THIAMINASE IN FISHERY PRODUCTS: A REVIEW

By Charles F. Lee

ABSTRACT

Severe losses of silver foxes due to paralysis led to the discovery of a thiamine-destructive substance which was present in the raw fish included in the diet. Only a few fresh-water species were found to contain thiaminase. More recent assays have shown that a few marine fish and shellfish also possess this thiamine-destructive capacity. The review of literature deals with the properties of thiaminase, its distribution in the fish body, and a listing of the various species tested for thiaminase. A similarly-active principle has also been reported in certain products of a plant origin. An attempt is also made to explain the numerous contradictions regarding thiaminase that are found in the literature.

INTRODUCTION

A new disease of foxes was first noted in 1932 on the fur farm of Mr. J. S. Chastek, in Glencoe, Minnesota. The foxes became paralyzed and, in most cases, soon died. Tests were not successful in determining the presence of any virus or bacteria responsible for this and subsequent outbreaks. The diet was then suspected, and the various ingredients of the diets fed were recorded and compared in outbreaks reported from Utah, Idaho, Wyoming, and other places in Minnesota.

It appeared then that onset of "Chastek" paralysis was related to the consumption of raw fish, when this was used instead of the meat which was usually fed. Green, et al, (1937, 1941, and 1942) studied the pathology of the disease, and eventually proved definitely that "Chastek" paralysis was caused by a deficiency of thiamine. Although diets were fed which should be adequate in thiamine, a deficiency was apparently caused by the raw fish which had also been fed in every case. Paralysis may be prevented by feeding large doses of thiamine orally or by injection of thiamine. It may be prevented by not feeding raw fish, by removal of the raw fish from the diet, or by feeding the raw fish on alternate days, or feeding fish at a different time of the day from rest of diet.

These investigators (1942-A) also found that both the viscera and trimmings were more active than the whole carp in producing the paralysis in foxes. The skinned fillets were found to be without effect during the period they were fed. Twenty percent raw, whole carp was mixed into the diet and supplements of 1, 2, 5, and 10 milligrams of thiamine were fed daily to 6 pups. The highest level gave complete protection; whereas, with the 5 milligrams level the thiaminase exerted a slight depressive effect on appetite. There was even some beneficial effect of the supplement at the 1 milligram level.

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THE ANTI-THIAMINE FACTOR

These papers stimulated interest in this thiamine-destructive factor which was the first "anti-vitamin" to be reported. The only previous comparable observation in the field of nutrition was the biotin-avidin relation when raw egg white was fed. Apparently the disease studied was an uncomplicated thiamine deficiency. This was confirmed by feeding the raw fish to cats (Smith and Proutt, 1944) and chicks (Spitzer, et al., 1941) with production of typical deficiency symptoms. Most of the work since 1941 has been directed towards a study of the active principle involved: methods of extraction and concentration, assay, properties, and the nature of its reaction with thiamine. To a lesser extent, its distribution in the body of the fish has been studied. A few investigators have sought to determine which of the many species of fish and shellfish contain the destructive factor.

Green, et al., (1942) have suggested the possibility that the reaction between thiamine and the destructive factor was enzymatic. Development of in vitro techniques greatly simplified the study of its various properties. The whole fish or viscera were finely ground and were either diluted with water to form a suspension, or the active principle was extracted with a 10 percent solution of sodium chloride. Thiamine was then added to the preparation and it was incubated for 2 hours or longer at 20° to 37° C. The thiamine remaining was determined by the thiochrome method or other chemical methods. Sealock, et al., (1943) standardized the assay and established as an empirical unit value for the thiamine-destructive principle, the amount which would destroy 1 micromole1 of thiamine under these standard conditions.

Woolley (1941) was probably one of the first investigators to study the properties of the destructive principle by chemical methods. He reported that 100 grams of ground whole carp would destroy 150 to 190 micrograms out of 200 micrograms of added thiamine. On dialysis, he found that the active principle was made up of two fractions. However, neither the dialysate nor the non-dialysable fraction exhibited thiamine-destructive activity when used alone. He estimated that one-fourth of the total activity in the carp was in the head, three-eighths in the viscera, and three-eighths in the rest of the body of the fish.

