Polyamines and their Biosynthesis in Ehrlich Ascites Cells

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The aliphatic polyamines, spermidine and spermine, are naturally occurring components of almost all living cells, whether of plants, microorganisms, or animal tissues.[1] However, the diamine putrescine, a potent precursor of polyamines also in animal tissues, has been found in considerable amounts only in microorganisms.[1-3]

Putrescine, which is the decarboxylated derivative of ornithine, is formed in microorganisms directly from ornithine or from arginine *via* agmatine.[4,5] Some evidence suggests that its biosynthesis is similar in animal tissues.[2,6]

Putrescine serves as the 4-carbon precursor in polyamine synthesis, being incorporated as a unit into spermidine both in microorganisms and in animal tissues.[1-3] The 3-carbon chain of the spermidine molecule is derived from the carbon chain of methionine.[1-3]

The above-mentioned precursors of spermidine and spermidine itself are also incorporated into spermine.[1,7,8] The converse reaction, formation of spermidine from spermine, has been reported in microorganisms [9] and recently in rat tissues.[10]

In our previous studies it was shown that the concentration of spermidine is highest in various rat tissues during the period of rapid growth.[11] Further, a marked increase in spermidine concentration was demonstrated in regenerating rat liver. It is obvious that during regeneration a real activation of spermidine biosynthesis occurs, because a marked increase in the incorporation of labelled precursors into spermidine has been demonstrated.[12-14] Unusually high concentrations of spermidine have also been found in tumour tissues, *e.g.* in some experimental tumours of the mouse.[1]

The present study deals with the occurrence, biosynthesis and interconversion of spermidine and spermine in Ehrlich ascites cells.

Material and methods. The Ehrlich ascites cells were maintained in male albino mice (obtained from Oy Orion Ab, Mankkaa, Finland). The tumour cells were used 14 days after intraperitoneal inoculation of 0.5 ml of ascites fluid. A pooled sample derived from five mice was centrifuged at 500 *g* for 5 min. The supernatant fluid was removed with a pipette and the cells resuspended with an equal volume of ice-cold Krebs-Ringer phosphate buffer (Ca^{2+} omitted, pH 7.4). The incubations for testing polyamine synthesis and interconversion were performed in 25-ml Erlenmeyer flasks containing cell suspension and the radioactive precursor in a total volume of 2 ml. The flasks were closed with rubber stoppers and the incubation was performed at 37°C with slow shaking. After the incubation the flasks were immediately placed on ice at 3°C. The cells were centrifuged at 500 *g*, washed twice with cold buffer and 4 ml of 0.1 N HCl was added to lyse the cells. After deproteinization with trichloroacetic acid the polyamines were determined according to Raina,[2] and the protein according to Lowry *et al.*[15] from the precipitate.

The radioactivity measurements were performed in a Packard Tri-Carb liquid scintillation spectrometer. The polyamines were counted directly from the paper strips after electrophoretic separation.

The decarboxylation of ornithine-1-^{14}C was measured by binding and counting the $^{14}CO_2$ evolved from the substrate. The incubation was carried out in 50-ml flasks with central wells closed with rubber stoppers. 2 ml of the cell suspension in the outer compartment and 1 ml of hyamine (Packard Instrument comp.) in the center well were added to each flask. The reaction was stopped by addition of 0.5 ml of 40 % trichloroacetic acid, and the flasks were then reincubated for 30 min to ensure that all the CO_2 was transferred to the hyamine. The hyamine was placed in 10 ml of Bray's scintillation liquid and counted in a Packard Tri-Carb scintillation spectrometer.

Results. The concentration of spermidine in washed ascites cells was 8.18 and that of spermine 8.16 μmoles per g of cell protein. Thus the polyamines occur almost equimolarly. The concentration of polyamines in these tumour cells is relatively high, *e.g.* the concentration of spermidine is just about twice that found in normal rat liver.[13]

In the electropherograms a weak ninhydrin-positive fraction could also be detected in the area corresponding to putrescine, but this fraction was too weak to identify. The ascites cells possess a marked ability to decarboxylate ornithine, as shown in Fig. 1. This decarboxylation reaction is known from microorganisms

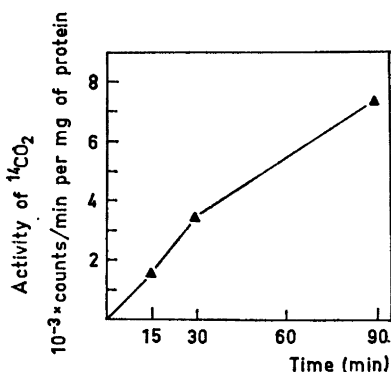

Fig. 1. The decarboxylation of DL-ornithine was measured under standard assay conditions, described in Material and methods, in the presence of 2 ml of ascites cell suspension (5 mg cell protein) and 0.4 μC (0.176 μmole) of DL-ornithine-1-^{14}C (New England Nuclear Corp.). Each point represents a mean of three assays.

and is one of the main pathways for the formation of putrescine.[4,5] In this study the corresponding formation of putrescine was not determined with ^{14}C-ornithine labelled in the carbon chain, but it seems probable that this reaction serves as a biosynthetic pathway for putrescine also in

