ELEVATED SERUM ENZYME ACTIVITY IN ACUTE RENAL FAILURE

E. Kemp, H. Lange, T. Laursen and V. Kamp Nielsen*

The observation of high serum activity of the enzyme lactic acid dehydrogenase in every case of severe acute renal failure provoked a more detailed study of this phenomenon.

In the literature only few and sporadic observations of elevated serum enzyme activities have been mentioned (1-4). The cause of the elevated activities of serum transaminases has been subscribed to multiple tissue injuries in some of these cases, while other authors have emphasized the occurrence of hemolysis (3, 4).

The possibility of a constant renal contribution to the augmentation of serum lactic acid dehydrogenase activity in the shock kidney disease is put forward in the present paper. It may answer part of the question of the degree of renal damage in this disease.

In our unit of nephrology, serum enzyme determinations have been performed in 36 cases of acute tubulo-interstitial nephritis during the last 15 months. The diagnosis has been made according to the criteria of Brun (5), and Balslev and Jørgensen (6). According to these authors, the diagnosis is based on (1) the development of anuria in the course of a few days; (2) a low ratio of urine: plasma urea (<5); (3) a twenty-four hour endogenous creatinine clearance of less than 5 ml. per minute; (4) a typical history with a recognized condition of shock or other precipitating factor and/or a typical clinical course with return of renal function; and (5) characteristic histologic changes in renal tissue obtained by kidney biopsy and/or from a specimen of renal tissue rapidly fixed post mortem.

The precipitating causes of the tubulo-interstitial nephritis is described in Figure 1, where the distribution of surgical, medical and obstetrical cases is shown (according to Lunding, Steiness, Hess Thaysen (7)).

Serum lactic acid dehydrogenase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase were determined by fluorometric methods (8-10).

Serum isoenzyme distribution was determined by an agar gel electrophoresis and subsequent fluorometric scanning (11).

All determinations of serum lactic acid dehydrogenase in the first month of the illness are shown in Figure 2. At the abscissa we have the days after the onset of anuria. At the ordinate serum activity. The stippled line indicates the highest normal value.

The tremendously high activity in several cases is far from the elevated activity usually seen in say heart or liver injuries. No special differentiation in surgical and medical cases was observed. This is seen from Figure 3 where mean values for these groups are plotted with the same abscissa as in Figure 2.

The same pattern as for lactic acid dehydrogenase was found concerning glutamic pyruvic acid transaminase and glutamic oxalacetic acid transaminase. This is seen in Figure 4 and 5, respectively.

The cause of the high serum enzyme activities seen in many cases is obvious: tissue damage in combination with or following grave shock (12). But not all the case histories showed evidence of these conditions and many

*From the Division of Nephrology, Medical Department P, Rigshospitalet, University Hospital, Copenhagen, Denmark.
other possible contributing factors exist - as shown in Figure 6. These enzymes are not normally excreted in the urine (13) and a failing renal degradation seemed improbable because such mechanism has not been demonstrated in normal humans and because no positive correlation was found between creatinine clearance and lactic acid dehydrogenase activity (Figure 7).

The possibility of a hepatic origin of the high serum activities is not probable in all the cases, and can be excluded as the only cause because of the results of the isoenzyme investigation (see below). The same arguments hold true concerning hemolysis as the only cause.

Neither augmentation of enzyme-accelerator concentrations nor lack of inhibitors seemed reasonable causes. No greater differences in serum enzyme activities were seen when samples were drawn just before and after hemodialysis. This indicates that eventually accelerators or inhibitors were not removed or introduced during this treatment.

The elevation was seen whether the patients were treated with drugs known to have potential influence upon serum enzymes (chlorpromazin, steroids) or not.