Spitzer, et al., (1941) found 100 percent destruction of lower levels of added thiamine within 15 minutes when only the entrails were used. The amount of thiamine added was varied from 100 to 600 micrograms and destruction of the larger quantities was found to be proportional, within certain limits, to time, and the amount of entrails. Their work with chicks verified the observation that the viscera had greater activity for destruction of thiamine. When flesh, skin, and heads and tails were fed, they also produced thiamine deficiency symptoms in all animals.

These investigators, as well as Green, et al., (1942), have reported that the substance was heat labile since a diet of cooked carp caused no thiamine deficiency symptoms. Also the rate of reaction, and the relation of quantity of thiamine destroyed to amount of destructive principle were all suggestive of an enzymatic reaction. Sealock, et al., (1943), made probably the most extensive chemical study of the "fish principle" reported in the literature. They determined the quantitative relation of the amount of destruction of thiamine to the pH in the range of 6 to 10.5. Thiamine loss was correlated to the temperature over the range 20° to 75° C. and the amount of active extract per 10 milliliters of incubated mixture. The rate of destruction of thiamine up to 5 hours of incubation at 37.5° C.,

1/One micromole equals one millionth of a molecular weight or 337 micrograms of thiamine.
and the rate of inactivation of the destructive principle in a boiling water bath were also determined. It was found that the rate of destruction of thiamine was maximal at a pH of 9.1 and temperature of 60° C. The results of all these studies strongly suggested the enzymatic nature of the reaction. The enzyme is of a protein nature, as indicated by its behavior with a number of protein precipitating reagents, for example, trichloroacetic acid, ammonium sulfate, etc. The reaction is different from the biotin-avidin relationship, as indicated by failure to release the thiamine in an active form by either hydrolysis, proteolytic digestion, or destruction with heat of the anti-vitamin principle.

Sealock, et al., (1943) suggested that the thiamine-destructive principle might be considered a "thiaminase," since it is an enzyme of which thiamine is the substrate, and this term has been frequently so used in recent literature. A more specific name would be desirable especially since Bonner and Buchman (1938) have already given the name "thiaminase" to an enzyme in pea roots which resynthesizes thiamine from its two component heterocycles. However, in the interest of simplicity, the destructive enzyme found in fish will be referred to as thiaminase in the balance of this review.

In 1944, Sealock and Goodland found further conclusive evidence of the enzymatic nature of thiaminase in the inhibiting effect on the rate of thiamine destruction of a number of the typical enzyme poisons. Inhibition was also produced by several derivatives of the thiazole and pyrimidine fractions of thiamine, particularly the aminobenzyl-thiazole derivative. The nature of the inhibition of thiamine-destruction by this compound was studied in detail.

Krampitz and Woolley (1944) demonstrated the presence of the two heterocyclic components of the thiamine molecule in the thiamine-thiaminase reaction mixture. The reaction, apparently a hydrolysis of the molecule, is complex in nature inasmuch as the free thiazole appears first, with the pyrimidine fraction being liberated much more slowly, 70 percent being freed in 8 days, in a sodium chloride extract. However, the pyrimidine moiety was freed as rapidly as the thiazole portion in a tissue suspension. A second enzyme not extractable with the salt solution appears to be involved. Use of mold cultures having specific requirements for each fraction of the molecule made it possible to follow the course of the reaction. Mucor ramannianus required either thiamine or the thiazole fraction while the pyrimidine moiety was essential to the growth of Endmyces vernalis.

The dual nature of thiaminase was shown by dialysis. The dialyzate contained only about 10 percent of the original activity, and the non-dialyzable portion slightly more, while almost the original strength could be restored by again mixing the two fractions. It was found that only the non-dialyzable fraction was heat labile, inasmuch as the boiled dialyzate was as effective as the unboiled in restoring activity when added to the solution remaining in the cell.

