Urinary Enzyme Measurements in the Diagnosis of Renal Disorders

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ABSTRACT

In view of Mattenheimer's comprehensive review of enzymes in renal disease in this journal only four years ago, major emphasis has been placed on findings in the newer literature. Especially important are those reported at the recent Symposium on Diagnostic Significance of Enzymes and Proteins in Urine held at Kanderstag, Switzerland in March of 1978.

The pathophysiology of enzyme release in renal disease is presented, the current status of enzyme measurement reviewed, and the laboratory findings in several main types of renal disease summarized. While the measurement of selected urinary enzymes has been found extremely useful in specific situations, most investigators agree that routine screening is not warranted at this time. Newer developments in the measurement of isoenzyme patterns hold promise of introducing increased diagnostic specificity and appear to be the wave of the future.

Introduction

Is it worthwhile to use urinary enzymes in the diagnosis and prognosis of renal disorders?

In 1959, Rosalki and Wilkinson described an increased urinary excretion of lactate dehydrogenase LDH (EC 1.1.1.27) (table I) in kidney disease and introduced the concept of urinary enzymes as a diagnostic tool. Wacker and Dorfmann's recommendation in 1962 of urinary LDH as a possible screening test for carcinoma of the kidney and bladder stimulated the investigation of a wide variety of urinary enzymes. Thus, less than ten years later a symposium devoted to "Enzymes in Urine and Kidney" was held at Rheinfelden, Switzerland and Raab's review on the subject in 1972 cites over 40 enzymes which have been investigated for the possible diagnosis of urorenal diseases. While the topic has been less actively explored over the past few years, a small number of clinical and research laboratories have contributed extensively, as evidenced by the proceedings of a second symposium under Drs. U. C. Dubach and U. Schmidt held at Kanderstag, Switzerland in 1978 on the
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**TABLE I**

Abbreviation of Enzymes with Classification Numbers (1978)

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Classification Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>EC 1.1.1.27</td>
</tr>
<tr>
<td>Malate dehydrogenase (MDH)</td>
<td>EC 1.1.1.37</td>
</tr>
<tr>
<td>t-GLUTAMYLTRANSFERSASE (GGT)</td>
<td>EC 2.3.2.2</td>
</tr>
<tr>
<td>Acid phosphatase (ACP)</td>
<td>EC 3.1.3.2</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>EC 3.1.3.1</td>
</tr>
<tr>
<td>Arylsulfatase A (ASA)</td>
<td>EC 3.1.6.1</td>
</tr>
<tr>
<td>Lysosome (LYS)</td>
<td>EC 3.2.1.17</td>
</tr>
<tr>
<td>a-Glucosidase (GLU)</td>
<td>EC 3.2.1.20</td>
</tr>
<tr>
<td>ß-Galactosidase (GAL)</td>
<td>EC 3.2.1.23</td>
</tr>
<tr>
<td>Trehalase (TRE)</td>
<td>EC 3.2.1.28</td>
</tr>
<tr>
<td>N-Acetyl-ß-glucosaminidase (NAG)</td>
<td>EC 3.2.1.30</td>
</tr>
<tr>
<td>ß-Glucuronidase (GRS)</td>
<td>EC 3.2.1.33</td>
</tr>
<tr>
<td>N-Acetyl-ß-galactosaminidase</td>
<td>EC 3.2.1.53</td>
</tr>
<tr>
<td>Alanine aminopeptidase (Cytosol)</td>
<td>EC 3.4.11.l*</td>
</tr>
<tr>
<td>Alanine aminopeptidase (Microsomal)</td>
<td>EC 3.4.11.2†</td>
</tr>
</tbody>
</table>

*Formerly Leucine aminopeptidase (LAP), (EC 3.4.1.11).
†Formerly Leucine arylamidase (LAS), (EC 3.4.1.12).

‘Diagnostic Significance of Enzymes and Proteins in Urine.’ Meanwhile, Werner has described comprehensively the methodological problems in the assay of urinary enzymes, and Mattenheimer reviewed for this journal only four years ago the more recent biochemical and clinical aspects of urinary enzymes. Hence, this paper will emphasize significant findings from the past three to four years, rather than provide a comprehensive review.

