On the Biosynthesis of Spermidine and Spermine in the Rat

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The biosynthesis of spermidine in several microorganisms has been demonstrated and shown to require methionine and putrescine or ornithine as precursors.[1-3] These substances have been shown to act as precursors in both spermidine and spermine synthesis in the chick embryo.[4,5] Furthermore, evidence has been obtained that spermidine is a precursor of spermine.[5] At the present time, however, there is only one report on the biosynthesis of these polyamines in mammals, indicating the incorporation of small amounts of ^{14}C-^{15}N-putrescine into the polyamines, chiefly into spermidine, in minced rat prostate.[6] In the present experiments it is shown that ^{14}C-labelled putrescine and methionine are incorporated into both the above-mentioned polyamines in several rat tissues.

Methods. The animals used were young albino rats about two weeks old and weighing 16 to 22 g. At the start of the experiments the young were removed from the litter and fed artificially. Newborn rats immediately after birth were used in short-term preliminary screening tests. The radioactive material, 1,4-^{14}C-putrescine dihydrochloride (New England Corp.) and 2-^{14}C-DL-methionine (Volk Radiochemical Co.) diluted with the corresponding unlabelled compound and dissolved in 0.9 % NaCl, was administered intraperitoneally. The polyamines were purified by paper electrophoresis and analysed for radioactivity in an Ecko type liquid scintillation counter by the method previously described in detail.[5]

Results. In preliminary experiments the young were removed from the litter immediately after birth to keep them as "sterile" as possible. Radioactivity was found in both spermidine and spermine isolated from extracts of whole animals 2 to 3 h after intraperitoneal administration of labelled putrescine or methionine. In the two subsequent experiments young about two weeks old were used. The polyamines from four different tissues were analysed for radioactivity 12 to 144 h after injection of the labelled compounds. The results are summarized in Table 1. Although there are appreciable quantitative differences between the activities of the polyamines in Expts. I and II, some general conclusions can be drawn. After administration of ^{14}C-labelled putrescine the incorporation of the label into spermidine was much greater and more rapid than into spermine. However, at 12 h the radioactivity of

Table 1. Incorporation of radioactivity into spermidine and spermine in rat tissues after intraperitoneal injection of 1,4-^{14}C-putrescine (6.5 μmoles) or 2-^{14}C-DL-methionine (24 μmoles). The weights of the animals used in experiment I were 20 to 22 g and in II 16 to 20 g. Most of the values are means from two animals. Spd, spermidine; Sp, spermine.

Exp. No.	Dose μC	Time h	Specific activity c.p.m. per μmole Liver Spd	Liver Sp	Kidney Spd	Kidney Sp	Muscle Spd	Muscle Sp	Brain Spd	Brain Sp
Putrescine										
I	5	12	7 060	500	10 700	1 260	4 950	250	—	—
	5	24	10 300	830	16 300	1 790	9 730	440	—	—
II	5	48	27 000	4 490	25 700	6 520	19 500	2 540	20 800	3 590
	5	96	22 000	7 430	23 000	8 790	22 600	4 290	17 100	4 920
	5	144	20 400	8 200	21 500	9 870	20 300	3 340	15 100	5 930
Methionine										
I	5	12	2 040	1 950	1 460	1 140	570	480	—	—
	5	24	1 510	1 320	1 640	1 750	1 560	1 360	—	—
II	10	48	5 980	8 350	6 470	9 450	6 830	4 480	12 000	12 000
	10	96	9 400	10 300	10 200	14 200	9 300	8 250	12 600	15 600

spermine significantly exceeded that of the background. The highest specific activities of spermidine were observed at 48 h, whereas those of spermine, except in muscle, increased continuously. Consequently, the ratio of the specific activities (spermidine/spermine) decreased from 14.1 (liver), 8.5 (kidney), and 19.8 (skeletal muscle) at 12 h to 2.5, 2.2, and 6.1 at 144 h. In contrast, after administration of ^{14}C-methionine the label was found in nearly equimolar amounts in both polyamines in the early phase and in somewhat higher amounts in spermine after 48 h. Thus the Spd/Sp ratio varied between 1.5 and 0.7. Neither after putrescine nor after methionine were there any marked differences in the specific activities of the polyamines from the various tissues analysed, excepting the somewhat lower values from the skeletal muscle, especially for spermine.

From the present data it can be concluded that spermidine and spermine are synthesized in rat tissues and that putrescine and methionine can act as precursors. In view of the fact that with putrescine, the source of the four-carbon chain of the polyamines,[6] the label in the early phase was found chiefly in spermidine whereas with methionine, which is shown to be the source of the three-carbon chain of spermidine [1] and likely of both the three-carbon chains of spermine,[5] the label was found in the two polyamines in roughly equimolar amounts, it seems obvious that spermidine is a precursor in spermine synthesis. These results agree with those obtained in previous experiments with chick embryos.[4,5]

Although the above results have been obtained in very young rats, it seems evident that spermidine and spermine are also synthesized in adult animals. This has been demonstrated indirectly with ethionine, a methionine analogue which causes remarkable changes in the polyamine concentrations in rat liver.[7]

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Purification of a Cholinesterase from Plaice *(Pleuronectes platessa)*

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Several attempts have been made to purify cholinesterase from different sources.[1-9] The present author has investigated two types of cholinesterases in the body muscles of fishes. A soluble acetylcholinesterase (acetylcholine hydrolase E. C 3.1.1.7) was the only one found in fresh water fish muscle.[10] In the body muscles of several species of salt water fishes were also found high concentrations of a structure bound cholinesterase [11] having properties distinguished from the more commonly known types of cholinesterases; *e. g.* it splits butyrylcholine rapidly at low substrate concentrations, the substrate concentration—activity curve giving evidence of self-inhibition at higher substrate concentrations.

When attempts were made to purify the latter enzyme, it was not possible to solubilize it by treatment with organic solvents or enzymes (protease, lipase, amylase, lysozyme) or by sonic disruption. Autolysis of a sonically treated homogenate, however, was effective. It also caused a remarkable increase in the enzymatic activity, (μmoles substrate split per hour by the same volume of homogenate before and after treatment, see Table 1). The following procedure was investigated and proved successful for the initial purification of the enzyme.

Commercially available, deep frozen filets from plaice (*Pleuronectes platessa*)