronidase excretion.

Despite the fact that the brush border enzymes are confined to an anatomically clearly definable and relatively small section of the nephron, the relations between alkaline phosphatase on the one hand and leucine arylamidase or γ-glutamyltransferase on the other hand are markedly less pronounced than in the case of the lysosomal enzymes. However, correlation is comparatively high ($r = 0.75$) between leucine arylamidase and γ-glutamyltransferase. Relatively weak, but still significant, correlations between these three enzymes were also reported in healthy subjects by *Szasz* (16) and *Thiele* (17).

The lack of correlation between the cytosolic enzymes lactate dehydrogenase, phosphohexoseisomerase, and glutathione-S-transferase could be causally related to their different localisation in the nephron. While glutathione-S-transferase is exclusively localised in the proximal tubule of rat, rabbit and man (33–35), lactate dehydrogenase is distributed over large parts of the nephron with high activities in the distal tubule (36). We have no information about the distribution of phosphohexoseisomerase in the nephron of the rat.

Acknowledgement

We thank Prof. Dr. *H. Mattenheimer*, Chicago, for stimulating discussion and critical comments on the manuscript.

References

1. Price, R. G. (1982) Urinary enzymes, nephrotoxicity and renal disease. Toxicology *23*, 99–134.
2. Braun, J. P., Rico, A. G., Bernard, P., Burgat-Sacaze, V., Ethbali, B. & Godfrain, J. C. (1978) La gamma-glutamyl transferase urinaire en toxicologic rénale chez la rat. Toxicology *11*, 73–82.
3. Ngaha, E. O. & Plummer, D. T. (1977) Toxic renal damage: changes in enzyme levels. Biochem. Med. *18*, 71–79.
4. Plummer, D. T. (1981) Urinary enzymes in drug toxicity. In: *Testing for Toxicity* (Gorrod, J. W., ed.) pp. 156–171, *1. Toxicology*, Taylor & Francis Ltd, London.
5. Stoykova, S., Philippon, C., Labaille, F., Prevot, D. & Manuel, Y. (1983) Comparative study of alanine-aminopeptidase and gamma-glutamyl-transferase activity in normal Wistar rat urine. Lab. Anim. *17*, 246–251.
6. Zbinden, G., Fent, K. & Thouin, M. H. (1988) Nephrotoxicity screening in rats; general approach and establishment of test criteria. Arch. Toxicol. *61*, 344–348.
7. Zekert, F. & Mautner-Markhof, C. (1976) Untersuchungen über die normale Enzymausscheidung im Harn des Menschen und der Ratte. Wiener Klin. Wochenschr. *88*, 467–469.
8. Groetsch, H., Hropot, M., Klaus, E., Malerczyk, V. & Mattenheimer, H. (1985) Enzymuria of the rat: biorhythms and sex differences. J. Clin. Chem. Clin. Biochem. *23*, 343–347.
9. Nakamura, M., Miyata, K., Tamura, T. & Kawabata, T. (1981) Sex difference in urinary excretion of alanine aminopeptidase in the rat. Jap. J. Nephrol. *23*, 209–212.
10. Burchardt, U., Hempel, A., Hoepfner, J. B. & Hempel, R. D. (1976) Alaninaminopeptidase- und Kreatininausscheidung unter verschiedenen Diuresezuständen beim Menschen. Zschr. Inn. Med. *31*, 550–552.
11. Joesch, W. & Dubach, U. C. (1967) Einfluß der Diurese auf die Enzymurie. Clin. Chim. Acta *15*, 325–330.
12. Jung, K., Schulze, G. & Reinholdt, C. (1986) Different diuresis-dependent excretions of urinary enzymes: N-Acetyl-β-D-glucosaminidase, alanine aminopeptidase, alkaline phosphatase, and gamma-glutamyltransferase. Clin. Chem. *32*, 529–532.
13. Thiele, K. G. (1973) Gamma-Glutamyltranspeptidase-Aktivität im Urin bei Gesunden und Nierenkranken. Klin. Wochenschr. *51*, 339–345.
14. Werner, M., Heilbron, D. C., Maruhn, D. & Atoba, M. (1970) Patterns of urinary enzyme excretion in healthy subjects. Clin. Chim. Acta *29*, 437–449.