**Methodological Problems**

The numerous technical problems which have arisen in the measurement of urinary enzymes include (1) activators, and especially inhibitors, (2) non-standardized methodologies, (3) diurnal variations, (4) urine specimen problems, such as reliability of random versus 24 hour specimens and collection and storage conditions, and (5) expression of units in relationship to volume and to creatinine or creatinine clearance. While interferences have traditionally been removed from urine specimens by dialysis, gel filtration and ultrafiltration are satisfactory alternative approaches. Although many methods have not been optimized, normal limits of urinary enzyme excretion have been published by Maruhn for 11 semi-standardized methods and modified microversions of nine standard procedures have been described by Werner. Very recently, Jung and Scholz have published an optimized kinetic method for alanine aminopeptidase (AAP) which fully meets the demand for an automated method in clinical chemistry. Thus, while improvements in methodology have been made in recent years, there has not been widespread employment of optimized methodologies. Although activities can be expressed as International units per liter of urine (U per l), it is probably soundest to report the values in terms of activity in International units per mg of creatinine excreted or as a “fractional clearance” in relation to creatinine clearance. The intent of such normalization procedures is to reduce variability, but they cannot correct for incomplete urine collection or loss of enzyme activity during long collection periods.

**Pathophysiological Rationale**

Under normal conditions, only few enzymatic activities of the urine derive from the serum, entering the urine by glomerular filtration. Enzymes exceeding a molecular weight of 70,000 are not normally excreted into the urine, at least in significant amounts; the vast majority of urinary enzymes must derive intrinsically from the kidneys. Renal tubular cells contain high activities of many enzymes, required to fulfill their biochemical functions. Normal turnover rate of the tubular cells and changes of their cellular permeability account for the renal enzymes excreted in normal urine. Genital secretions and bacteria usually contribute only in a minor way to urinary enzyme activity, except for acid phosphatase in males.
Thus, the urogenital tract is a closed compartment with only a limited number of potential enzyme sources to contribute to the enzymes present in the urine.

In the past, urinary enzymes have been used mainly to diagnose catastrophic and undifferentiated tissue damage, such as occurs in renal infarction or in the rejection of renal transplants, and to follow post surgical renal damage. Instead of considering the kidney simply as cortex and medulla, in 1974 Ceriotti proposed enzyme location, the possibility that enzyme mobilization and enzyme molecular dimensions be evaluated in selection of the choice of enzymes for investigating renal function. Thus, he suggested that (a) enzymes for the study of glomerular filtration should be present in blood, absent from renal tissue, and of such a molecular weight as to be normally non-filterable, (b) those for tubular reabsorption should be present in blood, absent from renal tissue, and of low molecular weight in order to be filtered freely and normally reabsorbed, and (c) those for evaluation of the anatomical and functional condition of the tubular epithelial cells should be present in renal tissue only. His criteria and rationale with suggested examples are summarized in table II.

Although alanine aminopeptidase (AAP) does not meet the criterion of being absent from renal tissue, it is present in blood and is not normally filtered by the glomerulus owing to its high molecular weight. The renal isoenzyme, AAP (microsomal), present in kidney tissue, has a slower electrophoretic mobility than the serum isoenzyme, AAP (cytosol).

While Ceriotti proposed a battery (table II) consisting of (1) alanine aminopeptidase (cytosol) as a measure of glomerular damage, (2) lysozyme and malate dehydrogenase (MDH) as a measure of tubular reabsorptive damage, and (3) α-glucosidase (GLU) for the measure of parenchymatous or lytic enzymuria, he omitted the measurement of urinary AAP from his experimental investigations. The serum and urine values for lysozyme and MDH were related to those for creatinine and expressed as fractional clearances, and α-glucosidase, which does not occur in the serum, as μU per min divided by creatinine clearance and multiplied by 100. His clinical findings are summarized in table III as percentage of patients showing a significant change.

Some patients with glomerular damage, such as occurs in chronic glomerulonephritis...
phritis and nephrotic syndrome, showed a decreased fractional clearance of the low molecular weight lysozyme (14,400), but the excretion of the higher molecular weight MDH (70,000) increased four to seven times. On the other hand, the urinary excretion of lysozyme increased three to five times in renal lithiasis and chronic pyelonephritis and 20 to 25 times in chronic uremia, whereas MDH increased 20 to 25 times in renal lithiasis and chronic uremia, but almost 50 times in chronic pyelonephritis. Ceriotti interpreted the increased enzyme clearances to be due to a deficiency of tubular reabsorption and, therefore, directly related in frequency and severity to the extension of the tubular atrophy. The lysosomal α-glucosidase (GLU) enzyme does not usually occur in serum but normally does so in urine. It is present in renal tubular epithelium and may be found in the blood in uncommon renal disorders such as anuria. It was found to increase two to five times in the renal disorders previously cited, with the highest (five times) urinary levels occurring in chronic uremia and acute renal failure.