It seems after these considerations justified to look more carefully at the target organ: the kidney, remembering the descriptive names of shock-kidney: acute renal tubular necrosis. Of course many of the patients had well known tissue necrosis in organs other than the kidneys. But we suggest that this has only partly contributed to the serum enzyme augmentation. Support of this view was found in the isoenzyme investigations. Figure 8 shows typical lactic acid dehydrogenase isoenzyme patterns for homogenized human heart muscle, serum from a case of coronary occlusion; homogenized human liver, serum from a case of hepatitis; homogenized human kidney and serum from a case of renal tubular necrosis. While liver and heart show activity in the gamma-globulin and in the alfaglobulin fractions, respectively, kidney shows activity in all five fractions separated by the technique employed.

During the first week of the disease, 22 sera from 15 patients were investigated with regard to isoenzymes. In 12 cases (16 sera) the 'kidney pattern' with all five isoenzymes was present, while it was not in 3 cases (5 sera). In one case we saw change from 5 to 4 isoenzymes from one day to the other. This pattern is unusual except in these cases, but we have seen it in a case with elective kidney damage after an incorrect ureteric catheterization with damage of kidney tissue.

As possible causes of renal tubular cell damage in acute anuria resulting in enzyme movement from cells to blood, two factors may be mentioned: first nephrotoxins and secondly renal ischemia.

In cases with a known nephrotoxin, as probable precipitating cause, renal tubular cell damage leading to enzyme 'excretion' is very likely.

The occurrence of renal ischemia in a degree that could be responsible for an enzyme leakage is questionable (14) except in cases of cortical necrosis (15). Renal blood flow is reduced in the anuric phase of the disease as shown by Munck and collaborators (16), but not to a degree sufficient to provoke tubular necrosis, and possibly not sufficient to cause enzyme liberation. In one of the patients in the present series we measured the renal blood flow by an inert radioactive gas diffusion method (17) and found a renal blood flow of 0.8 ml/g. kidney tissue/min. At that time,
lactic dehydrogenase activity in serum was still elevated, while the activities of the transaminases were normalized.

Nobody knows, however, the actual renal blood flow during the beginning of the disease. Renal blood flow may be very low in the first hours to days of the illness.

Our program for future investigations is therefore to measure renal blood flow and follow serum and urine enzyme activities from the very onset of the disease.

REFERENCES

THE PRECIPITATING CAUSE OF THE TUBULO-INTERSTITIAL NEPHRITIS

"SURGICAL" CASES
POST TRAUMATIC 4
BILIARY TRACT DISEASE 5
OTHERS 9

"MEDICAL" CASES
NEPHROTOXINS 3
CORONARY THROMBOSIS 2
SEPTICEMIA 2
NARCOTIC POISONING 1
HÆMOLYSIS 1
OTHERS 6

"OBSTETRICAL" CASES
POST PARTUM 2
POST ABORTUM 1
TOTAL 36

Figure 1.

Figure 2. Serum lactic dehydrogenase activity related to number of days after onset of acute anuria. Ordinate: Enzyme activity. Broken line indicates mean value for the individual days. Continuous line indicates mean values over a two day's period.

Figure 3. Mean values of serum lactic dehydrogenase activity in surgical and medical cases of acute anuria related to number of days after onset.

Figure 4. Serum glutamic pyruvic transaminase activity related to number of days after onset of acute anuria. Ordinate: Enzyme activity. Broken line indicates mean value for the individual days. Continuous line indicates mean values over a three day period.
Possible causes for elevation of serum enzyme activities in acute anuria:

1. Multiple tissue injuries causing, complicating or following shock.
2. Impaired renal excretion or renal break-down.
3. "Hepato-renal syndrome."
4. Greater concentrations than normal of enzyme-accelerators or lack of inhibitors. Influence of hemodialysis.
5. Medical treatment Chlorpromazin Steroids.
6. Acute renal tubular necrosis.

Figure 5. Serum glutamic oxalacetic transaminase activity related to number of days after onset of acute anuria. Ordinate: Enzyme activity. Broken line indicates mean value for the individual days.

Figure 6. Lactic dehydrogenase activities plotted against 24-hours endogenous creatinine clearance values. Broken lines indicate normal values.

Figure 7. Pattern of isoenzyme fractions of LDH in tissue-homogenates from (1) heart, (2) liver and (3) kidney compared with the typical serum patterns of (1) myocardial infarction, (2) hepatitis and (3) acute renal tubular necrosis.