These investigators used viscera from freshly-caught carp in preparing their extracts and suspensions, and they reported an unusually high potency for this raw material. They isolated and identified chemically the thiazole and pyrimidine fractions and in one such preparation, 900 grams of viscera completely split 1 gram of pure thiamine, while standing overnight. This is equivalent to the destruction of 1,100 micrograms of thiamine per gram of fresh viscera. They found thiaminase active over a pH range of 1 to 8, with about a fourfold increase in rate of action at pH 8. Within a temperature range of 0° to 37° C., the rate of destruction was increased by about one-half.
Bhagvat and Devi (1944) reported thiaminase to be about 25 times as concentrated in the viscera and blood of carp as in the flesh. They studied its action under dialysis and the stability of the fractions thus separated, their conclusions being in agreement with the work of Kramplitz and Woolley. They compared the thiaminase of carp with a similar substance which they found in a number of oil seeds native to India: ragi (Eleusine coracona), linseed, cottonseed, and mustard seed, also in rice polishings and mung bean (Phasealus aureus). The destructive principle was similar to fish "thiaminase" in that it was made up of two fractions separable by dialysis, which differed in stability to heat. The different sources varied in the proportions of the two fractions which were present. Ragi and cottonseed have predominantly the heat stable fraction which showed no loss in activity on autoclaving at 15 pounds pressure for 15 minutes or boiling for one-half hour. These investigators assumed that the reaction in this case was not enzymatic because of the stability to heat of the active principle and also the relative rapidity of the thiamine-destructive action. There is evidence, however, in the results of growth tests using mosquito larvae (Aides albopictus) that the reactions are chemically similar, that is, there is splitting of the molecule in each case. The thiamine-free product of ragi-thiamine digestion was as effective for growth as an equivalent amount of pure thiamine. However, the larvae were unable to utilize the decomposition products of thiamine produced by autoclaving, or treatment with sodium sulfite or sodium hydroxide. Pigeons and rats were unable to utilize the products of the ragi-thiamine digestion nor could thiamine be eluted from the solid residue by any solvents. The active principle was almost insoluble in salt solutions but easily extractable with a water-chloroform mixture.

Sure and Ford (1943) also report a substance in milk which destroyed 32 to 65 percent of added thiamine during incubation at 37° C. for periods up to 48 hours. This rate of reaction is much slower than that of the destructive enzyme of fish.

Weswig, et al, in 1946, reported the presence of a thiamine-destructive principle in yet another substance, not of aquatic origin. In a study of "fern poisoning" of horses and cattle that had eaten the fern, Pteris aquilina, the authors fed rats rations containing 40 percent of ground, air-dried fern. The thiamine content of the ration was estimated at 0.2 to 0.6 milligram per 100 grams but the rats lost weight in 10 days, and most of them died in another 20 days with symptoms characteristic of thiamine deficiency. When the animals were fed 0.5 milligrams of thiamine per day, all rats made good gains in weight over a 5-week period, proving the diet mixture with 40 percent fern to be otherwise innocuous. In stability towards heat, the active principle in fern resembles that found in oil seeds by Bhagvat and Devi (1944), rather than fish thiaminase. There was little decrease in activity when the dry fern was heated for 18 hours at 105° C.

It would appear that fish are not unique in the possession of a thiamine-destructive principle. Similarly acting, if not chemically identical, substances have been found in several materials of unrelated plant origin, and it seems probable that continued investigation will demonstrate that these or other anti-vitamins are more prevalent than has hitherto been considered.

Aside from the papers by Bhagvat and Devi, the only reports originating outside the United States of the occurrence of thiaminase have been from the Scandinavia-
Green, et al, (1942-A) remarked that the same type of paralysis that they had studied had been reported in Norway in 1938 and in Sweden in 1939, on commercial fur farms. It had not been recognized at that time as a nutritional deficiency disease related to consumption of raw fish. However, in 1944, Lieck and Agren report the presence of a thiamine destructive substance in 10 out of 21 species of fresh-water fish in Sweden. Nine of the ten species containing thiaminase were of the carp family. They also found that 9 salt-water species did not contain thiaminase.

