Biosynthesis of Spermine and Spermidine in the Developing Chick Embryo

AARNE RAINA

Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Although the occurrence of spermine and spermidine in the animal organism has been known for a long time, very little has been reported about the biosynthesis of these amines until recently. In the last few years much work has been done to elucidate their biosynthesis in micro-organisms. Studies with labelled putrescine have indicated that putrescine is incorporated as a unit into polyamines in growing cultures of *Escherichia coli* and *Aspergillus nidulans* [1]. Greene [2,3] demonstrated that in cultures of *Neurospora crassa* 2-^{14}C-DL-methionine added to the growth medium is incorporated into spermidine, being the source of the three-carbon chain of spermidine. Subsequent experiments [4-7] with whole cells and with cell-free preparations and purified enzyme systems of *E. coli* have confirmed that putrescine, and in one report [5] ornithine, is the source of the four-carbon chain of spermine and spermidine. Similarly, the important role of methionine in the biosynthesis of spermidine has been proved.

The studies cited above still leave open the question of whether the mechanism is the same in animal tissues, except for the preliminary note by Tabor *et al.* [1], which indicates a small incorporation of labelled putrescine into the polyamine fraction in minced rat prostate.

In the present work, incorporation studies have been carried out with developing chick embryos, because the synthesis of spermine and spermidine seems to be very rapid in growing organisms. With the method [8] used in this study the polyamines from the embryos could already be determined on the 3rd day of development, whereas none could be detected in unincubated eggs or their hydrolysates.

Methods. Of the radioactive material (putrescine-1,4-^{14}C dihydrochloride, specific activity 4.17 mC/mM, NEC—150; DL-ornithine-2-^{14}C hydrochloride, specific activity 1.0 mC/mM, California Corporation, Los Angeles; DL-methionine-2-^{14}C, specific activity 1.27 mC/mM, Volk Radiochemical Company, Illinois), 1—10 μC was injected onto the chorioallantoic membrane of developing chick embryos on the 9th day of development. The embryos were killed 5, 15, 30 or 60 h after administration. The radioactivity of the spermine and spermidine was determined in an Ecko type 664 A liquid scintillation counter directly from paper [9], after partial purification by extraction into butanol and paper electrophoretic separation of the amines [8].

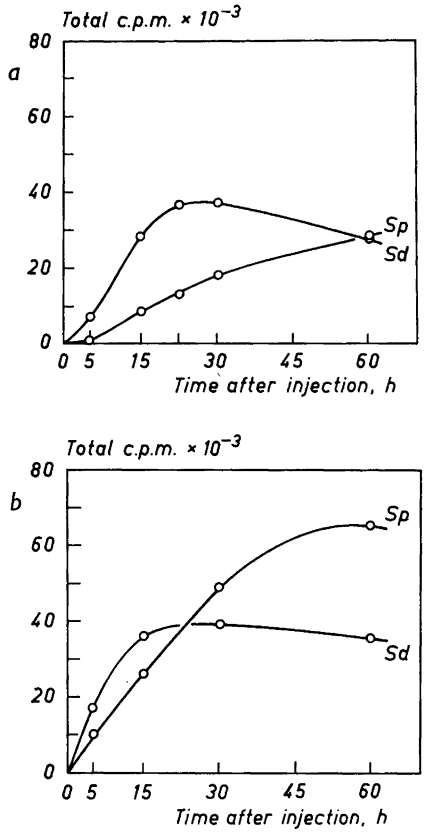


Fig. 1. Incorporation of radioactivity into spermine and spermidine in the developing chick embryo after injection of 5 μC of 1,4-^{14}C-putrescine (1a) or 5 μC of 2-^{14}C-DL-methionine (1b) onto the chorioallantoic membrane (total counts per minute per embryo). Sp = spermine, Sd = spermidine.

Results. The incorporation of labelled putrescine into these polyamines is seen in Fig. 1a. The total activity in the sper-

midine fraction reached its maximum about 20 h after administration of 5 μC of putrescine. Incorporation into spermine was slower. After a lag period, the total activity seemed to increase up to at least 60 h. Of the total radioactivity injected into the egg, about 1 % could be detected in the polyamines 60 h after treatment.