Dubach has reported a quantitative histochemical investigation of the metabolic differentiation of the kidney which shows a complicated architecture and the coexistence of differently structured epithelia. Furthermore, the enzyme pattern of the distal tubule, including the thick ascending limb of Henle’s loop located in the medulla, approximates to that of the proximal tubule convolutions located in the cortex; only quantitative and not qualitative differences in enzymes exist between the two tubules of the nephron. Thus, the excreted enzymes cannot be expected to indicate which part of the nephron has undergone damage.

Clarification of the pathophysiological mechanisms for the increased or occasionally decreased excretion of urinary enzymes may be the main hindrance to their broader diagnostic application. Such clarification is difficult because of the numerous problems in undertaking experiments with human patients. Nevertheless, Hautmann has carried out a unique study in which he compared urinary patterns for 11 enzymes with the corresponding tissue patterns from the healthy kidney versus the damaged kidney in patients with unilateral renal disease, the diagnosis of which was confirmed subsequently by histological examination. These comparisons were made for 23 healthy kidneys, 16 malignant kidney tumors, four adenomas, nine papillomas, and 88 cases of specific and non-specific renal inflammation (table IV).

The LDH activity in the urine of tumor patients was significantly increased in every renal disorder investigated although the tissue activities were relatively unchanged. Malate dehydrogenase activity decreased somewhat in the tumor tissue. On the other hand, urinary MDH increased (two to three times) in every investigated type of renal disorder, but was much higher (eight to 23 times) in patients with tumors. For GGT, both the malignant tissue and the corresponding urine showed a significant drop in activity which was not observed with benign tumors. Using this enzyme pattern for malignant tumors of increased tissue and urinary LDH, decreased tissue MDH but increased urinary MDH and decreased tissue, and urinary GGT in comparison with clinical diagnoses, Hautman cites correct enzymatic diagnoses as shown in table V.

Hautmann claims 129 of the 141 malignant tumor patients subsequently investigated to have been correctly diagnosed, with 12 false negatives for a sensitivity of 91 percent. Also, false positives were given by four of 13 patients with benign renal tumors and by two of 86 patients with various inflammatory renal diseases, and in six of 47 healthy patients for a specificity of 92 percent. Since, in contrast, all other patients showed an increase
TABLE IV
Enzymes in Tissues and Urine*

<table>
<thead>
<tr>
<th></th>
<th>LDH N</th>
<th>LDH Urine</th>
<th>MDH N</th>
<th>MDH Urine</th>
<th>GGT N</th>
<th>GGT Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Healthy</td>
<td>23</td>
<td>197</td>
<td>22</td>
<td>135</td>
<td>16</td>
<td>22</td>
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<tr>
<td>Malignant</td>
<td>16</td>
<td>233</td>
<td>225</td>
<td>78</td>
<td>209</td>
<td>5</td>
</tr>
<tr>
<td>Tumours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign adenomas</td>
<td>4</td>
<td>204</td>
<td>210</td>
<td>118</td>
<td>165</td>
<td>24</td>
</tr>
<tr>
<td>Benign papilloma</td>
<td>9</td>
<td>169</td>
<td>181</td>
<td>126</td>
<td>127</td>
<td>27</td>
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<tr>
<td>Tuberculosis</td>
<td>7</td>
<td>274</td>
<td>220</td>
<td>154</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Non-specific inflammation</td>
<td>21</td>
<td>254</td>
<td>177</td>
<td>140</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>56</td>
<td>280</td>
<td>189</td>
<td>145</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Cystic</td>
<td>4</td>
<td>248</td>
<td>165</td>
<td>155</td>
<td>39</td>
<td>29</td>
</tr>
</tbody>
</table>


**Tissue mean levels in U/g wet tissue.
**Urine mean levels in U/l of urine.
*:All urine values statistically significant at \( P = 0.0005. \)

N = Number of patients.

LDH = Lactate dehydrogenase.

MDH = Malate dehydrogenase.