Sealock, et al, (1943) have made the only quantitative study of the distribution of thiaminase in the organs of carp. In some cases, the same organs from different fish were assayed separately and a considerable range in values was observed with no apparent correlation to sex or size. The data proved little more than that the quantity of thiaminase in members of a species will show a large degree of variation. The thiamine content of species containing the vitamin has been observed to be as widely variable. The spleen was found to contain the greatest concentration of thiaminase, amounting to as much as 25 units per gram. The liver, pancreas, gastro-intestinal tract, and gills (3 samples of each) contained from 1.5 to 8.7 units per gram. The kidneys and blood contained 0.75 to 1.4 activity units per gram; ovaries, 0.6 unit, and the heart, testes, brain, gall bladder, bile, mucous, and eyes contained less than 0.3 unit per gram. The swim bladder and muscle tissue were reported to contain no thiaminase.

The thiaminase is seen to be very widely distributed in the body but its function in the metabolism of the fish is unknown. Also, there is no explanation for its presence in some species and absence from others, often of rather closely related genera.

Numerous contradictions are to be found in the literature concerning the presence or absence of thiamine or thiaminase in the different species of fish, both from fresh and salt water. There are perhaps four possible explanations for this. The portion of the fish used for analysis is certain to influence results. There are unpublished data (Deutsch and Halser, 1943) which indicate that both thiamine and thiaminase may exist simultaneously in the living tissue, and other sources indicate that in some species, for example, burbot, thiamine may be present in the flesh, and thiaminase in the viscera. In this case, assays of the viscera, the edible portion, or the whole fish, would show respectively, thiaminase, thiamine or either one, depending on the proportion of each present.

The method of assay is an important factor. The chemical methods are much more sensitive in detecting small amounts of thiamine than are the animal tests with foxes, chicks, rats, and cats. However, Myers of this laboratory (unpublished data, 1946) has shown that the thiochrome method as used in the assay of cereals cannot be directly and indiscriminately applied to the assay of thiamine in fish. Interfering substances, which are difficult or impossible to eliminate, may be encountered. For this reason, low values reported for thiamine, as for example, the values for starfish of 7 to 17 micrograms per 100 grams reported by Sautier (1946), may result from other substances and do not preclude the presence of thiaminase. Lastly, it seems quite possible that thiaminase may be present in one species of fish and absent in a closely related species from other waters or possibly even may be absent from the same species seasonally or at certain stages of development. The data at hand are not sufficiently complete to permit a final conclusion as to the significance of any of these possible variables.
SPECIES THAT CONTAIN THIAMINASE

With this explanation of the conflicting data, the species reported in the literature to contain thiaminase follow: Green, et al, (1942-A) reported thiaminase in frozen whiting (whole) and canned Pacific mackerel based on reports of Chastek paralysis of foxes which had been fed these fish. Carp has always been found to contain thiaminase. Raw smelt has been shown by feeding tests to contain this enzyme. Smith and Proutt (1944) fed cats a diet of raw fish exclusively, producing deficiency symptoms with carp and herring (salt-water species). Negative results were obtained when perch, catfish, butterfish, and spots were fed. The cat, however, is relatively slow to show symptoms of thiamine deficiency. Melnick, et al, (1945) found thiaminase in hard clams. One hundred grams of minced raw clams destroyed 8 milligrams of thiamine in vitro or several times the recommended daily allowance for adults. However, several human subjects ate the same amount of whole raw clams, with only about a 50 percent loss of thiamine intake. In vitro tests showed small amounts of thiaminase in marinated herring but none in oysters, smoked carp, or salmon.

Deutsch and Halser (1943) using a chemical method, tested 31 species of freshwater fish and 9 species of salt-water fish for thiaminase. They found thiaminase present in eviscerated whitefish and in the viscera of Menominee whitefish, carp, white bass, sauger, pike, and burbot. It was found in the whole fish in goldfish, smelt, chub, fathead, minnow, mud minnow, sucker, shiner, channel catfish, and bullhead. Fresh-water species free of thiaminase were herring, several varieties of trout, gar pike, and dogfish. None of the marine species tested showed the destructive enzyme. Whole fish were used in testing redfish, mackerel, whiting, lemon sole, blackback, yellowtail, and dab, but the cod and haddock tested were eviscerated.

