THE NITROGEN METABOLISM OF RAT TISSUE SLICES UNDER VARIOUS CONDITIONS

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When tissues are ground up and incubated aseptically, autolysis occurs and the proteolytic enzymes in the cell are considered responsible. It is assumed that after the death of an animal and while the cell structures are still intact similar autolytic action occurs. It is not, however, known whether autolysis begins immediately after death with the establishment of anaerobic conditions. It is possible that other changes in nitrogen metabolism and distribution precede autolysis. In order to investigate this possibility the changes in nitrogen metabolism of tissue slices during 3 or 4 hours post mortem were studied under various conditions and, in particular, the effects of anaerobiosis. It soon became evident that some of the changes observed under the conditions of the experiments could not be attributed to the result of autolytic action.

EXPERIMENTAL

200 gm. rats were killed by decapitation. The tissues were immediately removed and sliced as rapidly as possible in Krebs' bicarbonate solution. Before transfer into the experimental vessels the slices were washed in fresh solution and blotted on filter paper before weighing. 300 mg. of tissue (wet weight) were placed in each 50 cc. Erlenmeyer flask containing 4.0 cc. of solution which was incubated at 37° with either 95 per cent O₂ and 5 per cent CO₂, or 95 per cent N₂ and 5 per cent CO₂. The anaerobic condition produced by the latter mixture was sufficient to prevent the oxidation of p-phenylenediamine; i.e., the cytochrome oxidase system was completely inactive. The procedure for analysis was as follows. An aliquot was removed for the determination of total nitrogen by the micro-Kjeldahl technique. To the remainder, including the slices, 1.0 cc. of 20 per cent trichloroacetic acid was added. The solution with the precipitated protein was poured off from the slices and centrifuged. An aliquot of the supernatant fluid was analyzed for non-protein nitrogen by micro-Kjeldahl, and another (after autoclaving) for ammonia nitrogen by vacuum distillation for 10 minutes from 20 per cent sodium hydroxide at 60°. The values from these procedures were obtained by nesslerization and the estimation of the color in the Evelyn colorimeter. Finally, an
aliquot was used for the determination of amino nitrogen by the Van Slyke nitrous acid method. Before this could be done, however, it was necessary to autoclave the solutions for 15 minutes at 20 pounds because, aerobically, liver and to some extent kidney slices produce acetoacetic acid, which liberates nitrogen from nitrous acid. The autoclaving in acid destroys the acetoacetic acid and does not apparently hydrolyze peptide bonds, for this treatment causes no increase in amino nitrogen values from tissues incubated anaerobically. In fact, after autoclaving, the solutions are often cloudy, probably from a slight further precipitation of protein. The amount, however, is negligible and is included in the non-protein nitrogen values. By the Van Slyke procedure, under our conditions, 40 per cent of the nitrogen present as ammonia is estimated. It was therefore necessary to subtract 40 per cent of the ammonia nitrogen to obtain the true amino nitrogen values. The protein nitrogen listed in Table I was obtained by subtracting the non-protein from the total nitrogen, and represents the amount precipitated by the trichloroacetic acid at room temperature. 30 per cent of this protein nitrogen is due to red cells and a few liver cells which separate from the slices. 70 per cent is due to protein in solution.

Fig. 1 shows the change in all these values with time for kidney and liver slices, both anaerobically and aerobically. To obtain the initial values, the contents of one flask were removed without incubation and analyzed. Certain effects, which can also be seen in Table I, are immediately obvious, and they can be summarized as follows. The anaerobic condition increases the protein loss from the cells of both kidney and liver; it increases slightly the non-protein nitrogen in kidney and decreases it slightly in liver; it markedly increases the amino nitrogen in kidney and decreases it in liver; and finally, it decreases the ammonia nitrogen in kidney and increases it in liver. No variation from this pattern was observed in over forty experiments with as many animals. The aerobic

1Hamilton (1) has shown that both liver and kidney contain considerable quantities of glutamine. It is difficult to estimate the exact contribution of glutamine to the ammonia and amino nitrogen values. Glutaminase should be active aerobically and anaerobically and therefore some of the estimated ammonia undoubtedly comes from glutamine hydrolyzed during the course of the experiment. Any unchanged glutamine would be converted to pyrrolidonecarboxylic acid by heating in acid and thus the amide nitrogen would also be estimated as ammonia. Therefore the ammonia nitrogen values listed in Table I include all the amide nitrogen of glutamine except that fraction which, after hydrolysis by glutaminase, has been converted to other compounds. Any glutamic acid formed during the experiment will participate to an unknown extent in transamination. Its contribution to the amino nitrogen values is not predictable. It should be emphasized that the ammonia and amino nitrogen values are the result of a large number of metabolic processes.
Table I

Effect of Various Substances on Aerobic and Anaerobic Nitrogen Metabolism of Kidney and Liver

The slices were incubated 3.5 hours at 37°. The volume in each flask was 4.0 cc. The results are expressed in mg.