GGT = \( \gamma \)-Glutamyltransferase.

in GGT activity, Hautmann has found a decreased urinary GGT to be very useful in the diagnosis of malignant renal tumors. So far, it is unknown whether the drop in GGT activity in kidney tumor tissue and urine is caused by a decrease in activity or by an inhibitor, or whether one form of GGT is converted to another under certain circumstances.

Bilateral ureteric catheterization does not lend itself as a routine diagnostic collection procedure even though renal split function procedures are done occasionally. As a matter of fact, Dr. Hautmann recommends the procedure only in a search for malignant tumors or when the patient’s life depends on the correct diagnosis. Unfortunately, even this sophisticated study sheds little light on our understanding of renal pathophysiology aside from showing that the MDH and GGT enzyme composition of malignant tumor tissue differs from that of normal renal tissue and their urinary excretion levels are altered.

Several experimental approaches are available for the study of renal pathophysiology and urinary enzymes in various types of animal models ranging from nephrotoxic heavy metals such as cadmium, lead, and mercury to the nephrotoxic aminoglycoside antibiotics and certain drugs such as aspirin and phenacetin.

### Clinical Interpretations

Urinary enzymology, like other clinical laboratory diagnostic procedures, should aim at finding characteristic signs of alteration for specific renal diseases or groups of renal diseases. Like many analytical test methods, assays of urinary enzymes have a limited field of indication, and in general are not recommended as screening procedures. This is especially true since not only certain drugs, x-ray contrast media, and other diagnostic drugs but also extrarenal diseases such as myocardial infarction and hyperthyroidism may influence the enzymes present in urine and their activities. However, at the same time the possibility exists of utilizing urinary enzymes excretion for the evaluation of functional nephrotoxic damage by new drugs. In view of several recent reviews of the biochemical, methodological, and clinical aspects of urinary enzymes, this presentation will emphasize significant clinical studies carried out during the past three to five years with no attempt at being comprehensive.
Acute Renal Failure

For the evaluation of acute renal failure, Haschen and his colleagues developed a panel of ten different urinary enzymes but experience led them to diminish their set to three enzymes and to add the evaluation of the urinary proteins. Alanine aminopeptidase (AAP-microsomal) was selected as a typical tubular brush border enzyme, beta-glucuronidase (GRS) as a lysosomal enzyme, and lysozyme (LYS) as a low molecular weight enzyme which passes the glomerular membrane and is usually reabsorbed by the tubules. The data were normalized by being expressed in terms of the multiple of standard deviations above the normal mean value observed for each parameter. Their findings in various renal diseases and in hyperthyroidism are summarized in table VI.

Unlike the other renal diseases, acute renal failure results not only in an excessive increase of the brush border enzymes such as AAP (microsomal), (mean increase of 34 SD), but also in a very high level of the lysosomal GRS (mean increase of 46 SD). On the other hand, the low molecular weight lysosomal LYS increased by 12 SD, only one-fourth as much, perhaps due to better tubular reabsorption of the smaller molecule. In acute renal failure, proteinuria was very high. In contrast, chronic pyelonephritis without retention resulted in almost negligible increases in all parameters. However, chronic pyelonephritis with retention showed an entirely different pattern with a mean increase of 28 SD in protein, less than 5 SD change in AAP, no change in GRS but a 20 SD change in lysozyme excretion, in some patients exhibiting up to a 320 SD increase. Thus, the large increase in proteinuria correlates with the large increase in urinary LYS in chronic pyelonephritis and assists in distinguishing it from chronic pyelonephritis without retention.

Some increase in urinary AAP was observed in patients with non-renal diseases, such as hyperthyroidism and early infective hepatitis. Interestingly enough, Burchardt et al have found AAP excretion to be a useful indicator of therapy control in hyperthyroid patients receiving thyrostatic drugs, even though the excretion level of AAP did not prove helpful for the diagnosis of hyperthyroidism because of other occasional factors influencing urinary enzyme excretion.

Another clinical application of urinary enzyme estimation by Haschen's group was the finding that patients with renal disease develop osmotic nephrosis more intensively than subjects without renal infections. According to their data, osmotic nephrosis results in an increased urinary output of brush border enzymes such as AAP (microsomal). The clinical significance lies in the possibility of distinguishing patients suffering from latent pyelonephritis without retention, without bacteriuria and without an increased cell content of the urine from those patients suffering from active chronic pyelonephritis with retention and with bacteriuria.