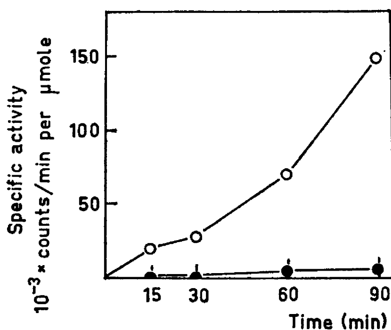

Fig. 2. Incorporation of radioactivity from putrescine-^{14}C into spermidine and spermine. The formation of polyamines from putrescine was measured under conditions, described in Material and methods, in the presence of 2 ml ascites cell suspension (5 mg cell protein) and 0.4 μC (0.044 μmole) of putrescine-1,4-^{14}C (New England Nuclear Corp.). Each point represents a mean of three assays. Open circle: spermidine, filled circle: spermine.

ascites cells. This hypothesis was supported by the finding that Ehrlich ascites cells decarboxylate arginine-U-^{14}C, the radioactivities found in CO_2 and in the putrescine fraction being approximately equal.

The total radioactivity in the CO_2 evolved from ornithine after 90 min incubation represented about 5 % of the activity of the added substrate.

Fig. 2 illustrates the biosynthesis of spermidine and spermine from putrescine. As seen in the figure labelled spermidine could soon be isolated from ascites cells incubated in a medium containing radioactive putrescine. On the other hand, the radioactivity in the spermine fraction barely exceeded that of the background after 90 min incubation.

The incorporation of label from putrescine to spermidine is a relatively rapid reaction. Preliminary experiments (unpublished) show that the rate of this reaction is much more rapid in Ehrlich ascites cells than in slices or homogenate of rat liver.

Interconversion between labelled spermidine and spermine also obviously occurs in ascites cells. These reactions are represented in Figs. 3 and 4. High specific activities of the one polyamine were found after addition of relatively small amounts of the other. It has been observed that in rapidly growing rat tissues the formation of spermidine from spermine is more rapid than the converse reaction. This is true in the tissues of young rats, and in the rat liver during the early stages of regeneration.[10] In Ehrlich ascites cells the con-

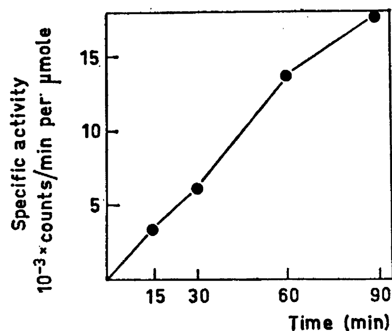

Fig. 3. Formation of spermine from spermidine. Incorporation of radioactivity from spermidine-^{14}C into spermine. 0.2 μC (0.030 μmole) of spermidine-1,4-^{14}C (NEN) was incubated in the presence of ascites cells. Details in Material and methods and in the legend to Fig. 2.

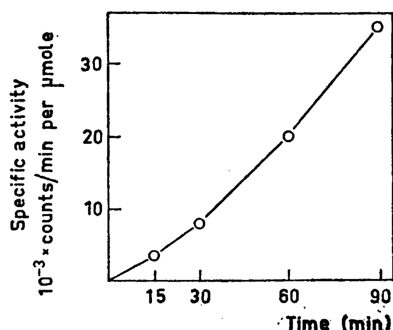

Fig. 4. Formation of spermidine from spermine. Incorporation of radioactivity from spermine-^{14}C into spermidine. 0.1 µC (0.024 µmole) of spermine-1,4-^{14}C (NEN) was incubated in the presence of ascites cells. Details in Material and methods and in the legend to Fig. 2.

version of spermine to spermidine also seems to be the more rapid reaction.

The physiological function of polyamines, spermidine and spermine is not yet understood. However, we now have increasing evidence of their association with certain cellular components, especially nucleic acids. They exert many stabilizing effects on cellular polyanions and can directly stimulate nucleic acid synthesis in microorganisms.[1]

The occurrence of polyamines and their biosynthetic pathways in rapidly growing cells and tissues suggests that they may play an essential role in cell growth.

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Germanium Alkoxides
Attempt at Synthesis by Photochemical Coupling

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A number of alkoxy-tin compounds have been prepared earlier by photochemical coupling between tin tetrahalides and simple halogenated aliphatic compounds.[1] Corresponding reactions between germanium tetrahalides and a number of the same organic reactants do not give alkoxides, but germanium dioxide, as a final product.

The reactions were performed in carbon tetrachloride solutions which were saturated with oxygen gas at a pressure of 1 atm. Small amounts of free halogen were added to initiate the reactions. A 125 W mercury-vapour lamp was used as a light source. All reactions were carried out under extremely dry conditions. Constant temperatures were obtained in a thermostated bath.

The different reaction experiments are specified in Table 1.

The germanium dioxide was identified by X-ray powder analysis and by infrared spectroscopic examinations.[2] The hexagonal modification was found in all cases. All reaction mixtures were filtered and