In summary, erythropoiesis may be considered to involve initially the stimulation of committed erythroid stem cells by erythropoietin. It is postulated that during this phase of active cell division, erythropoietin also gives rise to the production of an intracellular regulatory substance which is able (a) to switch on selectively the transcription of new genes (globin) and (b) to increase the previously limited transcription of certain other genes (carbonic anhydrase, adenylate kinase, catalase). Subsequently, the increase in the intracellular protein concentration (mainly haemoglobin) results in the condensation and expulsion of the nucleus as well as in rupture of lysosomes. Degradative enzymes released from the lysosomes may then inactivate mRNA species, the extent of this effect depending on the stability of the relevant mRNA. Consequently, relatively stable mRNA (globin) would continue to be available for protein synthesis for a considerable period after inactivation and loss of the nucleus. Eventually, however, all protein biosynthesis stops because of the degradation of ribosomes and other components of the protein-synthesizing system.


Biosynthesis and Metabolism of Polyamines and S-Adenosylmethionine in the Rat

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A number of observations (see Raina & Jänne, 1970; Stevens, 1970; Bachrach, 1973; Raina & Jänne, 1975) seem to indicate that the polyamines putrescine, spermidine and spermine play an essential role in cellular metabolism, even though the precise mechanism of their action is not well defined at the present time. The best evidence supporting this view comes from results obtained with polyamine-requiring bacterial mutants and with inhibitors of polyamine synthesis in various systems. Although it appears probable that the physiological function of polyamines largely relates to their interaction with nucleic acids, thereby affecting the synthesis, degradation and function of the nucleates, they can also affect other types of cellular processes which may be crucial for growth and differentiation (see Raina & Jänne, 1975).

Increased synthesis and accumulation of polyamines has been found to occur in a number of systems characterized by rapid tissue growth. High concentrations of spermidine and spermine were found in the developing chick embryo (Raina, 1963). In the rat the total concentration of polyamines varied from 1 to 2 mm in several tissues (Jänne 1976.
The concentration of polyamines, in particular of spermidine, was highest in young animals and decreased with aging. Partial hepatectomy of the rat caused an early stimulation of spermidine synthesis, leading to a parallel accumulation of this polyamine and ribonucleic acid (Raina et al., 1966).

Although the initial experiments in vivo indicated that the biosynthesis of polyamines in animal tissues occurs through the same general pathway as in prokaryotic organisms, it was not until 1968 that the enzymic mechanism of polyamine synthesis in mammalian tissues began to be unravelled. It now appears that at least four different enzymes are involved in the synthesis of polyamines in animal cells (Raina & Jänne, 1975), namely L-ornithine decarboxylase, catalysing the formation of putrescine, S-adenosyl-L-methionine decarboxylase, catalysing the decarboxylation of S-adenosylmethionine, and two propylamine transferases, one catalysing the transfer of the propylamine group of decarboxylated S-adenosylmethionine to putrescine (spermidine synthase), the other catalysing the transfer to spermidine (spermine synthase). It appears that ornithine decarboxylase, a pyridoxal phosphate-requiring enzyme having an extremely short biological half-life (Russell & Snyder, 1969), plays a crucial role in the regulation of polyamine synthesis in mammalian tissues. Ornithine decarboxylase activity rapidly increases in target tissues in response to various growth stimuli and hormonal treatments. This has been demonstrated in the regenerating rat liver after partial hepatectomy, in the liver after administration of growth hormone, glucagon, cortisol, thyroxine or dibutyryl cyclic AMP, in the prostate after androgen treatment, in the ovaries after injection of lutropin, in the oviduct and uterus after oestrogen treatment etc. (see Hölttä & Raina, 1973). The short half-life permits rapid fluctuations in the ornithine decarboxylase activity. Most of the evidence indicating that the synthesis of the enzyme de novo is needed for stimulation is based on the use of inhibitors of RNA and protein synthesis. It has been demonstrated that after partial hepatectomy or growth-hormone treatment, the ornithine decarboxylase activity and the immunoreactive enzyme protein increase concomitantly (Hölttä, 1975).

S-Adenosylmethionine decarboxylase isolated from eukaryotic sources has some unique characteristics. It is intensively and specifically stimulated by putrescine and strongly inhibited by decarboxylated S-adenosylmethionine, the product of the reaction. The identity of the cofactor of this enzyme is still a matter of controversy (see Raina & Jänne, 1975). Although some indirect evidence suggests that the cofactor might be pyridoxal phosphate, no direct evidence to prove this has been obtained with purified enzyme preparations (Pegg, 1974; Hannonen, 1975). Mammalian S-adenosylmethionine decarboxylase also has a very short half-life of only 20–60 min. Although the activity of this enzyme can rise in response to various stimuli, such as partial hepatectomy or some hormonal treatments, the changes are usually considerably smaller than those observed in ornithine decarboxylase activity (Raina & Jänne, 1975).