Acute Renal Damage

Feinfeld et al have investigated ligandinuria as a specific marker of renal
Ligandin, a dimer of molecular weight 46,000 daltons, has two functions: first, it binds many organic anions and several hormones, functions in the transfer of organic anions from plasma into the liver, and may have a similar role in organic anion transport in the renal tubule. Second, ligandin is the major glutathione-S-transferase enzyme and catalyzes the conjugation of many ligands with the sulfhydryl group of glutathione. Immunofluorescence studies in the kidney showed ligandin in the cytoplasm of the proximal tubular cells with no staining in the glomeruli or interstitium. Ligandinuria was measured both enzymatically and by immunodiffusion on agarose plates with agreement in results by the two techniques. Feinfeld's findings are summarized in table VII.

Ligandinuria was not found in normal patients, patients with chronic renal failure of various etiologies, nor in patients with acute renal failure without tubular necrosis, such as glomerulonephritis and hepatorenal syndrome. Ligandinuria was observed in two renal patients with acute tubular necrosis in whom urine collections were obtained within 24 hours of the precipitating event but was negative for 10 other patients from whom the urine was not obtainable prior to 96 hours after the acute injury. Of 17 patients studied before and after renal arteriography, six developed transient ligandinuria. None of these patients subsequently developed acute renal failure by clinical or laboratory criteria.

However, Feinfeld's most significant findings were with perfusates from 13 kidneys which were obtained from cadaver donors, stored prior to transplantation, and examined for ligandin. Both assays were used, and the analyst was unaware of the post-implantation clinical course. Subsequently, the results and clinical course were correlated. All eight transplant patients receiving kidneys which had showed ligandinuria developed post-transplant acute oliguric renal failure, but none of those receiving kidneys in which the perfusates were negative for ligandinuria developed acute oliguria. Thus, ligandinuria indicates acute rather than chronic renal tubular damage, and Feinfeld's group believes examination of perfusates from stored cadaver kidneys prior to their transplantation may predict tubular damage and the likelihood of post transplantation acute oliguric renal failure.

### Table VII

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Ligandinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>12</td>
<td>Negative</td>
</tr>
<tr>
<td>Acute renal failure</td>
<td>12</td>
<td>Negative</td>
</tr>
<tr>
<td>Acute tubular necrosis</td>
<td>12</td>
<td>Before 24 hours - 2 of 2 positive. After 96 hours 10 of 10 negative.</td>
</tr>
<tr>
<td>Renal arteriography</td>
<td>17</td>
<td>6 transient positives</td>
</tr>
<tr>
<td>Perfusates of kidneys</td>
<td>13</td>
<td>8 positive and showed acute oliguria; 5 negative and none with acute oliguria.</td>
</tr>
<tr>
<td>with transplantation subsequently</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


1One showed a trace.  
2N = Number of patients.

Various Renal Diseases and Hypertension

Maruhn and his associates have evaluated ten urinary enzymes (LDH, GGT, ALP, ASA, GLU, GAL, TRE, NAG, GRS, and AAP-microsomal) in patients with various kidney diseases and six of these in renal artery stenosis and hypertension. The 68 patients with biopsy proven glomerulopathies consisted of 63 with chronic glomerulonephritis and five with amyloidosis. The interstitial renal disease group (n = 54) was made up of 36 patients with radiographic chronic pyelonephritis and 18 with analgesic abuse syndrome. Discriminant
analysis was used in the statistical evaluation of the very large data base obtained. The analysis showed that two carefully selected test parameters were adequate to diagnose patients with interstitial disease with either normal or reduced renal function, and that extending the test variables from three to eight only increased from 60% to 70% the diagnostic effectiveness for detection of glomerular disease in the presence of reduced renal function.

However, for the detection of glomerular disease in patients with normal renal function there was a linear correlation with the number of test parameters measured, the effectiveness increasing from 52 percent with one parameter to 95 percent with 12 parameters, including urinary proteins. Of the ten enzymes measured, NAG was the most sensitive parameter for the detection of chronic renal diseases. In general, Maruhn’s findings in glomerular disease agree with those of Price’s group but, in contrast, they observed an elevated output of NAG in a considerable number of patients with pyelonephritis and with analgesic abuse syndrome with normal renal function. However, they feel the diagnostic specificity of urinary NAG to be low since an increased output was also found in non-renal diseases, e.g., primary hypertension and diabetes mellitus without complications. On the other hand, these increases might be due to hypoxic damage from altered renal tubular flow.