Wolf, in 1942, was probably the first investigator to report thiaminase in a salt-water fish, namely, the Atlantic herring (Clupea harengus). He also reported its presence in the buckeye shiner (Holotopus atherinoides). These results were obtained from feeding experiments in which hatchery-bred trout were the experimental animals. The symptoms of thiamine deficiency exhibited were not unlike those of higher animals, being mostly of a neurological nature, and similar brain lesions were observed on histological examination.

Yudkin (1945) in the course of an investigation as to the possibility of utilizing certain "trash" or undesirable fish of the Long Island Sound area, tested whiting, sea robin, cunner (Tautogolabrus adspersus), and tautog or blackfish (Tautoga onita) using the viscera only. None was found to contain thiaminase. He concluded that thiaminase occurs very rarely in strictly marine species, only herring having been reported to contain it at that time. It has since been reported in the hard clam of the Atlantic Coast (Melnick, et al, 1945).

Sautier (1946), on the other hand, reports from 68 to 140 micrograms of thiamine per 100 grams of sample in 5 genera of clams from Alaskan waters. Of most interest among the numerous other species he reports containing thiamine is the Pacific herring (Clupea pallasia). The flesh contained small amounts, ranging from 11 to
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40 micrograms per 100 grams, with larger amounts in the herring milt, roe, and viscera. A single sample of mussels (Mytilus edulis) was reported to contain 162 micrograms per 100 grams. All these data were obtained with the thiochrome method.

Goldbeck, of this laboratory (1947), assayed a number of fresh and canned fish for thiamine by the thiochrome method. When low values or no thiamine were found, the material was tested for the presence of thiaminase by addition of thiamine. She found thiamine present in the edible portions of burbot, lake herring, whiting, and in Boston, Spanish, and king mackerel, but reports thiaminase in carp, smelt, menhaden, and mussels. The latter two marine species had not previously been reported as containing thiaminase.

The mussels (Mytilus edulis) were of the same species that had been reported in the data from the Alaskan sources (Sautier, 1946) as containing thiamine. The whole mussel meat was used in both instances so that it is difficult to account for the opposite results obtained.

From experimental data obtained by the author, two additional marine animals can be added to the list of species which contain the thiamine-destructive enzyme. The ocean or black quahog (Arctica islandica) was found to contain this enzyme by chemical methods. Earlier, the common starfish (Asterias forbesii) of the shore waters of the Atlantic Coast had been shown to contain thiaminase by several series of feeding tests with both rats and chicks. Previously, Sautier (1946) reported very small amounts of thiamine in 3 Pacific species of starfish, using chemical methods. Hutchinson, et al., (1946) reported similar observations on the starfish, using microbiological methods.

It was noted in an assay for thiamine by the rat growth method that rats receiving ground raw starfish as a supplement to the thiamine deficient basal diet were in much worse condition than the control group fed only the thiamine deficient diet. Two-thirds of the rats on the highest level, 1 1/2 grams starfish per day, developed severe polynuritis, while the rats fed the control diet averaged 8 grams a week gain. The addition of 150 micrograms of thiamine per 100 grams of basal diet stopped the loss in weight of the rats receiving starfish, and in a short time, resulted in these rats making large gains in weight.

Quantitative estimation of the thiaminase present was made difficult by the refusal of the rats to eat the raw starfish supplement completely or consistently. In later tests, it was mixed directly into the diet at a 10 percent level, but even this was not entirely satisfactory as the starfish probably still had a depressing effect on the appetite of the rats. It was concluded after running 4 series of test animals that 1 gram of raw starfish destroyed about 4 micrograms of added thiamine, when both were mixed in a thiamine deficient diet. It seems probable that in vitro tests would show an even greater concentration of thiaminase but at that time (1942), facilities for the assay of thiamine by the thiochrome method were not available.