15. Paigen, K. & Peterson, J. (1987) Coordinacy of lysosomal enzyme excretion in human urine. J. Clin. Invest. *61*, 751–762.
16. Szasz, G. (1970) Gamma-Glutamyltranspeptidase-Aktivität im Urin. J. Clin. Chem. Clin. Biochem. *8*, 1–8.
17. Thiele, K. G. (1974) Enzymausscheidung bei eingeschränkter Nierenfunktion. Verh. Dtsch. Ges. Inn. Med. *80*, 774–776.
18. Maruhn, D. (1979) Preparation of urine for enzyme determinations by gel filtrations. Curr. Probl. Clin. Biochem. *9*, 22–29.
19. Maruhn, D., Fuchs, I., Mues, G. & Bock, K. D. (1976) Normal limits of urinary excretion of eleven enzymes. Clin. Chem. *22*, 1567–1574.
20. Bueding, E. & McKinnon, J. A. (1955) Studies of the phosphoglucose isomerase of schistosoma Mansoni. J. Biol. Chem. *215*, 507–513.
21. Recommendations of the German Society for Clinical Chemistry (Empfehlungen der Deutschen Gesellschaft für Klinische Chemie) (1972). Z. Klin. Chem. Klin. Biochem. *10*, 182–192.
22. Maruhn, D. (1976) Rapid colorimetric assay of beta-galactosidase and N-acetyl-β-glucosaminidase in human urine. Clin. Chim. Acta *73*, 453–461.
23. Feinfeld, D. A., Fleischner, G. M., Goldstein, E. J., Levine, R. D., Levine, J. D., Avram, M. M. & Arias, I. M. (1979) Ligandinuria: an indicator of tubular cell necrosis. Curr. Probl. Clin. Biochem. *9*, 273–280.
24. Maruhn, D., Wehling, K. & Metz, U. (1986) A stable liquid control material for urinary enzyme assays. Clin. Chim. Acta *160*, 119–122.
25. Chasson, A. L., Grady, H. J. & Stanley, M. A. (1961) Determination of creatinine by means of automatic chemical analysis. Am. J. Clin. Pathol. *35*, 83–88.
26. Plummer, D. T., Noorazar, S., Obatoni, D. K. & Haslam, J. D. (1985–1986) Assessment of renal injury by urinary enzymes. Uremia Invest. *9*, 97–102.
27. Planas-Bohne, F. (1977) The effect of mercuric chloride on the excretion of two urinary enzymes in the rat. Arch. Toxicol. *37*, 219–225.
28. Raab, W. (1967) Experimentelle Untersuchungen zur Steigerung der Laktatdehydrogenase-Aktivität des Harnes. Experientia (Basel) *23*, 833.
29. Raab, W. & Hohenegger, M. (1968) Renale Enzymausscheidung bei Ratten nach hohen Dosen von Penicillin, Kanamycin und Sulfonamiden. Clin. Chim. Acta *20*, 95–103.
30. Bogatzki, M. (1965) Protein- und Fermenturie bei experimenteller kompensatorischer Nierenhypertrophie und experimentellem renalen Hochdruck. Fortschr. Med. *83*, 859–863.
31. Feinfeld, D. A., Bourgoignie, J. J., Fleischner, G., Goldstein, E. J., Biempica, L. & Arias, I. M. (1977) Ligandinuria in nephrotoxic acute tubular necrosis. Kidney Int. *12*, 387–392.
32. Burchardt, U., Peters, J. E., Neef, L., Thulin, H., Gruendig, C. A. & Haschen, R. J. (1977) Der diagnostische Wert von Enzymbestimmungen im Harn. Zschr. Med. Labor.-Diagn. *18*, 190–212.
33. Fine, L. G., Goldstein, E. J., Trizna, W., Rozmaryn, L. & Arias, I. M. (1978) Glutathione-S-transferase activity in the rabbit nephron: segmental localization in isolated tubules and formation of thiol adducts of ethacrynic acid. Proc. Soc. Exp. Biol. Med. *157*, 189–193.
34. Fleischner, G. M., Kamisaka, K., Gatmaitan, Z. & Arias, I. M. (1976) Immunologic studies of rat and human ligandin. In: *Glutathione: metabolism and function* (Arias, I. M. & Jakoby, W. B., eds.), pp. 259–265, Raven Press, New York.
35. Harrison, D. J., Kharbanda, R., Cunningham, D. S., McLellan, L. I. & Haves, J. D. (1989) Distribution of glutathione S-transferase isoenzymes in human kidney: basis for possible markers of renal injury. J. Clin. Pathol. *42*, 624–628.
36. Schmidt, U. & Dubach, U. C. (1971) Quantitative Histochemie am Nephron. Prog. Histochem. Cytochem. *2*, 185–298.

Dr. E. Bomhard
Institute of Industrial Toxicology
Friedrich-Ebert-Straße 217–333
W-5600 Wuppertal 1
Germany