Subsequently, Maruhn, Paar and Bock decreased their enzyme panel to three lysosomal enzymes (GRS, GAL, NAG) and three brush border enzymes (GGT, ALP, AAP-microsomal) for the evaluation of patients with renal artery stenosis and with essential hypertension. In benign essential hypertension, the patients (n = 84) showed less than a 23 percent increase in all six enzymes. While some changes are statistically significant, the values are much lower than those observed in either malignant essential hypertension (n = 13) where ALP, AAP-microsomal, and NAG all showed changes in excess of 40 percent and in renal artery stenosis (n = 16) where all of the enzymes in the panel except GGT showed an average increase in excess of 40 percent and most changes were in excess of 50 percent. The application of discriminant analysis to the data resulted in an overall correct classification for 86 percent of the patients in the study. Urinary NAG offered the greatest diagnostic sensitivity and was increased in more than 88 percent of the cases with primary malignant or reno-vascular hypertension but in only 23 percent of patients with benign essential hypertension. Maruhn suggests that in the absence of significantly altered permeability of the glomerular membrane to macromolecules, hypoxically damaged renal cortical tubular cells of the affected kidney are the principal source of the urinary enzyme. Their view is supported by enzyme studies of split renal function tests and by the fact that surgical correction of the stenosis led to normalization of enzymuria in those cases studied after operation.

N-Acetyl-β-glucosaminidase as an Indicator of Renal Disease

The role of urinary NAG in relation to renal disease has been studied extensively by Price and his associates over the past ten years, both in experimental animals and patients. They analyzed NAG by an automated fluorometric method which permits up to 100 urine samples per day to be analyzed. The method showed excellent correlation (r = 0.98) with a manual method utilizing 4-methylumbelliferyl-N-acetyl-glucosaminide as substrate. While the excretion levels of NAG are low in normal individuals, they have been found to be age dependent, and minor differences exist between the sexes when reported in nanomoles of substrate hydrolyzed per hour per mg of creatinine. Increased urinary NAG appears to be de-
dependent both on the activity of the disease process and the functioning renal cell mass. Since the renal cell mass decreases in older individuals and there is a lower excretion of creatinine, an increased excretion of NAG in the over 70 years age group may be partially a result of the lower excretion of creatinine. Urinary NAG activity was found elevated during the active phase of a wide variety of renal diseases and, in particular, in acute tubular necrosis, active glomerular nephritis, analgesic nephropathy, and chronic pyelonephritis, and in patients with renal injury following episodes of hypotension.

Price suggests NAG estimations to be of particular value in the management of patients who have undergone a hypotensive episode. Persistently raised NAG levels after the acute phase of the disease has subsided suggest that the patients are in risk of relapse. There are two major tissue isoenzyme forms of NAG, A and B, plus the serum form, A*. In active renal disease, Price found the B form of NAG to be increased much more than the A form. The appearance of the B form probably indicates tubular damage whereas the appearance of the serum A* form possibly indicates glomerular damage. This has been confirmed by renal biopsy of a patient with Goodpasture’s syndrome, with only glomerular damage where the A* form was recovered from the urine.

The potential value of the assay of NAG in the detection of rejection in transplant patients was suggested from studies by Wellwood et al. in the early 1970’s when they monitored NAG in a small number of transplant patients. They reported that increased urinary NAG activity preceded the increase in serum creatinine and provided the earliest possible warning of rejection. Subsequently, Thompson and coworkers investigated some 200 transplant patients using the automated fluorometric procedure for NAG. In about 70 percent of the acute rejection episodes, an increase in urinary NAG was observed one to three days before any of the conventional signs of rejection; in the remainder of the patients, the NAG values increased on the same day as the rise in serum creatinine. It should be noted that while transplant patients excrete a higher level of NAG than the normal population, nevertheless, it is the increase in urinary NAG that is considered a significant response. Price stated, “Urinary NAG has provided additional valuable information for the clinician and, provided the assays are carried out on a daily basis, an early warning can be detected. Decisions about anti-rejection treatment are based on clinical observations and conventional laboratory tests together with the results of the NAG assay.” On the other hand, nonspecific elevations of NAG may occur, for example, owing to gentamicin therapy, urinary tract infection, or thrombosis of either the donor renal artery or vein as a result of ischemia or nephrotoxicity. Price’s associates feel that the conditions associated with nonspecific elevations are usually self-evident, and they withhold treatment with immunosuppressive drugs unless there is collaborative evidence of rejection.