Spermidine synthase and spermine synthase have not been extensively purified and characterized from eukaryotic sources. The slow progress in this area is obviously due to laborious methods used for the assay of these enzymes. Therefore we have developed a rapid isotopic method for the assay of spermidine and spermine synthases (A. Raina, R.-L. Pajula & T. Eloranta, unpublished work). The method is based on the use of phosphocellulose ion-exchange paper for the isolation of the radioactive spermidine or spermine formed from radioactive decarboxylated S-adenosylmethionine labelled in the propylamine moiety.

No systematic study of the distribution of spermidine and spermine synthases in various tissues of the rat have been reported so far. Surprisingly little is also known about the relationship between the synthesis of polyamines and that of S-adenosylmethionine, a substrate used for both polyamine synthesis and for biological transmethylications. Table 1 shows the activities of polyamine-synthesizing enzymes and that of methionine adenosyltransferase in tissues of male rats at the age of 2 months. In agreement with earlier observations (Williams-Ashman et al., 1969), high activities of the four polyamine-synthesizing enzymes were found in the prostate. The highest specific activity of spermidine synthase was observed in the pancreas. This observation, and the
Table 1. Distribution of polyamine-synthesizing enzyme activities and methionine adenosyltransferase in rat tissues

Except for the uterus, tissues of male rats aged 10 weeks were used for analysis. For determination of ornithine decarboxylase activity, six tissue extracts were pooled. The values for the other enzyme activities represent the means (±S.D.) of two or three pooled samples, each obtained by combining three and two organs respectively.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ornithine decarboxylase (pmol of $^{14}$CO$_2$/30 min per mg of protein)</th>
<th>$S$-adenosylmethionine decarboxylase (pmol/30 min per mg of protein)</th>
<th>Methionine adenosyltransferase activity (pmol/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>14 ± 11</td>
<td>3090 ± 150</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Heart</td>
<td>37</td>
<td>1200</td>
<td>41</td>
</tr>
<tr>
<td>Kidneys</td>
<td>75</td>
<td>1450</td>
<td>307</td>
</tr>
<tr>
<td>Liver</td>
<td>7</td>
<td>3220 ± 200</td>
<td>5610 ± 260</td>
</tr>
<tr>
<td>Lungs</td>
<td>20 ± 8</td>
<td>1980</td>
<td>38</td>
</tr>
<tr>
<td>Pancreas</td>
<td>7</td>
<td>37800</td>
<td>558</td>
</tr>
<tr>
<td>Prostate</td>
<td>941</td>
<td>18100</td>
<td>141</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>8</td>
<td>250</td>
<td>14</td>
</tr>
<tr>
<td>Small intestine</td>
<td>201</td>
<td>5910</td>
<td>78</td>
</tr>
<tr>
<td>Spleen</td>
<td>12</td>
<td>4080</td>
<td>65</td>
</tr>
<tr>
<td>Testes</td>
<td>99 ± 21</td>
<td>2180 ± 230</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>Thymus</td>
<td>63</td>
<td>5710</td>
<td>212</td>
</tr>
<tr>
<td>Uterus</td>
<td>38</td>
<td>9170</td>
<td>96</td>
</tr>
</tbody>
</table>
high activity of S-adenosylmethionine decarboxylase, might explain the high content of spermidine found in this particular tissue (see Raina, 1963). It is noteworthy that spermidine synthase activity was high in the uterus, but very low in the skeletal muscle. The spermine synthase activity was low in most tissues when compared with spermidine synthase, but was remarkably high in the brain. In general, there was no parallelism in the activities of spermidine and spermine synthases in different tissues. Neither was there any correlation between the activity of S-adenosylmethionine decarboxylase and the activities of spermidine and spermine synthases. These results are in accordance with the view that separate enzymes catalyse the decarboxylation step and the subsequent transfer of the propylamine group (Raina & Jänne, 1975). Methionine adenosyltransferase activity was highest in the liver, but the pancreas and kidneys also showed considerable activities.

We have also studied the changes with age in the activities of polyamine-synthesizing enzymes and that of methionine adenosyltransferase in the brain, kidneys, liver and skeletal muscle of the rat (A. Raina, R.-L. Pajula & T. Eloranta, unpublished work). In general, the activities of these enzymes were highest in tissues of newborn animals. There were, however, some exceptions to this rule. For example, the activity of S-adenosylmethionine decarboxylase of the brain was low at the time of birth and increased considerably during the first month. Spermine synthase activity was very high in the brain throughout the observation period of 9 months. Also the activity of methionine adenosyltransferase of the liver stayed fairly constant.

In conclusion, high concentrations of polyamines and polyamine-synthesizing enzymes are found in tissues active in protein synthesis, such as pancreas, prostate and liver, and in tissues undergoing rapid growth. The evidence for the suggested regulatory role of polyamines in cellular metabolism, in the synthesis and function of macromolecules in particular, is mostly circumstantial. It must be hoped that work with polyamine-requiring bacterial mutants or inhibitor studies will clarify some of these problems in the near future.

Höttä, E. & Raina, A. (1973) Acta Endocrinol. 73, 794–800