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*)

*Table 1.* Purification of cholinesterase from body muscles of plaice (*Pleuronectes platessa*).

| Preparation | Specific activity ($\mu$moles $mg^{-1}h^{-1}$)[a] | Purification (×) | Yield[b] (%) |
|---|---|---|---|
| Homogenate before autolysis | 1.65 | | |
| Homogenate after autolysis | 2.16 | 1.3 | |
| Supernatant from autolysate | 6.14 | 3.7 | 109 |
| Last ammoniumsulphate precipitate | 34.5 | 21 | 18.5 |
| Eluate from Sephadex G-200: most active fraction | 400 | 240 | 12.0 |

[a] Calculated per mg dry weight of the homogenate before and after autolysis and in the other cases per mg protein determined by a modified Biuret method.[12] The enzymatic activity was determined by electrometric measurements at 25°C in the buffer mentioned in the text using butyrylcholine iodide (pS = 2.8) as a substrate.[13]

[b] Calculated on the homogenate after autolysis.

were finely minced with a pair of scissors in distilled water (10 g/100 ml). pH was adjusted to 8.2 with 1 N potassium hydroxide. The suspension was treated sonically (Branson SONIFIER celldisruptor, Model S-100, 20 kc) twice, 1 min each time, correcting for pH-changes in between. The mixture was then kept at 30°C for 17—20 h and pH again adjusted. After rapid cooling to 5°C the homogenate was centrifuged at 2000 *g*. The supernatant was retained and fractionated stepwise by salting out with saturated ammonium sulphate solution at 5°C and pH 7.7—7.9. Using a centrifuge the first fractions were collected immediately. But the last fraction, obtained between final concentrations of 2.35 to 3.25 M ammonium sulphate, was allowed to form over night before being collected. This fraction was dissolved in a veronal buffer, pH 8.5, $\mu = 0.25$ and dialyzed against the same buffer at 5°C for 24 h. It was then further purified by gel filtration on Sephadex G-200 suspended in 0.1 M ammonium acetate, pH 8.2, the column being 1″ × 18″ (Pharmacia, Uppsala) and the flow rate 2 ml $cm^{-2}$ $h^{-1}$. The results of a typical experiment are summarized in Table 1, and shows that the enzyme has been purified more than 200-fold with a total yield of about 12 %. The further purification of the enzyme is in progress and will be fully reported elsewhere.


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