UPTAKE OF TRIMETHYLAMINE-N-OXIDE (TMAO) AND TRIMETHYLAMINE (TMA) IN SKELETAL MUSCLE AND RECTAL GLAND OF SHARK (SQUALUS ACANTHIAS)

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TMAO is a major osmolyte in elasmobranchs (Goldstein and Palatt, Am. J. Physiol. 227:1268, 1974). In the dogfish (Squalus acanthias) TMAO levels are close in the plasma and the rectal gland cells (70 mM; Kleinzeller, J. Exp. Zool. 236:11, 1985); however, the muscle cells accumulate TMAO against a 2.5 fold gradient (Robertson, Biol. Bull. 148:303, 1975). The dogfish lacks TMAO oxidase for the endogenous synthesis of TMAO from TMA (Goldstein and Dewett-Harley, Comp. Biochem. Physiol. 45 B:895, 1973); hence TMAO (pK_a=4.65) derived from the diet enters the cells by a poorly defined mechanism.

We compared the rate of TMAO uptake by cells of the rectal gland and skeletal muscle. [^{14}C] TMAO was prepared by Drs. M. Dinovi and R. Rafka (Monell Center, Philadelphia) by oxidation of [^{14}C] TMA with chloroperbenzoic acid (Craig and Purushothaman, J. Org. Chem. 35:1721, 1970). The handling of rectal gland slices was as described by Kleinzeller and J. Goldstein (J. Comp. Physiol. B 154:565, 1984). Bundles of skeletal muscle were dissected from the pelvic fins (Leech et al., J. Exp. Zool. 207:73, 1979). Tissues were incubated aerobically (air + 1% CO_2) at 15°C for 30-300 min in standard shark Ringer containing 1-10 mM TMAO. After incubation in the labeled medium, the tissue was blotted, weighed and extracted 24 h with 0.1 N

Radioactivity in the extract and the medium was determined, and ti: e TMAO was expressed in μmol/g wet weight (WW). Total tissue water was obtained from dry and wet weights. Apparent intracellular concentrations of TMAO were compared after correction for the extracellular space: 20-25% of WW in the rectal gland (Kleinzeller and J. Goldstein, loc.cit.) and 9% in muscle (Forster et al., MDIBL Bull. 17:11, 1977).

TMAO uptake by slices of the rectal gland was slow. After 5 h incubation the cellular concentration of the label was only 1% that of the medium at both 1 and 10 mM external TMAO. Muscle uptake was appreciably faster: TMAO concentration in intracellular water was 25% of media concentration. Thus, while these data do not suggest that TMAO uptake is an active process, they do indicate that muscle uptake is more pronounced than gland uptake, which may contribute to the higher content of TMAO in muscle compared with the gland.

For contrast, [^{14}C] TMA uptake was very rapid in both tissues such that intracellular TMA was equal to media TMA at 7 min (the earliest period examined). While the 7 min uptake did not show saturation at various media TMA (0.02-10 mM), the 2 h uptake was saturated with an equilibration at 10 mM. Furthermore, the uptake of 0.1 mM TMA at 30 min was not affected by 5 mM choline, 0.1 mM dinitrophenol or 0.1 mM darstine, an inhibitor of amine uptake. The data suggest that TMA (pK_a=9.81) rapidly enters the cells by a predominantly passive mechanism (possibly in the undissociated form). The cellular accumulation and distribution of TMA may reflect the intracellular distribution of other ionic species. Hence, the cell membrane permeability to TMAO appears to be very low (albeit higher in muscle than in rectal gland) in contrast to the high permeability of other nitrogenous solutes e.g., TMA and urea (Kleinzeller and J. Goldstein, loc. cit.).

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