L.J. DENIS (Antwerp): I have been able to follow the total LDH in the serum and urine of 52 transplant candidates of Dr. Hume in Richmond, Virginia. So far as the total serum LDH is concerned, I don't consider it to be a very specific test for renal diseases. Among these 52 candidates there were all kinds of renal diseases, from cortical necrosis to acute tubular necrosis, and all these patients had more or less pronounced elevations of total serum LDH. Only rejection after renal homotransplantation seems to give high levels of total serum LDH with clinical application. This is reported by Prout G. R. Jr. et al. in Surgery 56: 283, 1964.

So far as the discrepancy is concerned that you observed between the LDH isozymes of kidney tissue homogenates and the serum LDH isozymes of patients with renal diseases where you observed a shift to the right, or in biochemical over-simplified terms a shift to anaerobic glycolysis, I should like to ask Dr. Kemp if he ever determined the LDH isozymes of tissue homogenates of a diseased kidney.

E. KEMP (Copenhagen): No, we have not done this.

L.J. DENIS (Antwerp): I think this is the next thing to do. I did an observation on the serum LDH isozymes of an artificial heart-lung preparation. These preparations while perfused stop most of the time after 45 minutes to 3 hours. Where normally in the serum of these preparations you do not find slow-moving fractions, they started coming up after 45 minutes and they were very marked and very high when the heart-lung preparation stopped. I think this also represents a shift to anaerobic glycolysis. I think this leaves us with still two solutions for this problem. Either we have in your case a non-specific shift to the right such as, for instance, in old age or in shock; or there is a specific shift due to a shift of the isozymes in the diseased kidney itself.

S. RINGOIR (Ghent): I would like to comment on Dr. Kemp's paper and the remarks of Dr. Denis.

I presented in Paris at the Société de Néphrologie in November 1963 a paper on the changes in the isoenzyme pattern of serum lactate dehydrogenase (LDH) during hemodialysis in acute tubular necrosis and in several other renal diseases (Journal d'Urologie et de Néphrologie, 70, 126-127, 1964). Up till now we examined serum changes in LDH during 42 hemodialyses in 34 cases of acute tubular necrosis. During 41 hemodialyses we found an elevation of the fifth fraction of LDH, the slowest fraction, at the end of the dialysis. Seven of these showed already an elevated LDH5 at the beginning of the dialysis (5 refers to the electrophoretic slowest LDH). Only in one case there was no elevation of LDH5 at all.

The total LDH level was elevated in all our cases of acute tubular necrosis.

We never found an elevation of LDH5 during hemodialysis in chronic glomerulonephritis but we found a slight elevation of LDH5 during hemodialysis in chronic pyelonephritis.

This first slide (Figure 1) shows an example of serum LDH isoenzyme pattern in a case of acute tubular necrosis before and immediately after hemodialysis. The elevation of LDH5 and LDH4 is clearly seen.
In a second slide (Figure 2) you see the figures for our first 9 cases of acute tubular necrosis as we presented them in Paris. It may be seen that they all had a high total serum LDH level which did not change with dialysis, while the pattern did.

In a third slide (Figure 3) we have a case of subacute glomerulonephritis. Serum was examined before dialysis and after one, two, three, four, five and six hours of dialysis. There are no significant changes of LDH5 during hemo dialysis.

I would like to make a comment on your pattern of normal kidney. You found 5 fractions of LDH in normal kidney. We examined different portions of normal human, rat and dog kidney in LDH tissue-electrophoresis. In normal rat and normal human kidney upon examination from cortex to medulla the first fraction of LDH (the 'heart' or 'aerobic' fraction) is diminishing while the fifth fraction ('liver' or 'anaerobic' fraction) is increasing. The deeper you go in the kidney the more fifth fraction you have; in dog kidney the third fraction behaves as the fifth in man and rat.

