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Occurrence of Thiaminase in Marine Teleosts.*

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Diets containing whole raw carp have been found to be responsible for deaths due to B_1 avitaminosis in silver foxes.[1,2] Available thiamine was rendered inactive by the raw carp while still in the feed mixture.[3] This destruction of thiamine has been shown to be a split of the molecule into its pyrimidine and thiazole portions; the split is catalyzed by an enzyme, thiaminase, present in the viscera of raw carp and soluble in 10% salt solution.[4,5]

Deutsch and Hasler[6] examined a total of 31 species of fresh-water fish in the Great Lakes region and found the thiaminase to occur in about half of them. Examination by these authors of 9 marine teleosts, however, did not reveal thiaminase in any. They determined the presence of the enzyme in fish homogenates by their power to destroy the thiamine of added brewer's yeast. Thiamine analyses were made by the thiochrome method.

The only marine teleost which has been demonstrated to contain thiaminase is the Atlantic herring, *Clupea harengus*.[7] Both feeding tests and incubation with thiamine have given ample proof of the high thiaminase activity of this fish.[7,8,9] Green *et al.*,[10] on the basis of observations on the effect of diets fed at fur farms, suggest that Atlantic whiting, *Merluccius bilinearis*, has the power to destroy thiamine. On the other hand, according to the assays of Deutsch and Hasler[6] the whiting contains no thiaminase.

Large portions of the catch in certain fisheries are usually discarded,[11] although in recent years there has been a decided trend towards fuller utilization of this material. The possibility exists that such occasionally marketable or unmarketable (*i.e.*, "trash") species may at times be used for livestock feed on coastal farms. The presence of thiaminase in such species would preclude their use if raw and unprocessed. This paper reports the results of assays for thiaminase in 4 species of marine teleosts, namely:

Merluccius bilinearis; whiting
Prionotus carolinus; sea robin
Tautogolabrus adspersus; cunner
Tautoga onita; tautog or blackfish.

Thiaminase activity was determined by the method used by Sealock *et al.*[5] The viscera of fresh fish were minced in a Waring blendor with an equal volume of 10% saline in 0.1M phosphate buffer at pH 7.4. Large particles were spun down by centrifugation and 2 ml of the supernatant suspension (equivalent to about 1 g of fish) was transferred to a centrifuge tube together with 750 γ of thiamine hydrochloride and 1 ml of a 0.2M phosphate buffer at pH 7.4. The contents of each tube were adjusted to a volume of 5 ml with distilled water and then incubated for 2 hours at 37.5°. At the end of incubation 5 ml of 20% trichloracetic acid were added and the

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protein allowed to flocculate with subsequent centrifugation. Standards received the same ingredients as the experimental tubes but the trichloracetic acid was added directly after the thiamine and incubation was omitted. Controls were run with the fish suspension subjected to 100° for 20 minutes prior to the addition of the thiamine followed by incubation. For assay two 4 ml samples of the resulting supernatant were used. A simplified modification[12] of the Melnick and Field[13] colorimetric diazonium method was employed to determine residual thiamine. It was found that the zeolite adsorption could be omitted without interference of color formation leading to erroneous analyses providing the absorption of light by the xylene extract was measured in a Klett-Duboscq visual colorimeter.

None of the 4 species of marine teleosts tested showed the presence of thiaminase in a saline suspension of its minced viscera. A similar suspension of whole minced quahogs (*Venus mercenaria*), which has already been shown to contain thiaminase,[9] destroyed 33% of the added thiamine in 2 hours. No appreciable heat-destruction of thiamine was found to occur under the conditions of the incubation.

The absence of thiaminase in most marine teleosts is still inexplicable. In the 13 species so far investigated only the herring, *Clupea harengus*, has been demonstrated to contain the enzyme. The conclusion of Deutsch and Hasler[6] that the whiting does not contain thiaminase has been confirmed. In their experiments with cod and haddock, Deutsch and Hasler[6] used eviscerated fish. Since the enzyme occurs primarily in the viscera, if at all, this leaves the status of these fish doubtful. Explanation of the occurrence of thiaminase in some fish and not in others may rest on a phylogenetic basis as has been suggested.[6] In that case extensive investigation of additional marine fish as well as freshwater, estuarine, and marine invertebrates and primitive chordates is requisite.

Summary. In 4 species of marine teleosts no thiaminase was found according to the procedure described. This brings to a total of 12 the marine teleosts in which there appears to be no thiaminase. In only one species, the Atlantic herring, has its presence been demonstrated.

I wish to thank Captain Jared Vincent for supplying me with the Atlantic whiting used in this investigation.

[12] Hoehberg, M., and Melnick, D., *J. Biol. Chem.*, 1944, **156**, 53.

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Utilization of Certain Rare Sugars by Microorganisms.

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The use of common sugars and alcohols in bacteriological work to differentiate and characterize species is well known. However, some of the so-called "rare" sugars and their derivatives have been used much less frequently for such purposes.[1] The failure to employ such substances in the past has been due in part to their high cost, or to the fact that they have been unavailable on the market.

In connection with other studies in the Division of Organic Chemistry at the State University of Iowa, several unusual sugars were prepared in sufficient quantities to permit their use in a few bacteriological studies. To

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[1] See for example, Koser, S. A., and Saunders, F., *J. Bact.*, 1933, **26**, 475; Sternfeld, L., and Saunders, F., *J. Am. Chem. Soc.*, 1937, **59**, 2653.