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Kidney</th>
<th></th>
<th></th>
<th></th>
<th>Liver</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic</td>
<td>An aerobic</td>
<td></td>
<td></td>
<td>Aerobic</td>
<td>An aerobic</td>
<td></td>
</tr>
<tr>
<td>Protein N</td>
<td>Non-protein N</td>
<td>NH₂-N</td>
<td>NH₄-N</td>
<td>Protein N</td>
<td>Non-protein N</td>
<td>NH₂-N</td>
<td>NH₄-N</td>
</tr>
<tr>
<td>4.0 mg. pyruvate</td>
<td>1.12</td>
<td>0.67</td>
<td>0.07</td>
<td>0.46</td>
<td>2.73</td>
<td>0.60</td>
<td>0.22</td>
</tr>
<tr>
<td>4.0 mg. glucose</td>
<td>0.81</td>
<td>0.66</td>
<td>0.11</td>
<td>0.38</td>
<td>0.81</td>
<td>0.66</td>
<td>0.26</td>
</tr>
<tr>
<td>4.0 mg. fumarate</td>
<td>1.23</td>
<td>0.49</td>
<td>0.09</td>
<td>0.28</td>
<td>2.17</td>
<td>0.61</td>
<td>0.25</td>
</tr>
<tr>
<td>4.0 mg. avertin</td>
<td>0.89</td>
<td>0.51</td>
<td>0.06</td>
<td>0.35</td>
<td>2.11</td>
<td>0.57</td>
<td>0.25</td>
</tr>
<tr>
<td>0.5 mg. p-phenylene-diamine</td>
<td>1.41</td>
<td>0.59</td>
<td>0.03</td>
<td>0.32</td>
<td>1.41</td>
<td>0.59</td>
<td>0.12</td>
</tr>
<tr>
<td>0.4 mg. NaCN</td>
<td>2.75</td>
<td>0.67</td>
<td>0.26</td>
<td>0.19</td>
<td>2.75</td>
<td>0.67</td>
<td>0.07</td>
</tr>
<tr>
<td>4.0 mg. l-alanine</td>
<td>0.98</td>
<td>0.46</td>
<td>0.05</td>
<td>0.36</td>
<td>2.37</td>
<td>0.65</td>
<td>0.25</td>
</tr>
<tr>
<td>0.9 mg. l-methionine</td>
<td>0.92</td>
<td>0.36</td>
<td>0.45</td>
<td>0.45</td>
<td>2.26</td>
<td>0.45</td>
<td>0.25</td>
</tr>
<tr>
<td>2.0 mg. (NH₄)₂SO₄</td>
<td>0.94</td>
<td>0.30</td>
<td>0.05</td>
<td>0.42</td>
<td>3.00</td>
<td>0.60</td>
<td>0.28</td>
</tr>
<tr>
<td>Hypertonic NaCl</td>
<td>1.36</td>
<td>0.61</td>
<td>0.07</td>
<td>0.37</td>
<td>2.60</td>
<td>0.60</td>
<td>0.26</td>
</tr>
<tr>
<td>Hypotonic</td>
<td>0.97</td>
<td>0.55</td>
<td>0.08</td>
<td>0.39</td>
<td>2.00</td>
<td>0.61</td>
<td>0.28</td>
</tr>
<tr>
<td>Fasted 4 days</td>
<td>1.09</td>
<td>0.64</td>
<td>0.02</td>
<td>0.47</td>
<td>2.11</td>
<td>0.70</td>
<td>0.24</td>
</tr>
</tbody>
</table>
protein loss is probably in part an artifact due to the absence of protein in the solution and the unavoidable trauma from shaking the vessels.

![Graph](http://www.jbc.org/)  
**Fig. 1.** The changes with time in the various nitrogen fractions of 300 mg. of liver and kidney slices under aerobic and anaerobic conditions.

It is very little affected by hypertonic and hypotonic solutions (1.8 and 0.45 per cent NaCl with all the other constituents present in normal concentrations); nor is it lowered appreciably by 4 days fasting of the
animal before removal of the tissues, when, presumably, some of the so-called labile protein of the liver has disappeared. Absence of calcium from the solution increases the aerobic protein loss, indicating that the permeability of the cell membrane is, in part, a limiting factor; and an increase in this permeability under anaerobic conditions probably accounts for the greater protein loss.

In kidney the non-protein nitrogen is slightly higher anaerobically, but the difference is so small that it would not be significant if it were not a consistent finding. On the other hand, the amino nitrogen is usually more than doubled anaerobically, while the ammonia nitrogen is markedly decreased. There is thus a reciprocal relation between these two and it is apparent that the precursor of most of the aerobic ammonia is a substance which requires oxidation before the ammonia is liberated. There is some anaerobic ammonia production but it is relatively small and may not be significant.

