Effect of Ethionine on Spermidine, Spermine and Adenine Nucleotides in Rat Liver

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Ethionine treatment seems to afford information on the biosynthesis and metabolism of the polyamines. Present experiments deal with changes in the polyamines and adenine nucleotides in rat liver after administration of ethionine.

Methods. The animals used were female albino rats of the Wistar strain aged seven months and weighing 160 to 190 g, fed with a standard diet ad libitum. DL-Ethionine in 0.9 % NaCl was administered intraperitoneally as indicated in Table 1. For nucleotide determination liver samples were frozen in situ under ether anesthesia, excised, weighed and homogenized in perchloric acid with an Ultra-Turrax homogenizer. The acid extracts were neutralized and evaporated under a vacuum at 3—5°C. ADP, ATP and S—AE* were determined from the crude extracts by paper electrophoresis, AMP after charcoal treatment. Enzymic methods for ADP and ATP gave parallel, though somewhat lower values. The polynucleotide were determined from the rest of the liver by paper electrophoresis.

Results. Table 1 demonstrates the marked effect of ethionine on the liver polyamine contents. With the larger dose there was at first a significant decrease and then an increase in the spermidine concentration, whereas spermine decreased continuously. With the smaller dose the changes were similar, though not so marked or rapid. Calculation per organ reveals similar changes as per unit of wet weight.

As previously reported, ethionine causes a decrease in the liver ADP and ATP, which is also seen in Fig. 1. With the larger dose ADP and ATP were at the level of 30—35 % of the control values at 24 h, but then increased, being near the controls 5 days later. A striking feature was the appearance of a UV-absorbing substance in the electropherograms from the ethionine-treated rats, not present in the control ones. By paper chromatography this fraction was identified as S-adenosylmethionine. The concentration of S—AE was highest, reaching almost the same value with both doses, at 24 h, after which it decreased rapidly with simultaneous increase in ADP and ATP.

Data obtained in isotope experiments with chick embryos indicate that the

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* Abbreviations: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; S—AE, S-adenosyl ethionine; S—AM, S-adenosylmethionine.
Table 1. Effect of ethionine on the polyamine concentrations of the rat liver. Daily dose of DL-ethionine, 0.2 or 0.6 mmole per 100 g animal weight divided into two portions, was administered intraperitoneally. Analysis 6 h after the last injection. Values are means ± standard deviation of the mean obtained from six animals in each group. Spd, spermidine; Sp, spermine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight</th>
<th>Polyamines mmol</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Dose mmol</td>
<td>Spd/g wet weight</td>
<td>Spm/g wet weight</td>
</tr>
<tr>
<td>Controls</td>
<td>saline</td>
<td>934 ± 95</td>
<td>787 ± 40</td>
</tr>
<tr>
<td>Ethionine</td>
<td>1 0.2</td>
<td>6.91 ± 0.50</td>
<td>932 ± 63</td>
</tr>
<tr>
<td></td>
<td>3 0.2</td>
<td>5.74 ± 0.34</td>
<td>833 ± 31*</td>
</tr>
<tr>
<td></td>
<td>6 0.2</td>
<td>5.20 ± 0.21**</td>
<td>1213 ± 98***</td>
</tr>
<tr>
<td></td>
<td>1 0.6</td>
<td>5.91 ± 0.52</td>
<td>716 ± 103**</td>
</tr>
<tr>
<td></td>
<td>3 0.6</td>
<td>7.03 ± 0.26</td>
<td>988 ± 97</td>
</tr>
<tr>
<td></td>
<td>6 0.6</td>
<td>5.21 ± 0.72</td>
<td>1499 ± 306**</td>
</tr>
</tbody>
</table>

* p < 0.05, **p < 0.01, ***p < 0.001. The significance of the differences as compared with the controls.

propylamine moieties of the polyamines, one chain in spermidine and two in spermine, are derived from methionine, and further that spermidine acts as a precursor in spermine synthesis. The decrease in spermine in the rat liver after ethionine treatment agrees with that found in the chick embryo, whereas spermidine only showed minor changes in the latter. The rapid decrease in spermidine in the early phase after a large dose of ethionine is probably due to inhibition of spermidine synthesis as a result of the decrease in the substrate concentration, e.g., ATP and S—AM. The subsequent accumulation of spermidine may be due to a block between spermidine and spermine, at the same time as spermidine synthesis is resumed. At present it is hard to interpret this difference, but it is possible that the sites

Fig. 1. Effect of ethionine on the adenine nucleotides of the rat liver. The analysis was performed from the same animals as indicated in Table 1. Dose of ethionine in A 0.2 mmole and in B 0.8 mmole per 100 g animal weight. C, controls.

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Oxidation of Heterocyclic Tertiary Bases by Quinones

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The present work is an extension of an earlier investigation concerning the reaction between p-benzoquinone and pyridine in an alkaline medium in the presence of dimethyl sulphate. In that paper it was suggested that this reaction could possibly be used for the oxidation of analogues of pyridine as well. The present work has been carried out in order to test this possibility.

In the previous work, p-benzoquinone and dimethyl sulphate were allowed to react with N-methylpyridinium methosulphate in alkaline methanol solution. In the present experiments the steps of the reaction took place separately in the following manner: (1) reaction between the tertiary heterocyclic base and dimethyl sulphate, (2) decomposition of excess dimethyl sulphate and neutralization of the resultant solution with aqueous potassium hydroxide, (3) reaction between p-benzoquinone and the quaternary N-methyl hydroxide* under basic conditions. Thus, the reaction product formed from p-benzoquinone was not hydroquinone dimethyl ether, but hydroquinone itself. This compound is not so easily isolated from the reaction mixture as its dimethyl ether, nor is it as stable in alkaline solution. However, as the chief aim was to isolate the oxidation products formed from the heterocyclic bases and as the yields appeared to be better, the reactions were carried out in the way described above.

The following compounds gave positive results: quinoline, isoquinoline, acridine,

* Probably better expressed, especially with higher pyridine analogues, as its isomeric equilibrium form, a N-methyl-dihydro-hydroxy compound or so-called pseudo-base.

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