I would like to add some comments on what Dr. Denis said. I had the opportunity this year to examine serum of patients who had a kidney transplant at the renal Division of Professor R.O. Morgen at Baylor University in Houston, Texas. Three patients who had a kidney transplant had a normal serum isoenzyme LDH pattern; they were doing well clinically and they were not rejecting. One patient was doing a rejection clinically which was proved by biopsy of the transplanted kidney; he showed very high LDH1 and LDH2 in his serum and no LDH5, although I was rather looking for an increase of that particular fraction. This is in contradiction with what you said about the patients of Dr. Hume where an elevation of LDH5 was observed during rejection.

E. KEMP (Copenhagen): I was very impressed by what you have said. I think you have the explanation here of what happens to the fifth fraction. We have been a little afraid of this fraction because, as you saw, we found it every time. I think that you are right and it may be the haemodialysis. Is this not your explanation?

S. RINGOIR (Ghent): No, I don't think it is the hemodialysis alone which is responsible for the elevation of LDH5. I really think the phenomenon is due to the fact that we have a tubulopathy here. We have too many cases of glomerulonephritis which do not show an elevation of this LDH5 during hemodialysis. It is not the hemodialysis per se nor the extracorporeal circulation which is responsible. We have observations on two or three heart-lung preparations and when they have no shock they do not show an elevation of LDH5. They only show an elevated total LDH but no fifth.

E. KEMP (Copenhagen): You saw it in the acute tubular nephrititis without hemodialysis?

S. RINGOIR (Ghent): Of 34 cases of acute tubular necrosis there were only 7 who showed at the beginning a high fifth fraction, but in those cases I think it originated, at least partially, in the liver and was produced by shock.

E. KEMP (Copenhagen): All I can think is that our cases are very severe and all have had several hemodialyses. This may be the cause. In one of
our patients we measured the blood flow and found it very low, below 1 ml/g/min., and it may be that in some of your patients the blood flow has been better and the medullary blood flow has been better. I do not know of course.

THE CHAIRMAN, G. RICHE (Paris): Le temps presse et je vous prie de m'excuser de limiter la discussion. Je voudrais simplement demander au Dr. Scheler si dans les urines il y a un rapport quelconque entre le taux des amino-peptidases particulières qu'il a dosées et la teneur des urines en protéines d'une part, en cellules d'autre part, globules rouges, globules blancs et cellules épithéliales.

F. SCHELER (Göttingen): These influences do not disturb our estimation.

THE CHAIRMAN: Parce qu'il y a un fait très remarquable et presque contradictoire : le Dr. Kemp trouve des taux d'enzymes élevés dans le sang et pas dans les urines, et le Dr. Scheler trouve des taux élevés d'enzymes dans les urines et pas dans le sang.

E. KEMP (Copenhagen): We have not measured it in the urine.

THE CHAIRMAN: Je vous demande une minute encore pour dire que, il y a quelques années à l'Hopital Necker à Paris, nous nous étions intéressés au dosage des aminopeptidases du plasma, mais d'autres amino-peptidases que celles du Dr. Scheler. Nous avions abouti à la conclusion que l'élévation dans le sang de l'activité de la glycyl-glycyl glycine peptidase, qui s'observe dans un grand nombre de maladies rénales ou autres, était directement en rapport avec l'intensité du catabolisme azoté et non pas avec l'atteinte d'un viscére ou d'un autre.
Figure 1. Serum LDH Isoenzyme pattern in a case of acute tubular necrosis before and immediately after haemodialysis.
### ACUTE TUBULAR NECROSIS