In the liver the situation is exactly the reverse. The non-protein and amino nitrogen are always less under anaerobic conditions. There is thus not only no hydrolysis of protein but actually an inhibition of processes which are involved in the formation of non-protein nitrogen material. The anaerobic ammonia nitrogen is always higher than the aerobic, for under these conditions urea production is stopped and any ammonia formed tends to remain as such. The source of the anaerobic ammonia is unknown but may be glutamine.

Table I summarizes the effect of a number of substances on the various nitrogen fractions. Pyruvate has two marked effects. It decreases the protein loss from the liver anaerobically and to a less extent also in the kidney. This decrease occurs whether the protein loss is high or low. (In the control experiments listed in Table I, it is the protein loss that shows the greatest variations. All the other figures are remarkably constant.) Pyruvate decreases both the aerobic and anaerobic ammonia nitrogen in kidney, causing a corresponding increase in the amino nitrogen, but also some decrease in non-protein nitrogen. Glucose, on the other hand, is without any significant effect. Pyruvate in its effect on anaerobic protein loss may be acting as a hydrogen acceptor. If that is so, its action is nevertheless highly specific, for fumarate has no effect on protein loss. The latter will, however, reduce the aerobic ammonia production in kidney and cause some increase in amino nitrogen and decrease in non-protein nitrogen. Amino acids and ammonia very definitely decrease the non-protein nitrogen. This result is obtained by subtracting the nitrogen added as amino acid or ammonium sulfate from the total non-protein nitrogen estimated. The reduction is significant in all cases except in the anaerobic liver. It is therefore probable that free ammonia as well as amino acids.
prevents the normal breakdown of protein. The corrections in the amino and ammonia nitrogen values when nitrogen compounds are added are so large that the results are not significant.

Avertin belongs to the class of halogenated organic compounds that may cause liver damage. Its main action is on the liver aerobic non-protein nitrogen and amino nitrogen, both of which are decreased. It causes a slight increase in aerobic protein loss in liver and kidney and a small depression of aerobic ammonia production in kidney. Its preferential action therefore is on the liver, where it suppresses the production of non-protein nitrogen and in particular the formation of amino groups. Cyanide has exactly the same effect as anaerobiosis, and when it is added to slices incubated anaerobically it has no added effect. Therefore, it does not increase catheptic activity in kidney or liver under these conditions. p-Phenylenediamine is also a respiratory inhibitor and its effects are similar to those of cyanide, but are less marked.

All the above experiments were done at pH 7.4 where autolysis occurs more slowly than in acid solutions. In order to test the extent of autolytic activity at pH 7.4 and under identical conditions liver and kidney cell suspensions were made by grinding in a mortar with sand. The initial amino nitrogen in the kidney suspension was 0.258 mg. and after 3 hours aerobic incubation rose to 0.528 mg. The corresponding values for liver were 0.226 mg. and 0.406 mg. Cyanide and anaerobiosis did not increase these figures and this indicates that they are the result of proteolytic activity not dependent on reducing agents such as sulfhydryl groups. The existence of this type of autolysis was shown by Bailey et al. (2) for hog liver. The protein hydrolyzed at pH 7.3 is, according to Luck et al. (3), a globulin II. Thus broken cell suspensions show definite autolysis under conditions in which tissue slices show little evidence of it.

Experiments with heart slices show that this tissue is much less active than liver or kidney in the production of ammonia, amino, and non-protein nitrogen. More protein is lost from the slices, possibly because of the syncytial structure. Anaerobic conditions, hypotonicity, absence of calcium, and avertin have minimal effects. If the cells are completely destroyed autolysis occurs.

DISCUSSION

Under the conditions of the experiments it is not possible to say how much the aerobic protein loss from the cells is due to trauma and how much is due to normal diffusion. It is, however, evident that anaerobic conditions greatly increase protein loss and that this is the only anaerobic effect common to both liver and kidney. It seems probable that a similar protein loss from cells to intracellular fluid occurs in vivo when the blood
supply to an area is occluded. This loss apparently is the first reaction, for it occurs \textit{in vitro} when there is no evidence of any autolysis. When autolysis occurs \textit{in vivo}, it probably does so only after a considerable portion of the protein has leaked out of the cell. In the above experiments about one-third of the dry weight of the tissue slice is lost as protein to the surrounding fluid under anaerobic conditions lasting 3 hours.

**Summary**

1. The protein, non-protein, amino, and ammonia nitrogen values have been estimated after incubating rat liver and kidney slices under various conditions.

2. Anaerobic conditions in the kidney cause an increased protein loss, a slight increase in non-protein nitrogen, a large increase in amino nitrogen, with a corresponding decrease in ammonia nitrogen.

3. Anaerobic conditions in the liver cause an increased protein loss, a decrease in non-protein nitrogen, a decrease in amino nitrogen, and an increase in ammonia nitrogen, which results, except for the protein loss, are exactly opposite from those in the kidney.

4. The effect of pyruvate, glucose, fumarate, amino acids, and various drugs on these patterns is described.

**Bibliography**

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