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Distribution of a Vitamin B_1 Destructive Enzyme in Fish.

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"Chastek paralysis," a polyneuritic condition in foxes, has been produced when certain raw fish are included in the diet. Green *et al.*[1] showed that it could be cured with thiamine and more recently[2,3] that the pathologic manifestations resembled those of Wernicke's disease.

The ability of carp tissues to destroy vitamin B_1 has been reported by various investigators. Of these Woolley,[4] Sealock *et al.*,[5] and Spitzer *et al.*[6] suggest or recognize the enzymatic nature of the destructive factor. The latter workers showed that the destruction took place within the feed mixture. Deutsch and Ott[7] reported that thiamine destruction by freshwater smelt is likewise of an enzymatic nature. Wolf[8] found that buckeye shiners and saltwater herring are active in this destruction. The "cold storage herring" reported by Alexander *et al.*[9] to destroy vitamin B_1, appears to be the saltwater herring *(Clupea harengus)*. When trout were fed a diet containing this fish, the pathology of the central nervous system was analogous to that usually encountered in avitaminosis B_1 in higher vertebrates.

The purpose of this investigation was to study the distribution of the enzyme in fresh and saltwater fish by assay of as many species as were readily available.

Procedure. Fresh whole fish* or fresh fish viscera were finely ground to give a homogeneous mixture. Two equal portions were weighed and one was heated at 100°C for 15 minutes to inactivate the vitamin B_1 destructive enzyme if present. Equal amounts of dried brewer's yeast were then added to the raw and the heated portions, sufficient to provide 420-450 γ of thiamine per 100 g of fish preparation. After thorough mixing, these preparations were spread out on a flat surface and air-dried at room temperature under forced ventilation. Under these conditions 4-5 hours elapsed after mixing before any appreciable drying took place and the total period of moist contact of fish and yeast was approximately 12 hours. Previous work had shown that this period was greatly in excess of that required for complete destruction of the added vitamin B_1 under these conditions when smelt *(Osmerus mordax)* were used.

After thorough trituration in a mortar, a portion of the fish-yeast mixture (1-2 g) was assayed for vitamin B_1 by the standard thiochrome method. The presence of the destructive enzyme was indicated by the disappearance of thiamine from the unheated portion; if a trace remained it was usually less than 5% of the original amount added.

Results. Typical assays are shown in Table I. In Table II are the results of the analyses for the distribution of the enzyme in the series of fish examined. The species analyzed are arranged in taxonomic order.

1 Green, R. G., Evans, C. A., and Carlson, W. E., *Minn. Wildlife Disease Invest.*, 1937, **3**, 173.

2 *Ibid.*, *J. Nutrition*, 1941, **21**, 243.

3 Evans, C. A., Carlson, W. E., and Green, R. G., *Am. J. Path.*, 1942, **18**, 79.

4 Woolley, D. W., *J. Biol. Chem.*, 1941, **141**, 997.

5 Sealock, R. R., Livermore, A. H., and Evans, C. A., *J. Am. Chem. Soc.*, 1943, **65**, 935.

6 Spitzer, E. H., Coombes, A. I., Elvehjem, C. A., and Wisnicky, W. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 376.

7 Deutsch, H. F., and Ott, G. L., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 119.

8 Wolf, L. E., Fisheries Research Bull. No. 2, N. Y. State Cons. Dept., Jan., 1942.

9 Alexander, L., Green, R. G., Evans, C. A., and Wolf, L. E., *Trans. Am. Neurol. Assn.*, 1941, **67**, 119.

* Most of the species common to the Great Lakes region were furnished through the courtesy of Smith Bros. of Port Washington, Wis. The saltwater species were provided by the Atlantic Coast Fisheries Co. of New York.

TABLE I.

Fish assayed	γ B_1/ g unheated fish-yeast mixture	γ B_1/g heated fish-yeast mixture	Presence or absence of the enzyme
Freshwater herring *(Leucichthys artedi arcturus)*	17.3	17.6	—
" " " " "	15.8	16.6	—
" " " " "	18.4	17.9	—
Crappie *(Pomoxis nigro-maculatus)*	11.8	12.9	—
Sauger pike *(Stizostedion c. canadense)*	0	21.9	+

TABLE II.