Studies with labelled ornithine showed a similar incorporation. It was interesting to note that after paper electrophoretic separation radioactivity was also found in the "putrescine area". In control experiments it was demonstrated that this unknown compound (putrescine?) was not produced during the isolation procedure.

Incorporation of 2-^{14}C-methionine into spermidine was quite similar to that of putrescine (Fig. 1b). After administration of 5 μC of methionine the maximum of total counts was about 40 000 c.p.m. in spermidine at 30 h after injection. The incorporation of methionine into spermine was more rapid than that of putrescine. 60 h after injection, about 65 000 c.p.m. could be detected in spermine and 35 000 c.p.m. in the spermidine fraction. Of the total radioactivity injected into the egg about 2.8 % was recovered in the polyamines 60 h after administration.

The results described here demonstrate that putrescine, ornithine and methionine can act as precursors in the biosynthesis of spermine and spermidine in the chick embryo. In addition, spermidine seems to be a precursor of spermine.

A more detailed report of the methods and results described here will be published in the near future.

1. Tabor, H., Rosenthal, S. M. and Tabor, C. W. *Federation Proc.* **15** (1956) 367.
2. Greene, R. C. *Federation Proc.* **16** (1957) 189.
3. Greene, R. C. *J. Am. Chem. Soc.* **79** (1957) 3929.
4. Tabor, H., Rosenthal, S. M. and Tabor, C. W. *J. Am. Chem. Soc.* **79** (1957) 2978.
5. Tabor, H., Rosenthal, S. M. and Tabor, C. W. *J. Biol. Chem.* **233** (1958) 907.
6. Tabor, H. and Tabor, C. W. *Federation Proc.* **19** (1960) 6.
7. Tabor, C. W. in Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Vol. V. Acad. Press, New York 1962, p. 756.
8. Raina, A. *Scand. J. Clin. Lab. Invest.* **14** (1962) 318.
9. Bosquet, W. F. and Christian, J. F. *Anal. Chem.* **32** (1960) 722.

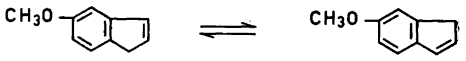
Received November 26, 1962.

On the Tautomerism of 5(7)H-1-Pyrindine

GÖRAN BERGSON
and ANNE-MARIE WEIDLER

Chemical Institute, University of Uppsala, Sweden

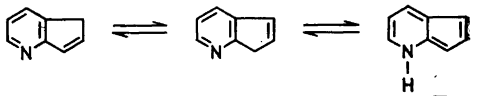
In 1923, Ingold and Piggott [1] investigated the tautomerism of the three-carbon system of the methoxy-indenes Ia and Ib.



Ia *Ib*

These two forms could not be separated, different synthetic methods only yielding identical samples. The single substance thus isolated gave, however, by oxidation a mixture of two methoxy-homophthalic acids, and on blocking the tautomeric system by condensation with piperonal, a mixture of two, separable, derivatives was obtained.

Robison [2] synthesized and investigated 5(7)H-1-pyrindine (II) in which the same kind of tautomerism is possible (IIa and IIb) as in the methoxyindenes. A third tautomeric form (IIc) with the proton attached to nitrogen is, however, also possible, and Anderson *et al.* [3] have suggested that this form is responsible for the



IIa *IIb* *IIc*

orange color of II as observed by Robison. Quite recently Reese [4] has confirmed this hypothesis in a very beautiful way, by synthesizing 1-methyl-1-pyrindine, discussed long ago by Armit and Robinson [5], and comparing its UV-spectrum with that of Robison's substance. The outcome of this investigation was that II contains 1.1 parts per 1000 of the coloured pseudoazulene IIc.

We have for some time been interested in the tautomerism of three-carbon systems, and in particular the tautomerism of 5(7)H-1-pyrindine and related compounds. Due to the current interest in this substance we wish to report the results