Innovative studies into the feasibility of using a newly synthesized substrate as a dip-stick urine test for NAG have been undertaken by Price. Untreated urine is allowed to flow slowly up a capillary tube containing the substrate and a chromophore is released from the substrate when NAG enzyme is present in the sample. The chromophore turns red when it reaches a layer of diethylaminoethyl (DEAE)-cellulose. These dip-sticks for NAG were reported to have detected rejection successfully in transplant patients and might be useful in other patients with renal diseases.

More recently, Price’s group has further characterized the human NAG isoenzymes in order to develop more specific indicators of tissue damage in renal diseases. The isoenzyme profiles of
NAG were determined using semi-automated DEAE-cellulose chromatography. Neonate and infant but not adult kidney tissue contain significant amounts of an intermediate form (I) of activity in addition to the A and B forms. No differences were found between the isoenzyme profiles of kidney cortex and medulla. The NAG isoenzymes were found to be affected by neuraminidase treatment in different ways. Renal tissue NAG isoenzyme A activity was unaffected by neuraminidase, but the intermediate forms (I₁ and I₂) present in neonate kidney were lost as the proportion of B activity increased. However, urinary NAG A activity was converted by neuraminidase to the B form and I², while the A² form of serum was recovered mainly as the B form. The urine from a patient with Goodpasture’s syndrome was stated to contain the serum A² form, suggesting the presence of glomerular damage and a renal biopsy demonstrated that the damage was restricted to the glomerulus. In contrast, the urine from a patient with nephrotic syndrome contained increased NAG isoenzyme A and B activity, probably resulting from damage primarily to the tubular epithelia.

Synovial fluid, seminal fluid, and cerebrospinal fluid were found to contain mainly the serum A² form of NAG. The isoenzyme profile of synovial fluid during active rheumatoid arthritis was characterized by the presence of both the serum A² and tissue A and B forms. Following drug treatment and remission, the profile of synovial fluid resembles that of normal serum. In summary, the Price group’s development of a simple dip-stick test for NAG may permit the screening of “at risk” populations for renal disease and the diagnostic potential of the estimation of urinary NAG activity may be enhanced by the separation of its isoenzymic forms by ion exchange chromatography.

Chronic Polyarthritis

Very recently, Knoll, Wisser, and Rautenstrauch¹⁷ completed a comparative study of the diagnostic value of disc electrophoresis of urinary proteins and the measurement of the excretion of NAG for the detection of renal tubular damage in 50 random urines from patients with chronic polyarthritis. Both methods were found to have equal diagnostic value.

Nephroptosis

Buser and colleagues² have investigated the diagnostic relevance of urinary lactate dehydrogenase in nephroptosis and as an indication for nephropexy. Previously reported experiments with animals had suggested that reduced renal arterial flow might be the actual cause for the pathogenicity of nephroptosis. Patients with a mobile kidney, verified by IV pyelography, were examined by an ¹³¹I-hippuran nephrogram (ING) and a one day test for urinary LDH. Total urinary LDH activity was measured in an eight hour urine volume collected in the supine position and then in an eight hour urine volume collected in the erect position for each patient. Results were expressed as percentage increase of LDH activity of the patient in the erect versus supine position and correlation was made with his or her isotope nephrogram pattern. In control patients, LDH 1 isoenzyme increased and LDH 5 isoenzyme decreased whereas in nephroptotic patients, LDH 1 decreased and LDH 5 increased. Of 45 patients with nephroptosis, 34 showed pathological isotope nephrograms (ING) and the same 34 patients showed greater than a 30 percent increase in LDH with a mean increase of over 100 percent. A study in dogs showed that the reduced arterial flow/hypoxia alone is responsible for a high release of urinary LDH. Eleven patients with anatomically (IV pyelography) verified ptosis, but normal nephrograms showed no increase in total urinary LDH nor did any of the 16 individuals in the control group. Buser and associates² report that the degree of kidney descent in centimeters does not correlate with the percentage increase of uri-
nary LDH and LDH excretion is, therefore, not a criterion for pathogenicity. Several patients were reinvestigated after nephropexy and all showed normal IV pyelograms, normal LDH, and no longer had clinical symptoms.