Family	Name	Presence or absence of the enzyme
Lepisosteidæ	*Garpike *(Lepisosteus osseus oxyurus)*	—
Ameidæ	*Dogfish *(Amia calva)*	—
Coregonidæ	*Lake Michigan chub *(Leucichthys* sp.)	—
	*Lake Superior herring *(Leucichthys artedi arcturus)*	—
	‡Whitefish *(Coregonus clupeaformis)*	+
	†Menomonee whitefish *(Prosopium cylindraceum quadrilaterale)*	+
Osmeridæ	*Freshwater smelt *(Osmerus mordax)*	+
Salmonidæ	†Lake trout *(Cristivomer n. namaycush)*	—
	*Rainbow trout *(Salmo gairdnerii irideus)*	—
	*Brown trout *(Salmo trutta fario)*	—
Cyprinidæ	†Carp *(Cyprinus carpio)*	+
	*Goldfish *(Carassius auratus)*	+
	*Creek chub *(Semotilus a. atromaculatus)*	+
	*Fathead minnow *(Pimephales p. promelas)*	+
	§Buckeye shiner *(Notropus atherinoides)*	+
Catostomidæ	*Sucker *(Catostomus c. commersonnii)*	+
Ameiuridæ	*Channel cat *(Ictalurus lacustris punctatus)*	+
	*Bullhead *(Ameirurus m. melas)*	+
Umbridæ	*Mud minnow *(Umbra limi)*	+
Esocidæ	*Pickerel *(Esox lucius)*	—
Serranidæ	†White bass *(Lepibema chrysops)*	+
Percidæ	†Wall-eyed pike *(Stizostedion v. vitreum)*	—
	*Perch *(Perca flavescens)*	—
	†Sauger pike *(Stizostedion c. canadense)*	+
Centrarchidæ	*Crappie *(Pomoxis nigro-maculatus)*	—
	*Largemouth bass *(Huro salmoides)*	—
	*Smallmouth bass *(Micropterus d. dolomieu)*	—
	*Pumpkinseed *(Lepomis gibbosus)*	—
	*Bluegill *(Lepomis m. macrochirus)*	—
	*Rock bass *(Ambloplites r. rupestris)*	—
Gadidæ	†Burbot *(Lota lota maculosa)*	+
	Saltwater Forms.	
	‡Cod *(Gadus morrhua)*	—
	‡Haddock *(Melanogrammus aeglefinus)*	—
Scombridæ	*Mackerel *(Scomber scombrus)*	—
Merluciidæ	*Whiting *(Merluccius bilinearis)*	—
Pleuronectidæ	*Lemon sole *(Pseudopleuronectes americanus dignabilis)*	—
	*Yellow tails *(Limanda ferruginea)*	—
	*Black backs *(Pseudopleuronectes americanus)*	—
Scorpaenidæ	*Red fish *(Sebastes marinus)*	—
Hippoglossidæ	*Dabs (unknown species)	—
Cupeidæ	§Herring *(Clupea harengus)*	+

* Whole fish.
† Viscera.
‡ Eviscerated fish. The whitefish showed 65% destruction.
§ From observations of Wolf.[8]

Discussion. The ability to destroy thiamine was found to occur fairly frequently among freshwater fish, but not in any of the saltwater species analyzed.

Green *et al.*[10] reported the enzyme present in herring from Lakes Michigan and Superior and in northern pike, whereas our results were negative with these species. Wolf[8] found that incorporation of the buckeye shiner *(Notropus atherinoides)* into the diet of the trout resulted in a B_1 deficiency. Smelt *(Osmerus mordax)*, however, showed no evidence of thiamine destruction under these conditions. Deutsch and Ott[7] proved by chick and thiochrome assay that smelt contain the destructive enzyme. However, it is noteworthy that Wolf's results on the buckeye shiner (Cyprinidæ) are consistent with our observations on this family.

In 3 analyses of the herring reported in Table II we were unable to show thiamine destruction. In addition, mink feeds containing 20-30% lake herring *(Leucichthys* sp.) were allowed to stand 24-36 hours before being assayed for thiamine. No vitamin B_1 destruction occurred.

Wolf's observation[8] on the presence of the factor in Atlantic herring *(Clupea harengus)* is the first clear-cut evidence of thiamine destruction by a saltwater fish. Green *et al.*[10] reported that Atlantic whiting and Pacific mackerel contained this factor. Our analyses showed no thiamine destruction by the Atlantic whiting *(Merluccius bilinearis)* and mackerel *(Scomber scombrus)*. Except for Pacific mackerel it is probable that the species were the same, but this is not certain because they failed to give scientific names. Moreover, their report on both fresh and saltwater species appears to be based on observations at different fur farms where these fish were fed rather than on controlled experiments.

Investigators have reported various amounts of thiamine in the edible portions of certain market fish. Their failure to consider the possibility of an enzyme in the tissues, that destroys thiamine, is fairly good evidence that the following varieties do not possess this enzyme:

Fish*	B_1/100 g	Investigator
Cod (fresh)	90	Munsell[11]
Haddock (fresh)	42	"
Cod (muscle)	120	Baker and Wright[12]
Halibut (fried)	180	" " "
Whiting (raw)	90	" " "
Sardine (tinned)	90	" " "
Herring (raw muscle)	9-12	Harris and Wang[13]
Halibut (edible portion)	84	Booker and Hartzler[14]
Trout (edible portion)	87	" " "

*Scientific names were not given.

Carp *(Cyprinus carpio)* and smelt *(Osmerus mordax)* have the destructive factor, and when they have undergone ordinary marketing or analogous handling no thiamine is found in the viscera or in the edible portions. On the other hand, tissues which are quickly inactivated with heat after removal from living carp and whole smelt contain thiamine.[15]

The taxonomic correlation of the presence or absence of the factor within certain families of fish is not constant; moreover, neither food habits nor environment appear to offer a basis of prediction as to whether a fish does or does not possess this enzyme. An extensive investigation of the saltwater fish and of the invertebrates may help in establishing any such relationship, should one exist.

Conclusion. Use of the described procedure permits easy determination of the presence of a vitamin B_1 destructive enzyme in fish tissues. The distribution of this enzyme in 31 species of fish in the Great Lakes region has been studied and its occurrence is rather common. On the other hand, our results with 9 species of saltwater fish all proved negative.

[10] Green, R. G., Evans, C. A., Carlson, W. E., and Swale, F. S., *J. Am. Vet. Med. Assn.*, 1942, **100**, 394.

[11] Munsell, H., *Milbank Mem. Fund. Quart.*, 1940, **18**, 311.

[12] Baker, A. Z., and Wright, M. D., *Biochem. J.*, 1935, **29**, 1802.

[13] Harris, L. J., and Wang, Y. L., *Biochem. J.*, 1941, **35**, 105.

[14] Booker, L. E., and Hartzler, E. R., Tech. Bull., 707, U. S. Dept. Agric., Dec., 1939.

[15] Unpublished data.