<table>
<thead>
<tr>
<th>Name</th>
<th>LDH₁</th>
<th>LDH₂</th>
<th>LDH₃</th>
<th>LDH₄</th>
<th>LDH₅</th>
<th>Total LDH I.U./ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.P.</td>
<td>50.7</td>
<td>37.0</td>
<td>6.8</td>
<td>2.7</td>
<td>2.7</td>
<td>2.0 after 2.2 x 2 before</td>
</tr>
<tr>
<td></td>
<td>33.9</td>
<td>25.0</td>
<td>9.8</td>
<td>10.7</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Ly.</td>
<td>28.16</td>
<td>23.02</td>
<td>18.5</td>
<td>14.54</td>
<td>15.71</td>
<td>0.45 x 10 before 0.55 x 10 after</td>
</tr>
<tr>
<td></td>
<td>31.90</td>
<td>23.94</td>
<td>13.5</td>
<td>10.28</td>
<td>20.91</td>
<td></td>
</tr>
<tr>
<td>La.</td>
<td>50.90</td>
<td>32.05</td>
<td>11.49</td>
<td>3.52</td>
<td>2.03</td>
<td>0.3 x 10 before 0.3 x 10 after</td>
</tr>
<tr>
<td></td>
<td>47.74</td>
<td>30.63</td>
<td>13.26</td>
<td>2.72</td>
<td>5.65</td>
<td></td>
</tr>
<tr>
<td>La.</td>
<td>47.55</td>
<td>39.26</td>
<td>11.04</td>
<td>1.4</td>
<td>0.74</td>
<td>1.7 before 2.8 after</td>
</tr>
<tr>
<td></td>
<td>44.50</td>
<td>30.67</td>
<td>13.05</td>
<td>4.97</td>
<td>6.34</td>
<td></td>
</tr>
<tr>
<td>Gou.</td>
<td>48.93</td>
<td>31.38</td>
<td>12.14</td>
<td>3.77</td>
<td>3.77</td>
<td>1.5 before 2.8 after</td>
</tr>
<tr>
<td></td>
<td>32.33</td>
<td>25.26</td>
<td>12.11</td>
<td>5.12</td>
<td>25.17</td>
<td></td>
</tr>
<tr>
<td>Gou.</td>
<td>57.63</td>
<td>34.40</td>
<td>7.97</td>
<td>0</td>
<td>0</td>
<td>1.6 before 2.3 after</td>
</tr>
<tr>
<td></td>
<td>45.58</td>
<td>28.86</td>
<td>9.85</td>
<td>3.23</td>
<td>12.46</td>
<td></td>
</tr>
<tr>
<td>Du.</td>
<td>42.43</td>
<td>31.93</td>
<td>15.21</td>
<td>5.71</td>
<td>4.71</td>
<td>2.3 before 2.8 after</td>
</tr>
<tr>
<td></td>
<td>37.58</td>
<td>27.58</td>
<td>19.28</td>
<td>7.06</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>Be.</td>
<td>50.6</td>
<td>40.4</td>
<td>8.9</td>
<td>0</td>
<td>0</td>
<td>1.5 before 1.5 after</td>
</tr>
<tr>
<td></td>
<td>46.8</td>
<td>33.8</td>
<td>11.7</td>
<td>3.1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Ni.</td>
<td>43.8</td>
<td>30.4</td>
<td>14.5</td>
<td>6.1</td>
<td>5.1</td>
<td>1.9 before 1.8 after</td>
</tr>
<tr>
<td></td>
<td>38.8</td>
<td>31.2</td>
<td>14.1</td>
<td>7.3</td>
<td>8.4</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 2.*

### SUBACUTE GLOMERULONEPHRITIS

<table>
<thead>
<tr>
<th>Name</th>
<th>LDH₁</th>
<th>LDH₂</th>
<th>LDH₃</th>
<th>LDH₄</th>
<th>LDH₅</th>
<th>Total LDH I.U./ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.A.</td>
<td>40.2</td>
<td>43.1</td>
<td>12.7</td>
<td>2.9</td>
<td>0.9</td>
<td>1.0 x 2 before 1.0 x 2 after 1 hr.</td>
</tr>
<tr>
<td></td>
<td>49.5</td>
<td>37.5</td>
<td>10.2</td>
<td>1.7</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.5</td>
<td>41.2</td>
<td>7.3</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.3</td>
<td>46.3</td>
<td>2.0</td>
<td>2.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.7</td>
<td>43.9</td>
<td>3.8</td>
<td>1.9</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.9</td>
<td>46.7</td>
<td>5.4</td>
<td>1.1</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Figure 3.*