Transplantation

The measurement of ligandinuria as a specific marker of renal tubular necrosis and as a predictor of the tubular damage in donor kidneys and the likelihood of post transplantation acute oliguric renal failure has been summarized under the section on Acute Renal Failure. This technique developed by the Feinfeld and Arias group holds promise of being a useful diagnostic tool prior to transplantation.

However, the use of urinary enzymes as an early predictor of transplant rejection has been controversial. The Price-Ellis-Tucker-Thompson-Wellwood groups in London have had much experience in the utilization of urinary enzymes and claim NAG to be of high diagnostic value in indicating acute renal graft rejection. Some of their findings have already been summarized in the section on NAG in Renal Disease. Several letters taking pro and con positions on the value of urinary enzymes in transplant patients were published in Clinical Chemistry between 1976 and 1978. In the experience of Horpacsy et al, GGT is the most sensitive indicator, followed by AAP, NAG, and lysozyme.

On the other hand, while Corbett, Thompson, and Price have not studied AAP, their comparative data for NAG and GGT in 20 rejection episodes shows NAG to be the more reliable parameter. Price also has pointed out that Horpacsy et al used a less sensitive colorimetric method for the measurement of NAG activity. More recently, Corbett, Wellwood, Tucker, and Thompson have reported on five years of experience with NAG in 231 transplant patients. A 50 percent increase above baseline was taken to indicate rejection, but interpretation was made in conjunction with all available information. There were 175 rejection episodes and NAG increased in 83 percent, but the test was reported to be most useful in the early period after transplantation when it correctly indicated 94 percent of rejection episodes. In 89 percent of episodes, NAG rose either the same day or one to three days earlier than serum creatinine. Other possible methods of monitoring rejection are the measurement of fibrinogen degradation products in the urine and the measurement of increased dopamine-B-hydroxylase in plasma. In summary, the value of urinary enzyme excretion in renal transplant patients has not been resolved, because different enzymes are measured by different methodologies in different patients under entirely different circumstances. However, most investigators agree that urinary enzyme excretion levels in individual transplant patients must be measured daily and comparison made with the patient’s baseline values and that interpretation must be made in conjunction with all other available clinical findings and factors.

Isoenzymes and the Future

The interesting findings of Price with forms A, B, I1, I2, and I5 of NAG have been briefly summarized in the section on NAG in Renal Diseases. Recently, Pfeiderer et al measured by immunochemical titration the isoenzymes of alkaline phosphatase in normal and pathological urine. Normal kidney tissue contains mainly liver type ALP plus some intestinal ALP whereas normal urine contains mainly intestinal ALP plus some liver ALP. However, the percentage of intestinal ALP in urine is reported to increase strongly in renal diseases and after renal transplantation. Nevertheless, while the major part of urinary ALP was reported to be immunologically precipitable, a distinct amount of enzymatically active but immunologically inactive ALP was always found present in urine. Pfei-
VANDERLINDE

TABLE VIII

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Total LDH*</th>
<th>LDH 5*</th>
<th>LDH 1 &amp; 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder infec-</td>
<td>22</td>
<td>27.0 ± 4</td>
<td>3.1 ± 0.8</td>
<td>Mainly</td>
</tr>
<tr>
<td>tions</td>
<td>Kidney infections</td>
<td>16</td>
<td>226 ± 67</td>
<td>120 ± 39</td>
</tr>
<tr>
<td>Healthy</td>
<td>24</td>
<td>10.6 ± 1</td>
<td>Trace</td>
<td>Mainly</td>
</tr>
</tbody>
</table>

LDH = Lactate dehydrogenase

3. Carvajal et al. found by Carvajal et al. to be useful in the differential diagnosis of renal and bladder infections but not bladder infections, as stated by Schöderer and associates. Their intention was to exploit these interesting findings.

The isoenzyme of LDH 5 has been found by Carvajal et al. to be useful in the differential diagnosis of renal and bladder infections. According to their research in children, LDH 5 is increased in kidney infections but not bladder infections, as shown in table VIII.

Carvajal reported a 94 percent correct diagnosis in children with pyelonephritis and 97 percent correct diagnosis in the total patient population with kidney and bladder infections.

These newer developments in the identification of isoenzymes in the urine in renal disease appear to signal the wave of the future. In this important area of research, the isoenzyme approach seems potentially capable of adding a dimension of specificity not previously available.

References

18. Maruhn, D.: Evaluation of urinary enzyme patterns in patients with kidney diseases and