Feeding tests with chicks were conducted for the purpose of finding the value of starfish meal as a protein supplement in a commercial-type mash mixture containing corn, bran middlings, soybean oilmeal, etc. This diet, therefore, contained considerable thiamine from natural sources. Abnormally high levels of starfish meal, amounting to 32 percent of the whole mash, were fed to two groups of the
first lot of chicks to study the effect of the excessive amounts of calcium thus introduced. This work will be reported in detail in another paper. Of interest, is the fact that chicks fed this diet grew very poorly, with 50 percent mortality at the end of 3 weeks. The remaining chicks in both groups showed considerable improvement and better growth with the addition, after 3 weeks, of 100 micrograms of thiamine per 100 grams of mash. After 2 more weeks, the amount of thiamine supplement was doubled for one group with a further improvement in rate of gain in liveweight. There were no more deaths after the addition of the thiamine.

This seemed conclusive proof that thiaminase remaining in the starfish meal had been the primary cause of mortality and poor growth, rather than the excess of calcium or any other factor.

The starfish meal had been prepared by drying the raw starfish in large galvanized sheet iron pans in a steam oven. The mass of starfish and design of the oven were such that temperatures rarely exceeded 50°C, and 5 to 7 days were required for adequate drying. For the first 48 hours, there was considerable enzymatic and bacterial action, usually with complete breakdown of the tough exoskeleton of the starfish. In spite of this prolonged exposure to moderately high temperatures and accelerated enzymatic, bacterial, and oxidative action, the indications are that the major part of the thiaminase present in the raw fish remained in the meal. This apparent stability is at variance with the report of destruction of thiaminase in finely ground raw smelt during drying at room temperatures (Deutsch and Ott, 1942). The presence of a stabilizing substance in starfish would seem indicated. Possibly, the enzyme is more stable at the higher pH maintained by the excess of calcium carbonate. On the other hand, smelt may contain a catalyst which destroys the destructive enzyme. This question of the stability of thiaminase at temperatures below 50°C has been almost unexplored up to the present time.

**SUMMARY**

It should be emphasized that the thiamine-destructive factor, no matter how widespread the occurrence, is of negligible importance in human nutrition in this country, because a very small proportion of fish is eaten raw. Cooking of the fish destroys thiaminase. Only two seafoods of those reported to contain the enzyme are occasionally eaten raw or pickled. These seafoods, namely, clams and herring, are not consumed raw in such quantity that they might justifiably be considered dangerous or undesirable foods. The thiamine-destructive principle might be of some significance, however, in some sections of China, Japan, India, Norway, Sweden, and other smaller countries and islands where large amounts of fish are eaten raw, dried, pickled, or otherwise preserved without cooking.

It is equally true that the possible presence of thiaminase should be taken into consideration when feeding pets, farm, or fur animals. Apparently, even fish themselves develop thiamine deficiency so that caution must be used in feeding fish to fish in hatcheries.

It has been suggested (Melnick, et al., 1945, and Weswig, et al., 1946) that practical use might be made of thiaminase in the preparation of thiamine-free
experimental diets. This suggestion would seem to have merit since present methods for thiamine destruction involve autoclaving or alkaline digestion. These techn­nics are not only involved but also require conditions which may destroy other factors of nutritional value. More important, these methods are not entirely effective as evidenced by the long preliminary feeding periods often required to produce even the initial symptoms of thiamine deficiency.

It is evident that a great deal of research work remains to be done before a clear understanding may be had of the value of thiaminase in biological processes.

At present, there seems to be a completely illogical distribution of thiamin­ase in various organisms. Those presently known to contain this enzyme consist of such diverse members as the fresh-water carp, a species of fern, the common starfish, seeds of native Indian plants, the salt-water menhaden, and the hard clam. Almost as difficult to understand are the great differences in heat stability of the destructive principle reported by different investigators and the large variation in amount of destructive activity.

In conclusion, it may be definitely stated that a few species of fish, mostly fresh-water forms, of those thus far reported, and some types of marine animals, contain an enzyme which is capable of splitting the thiamine molecule, rendering it unavailable for higher forms of life. The family of fish which contains this enzyme cannot be predicted with any certainty, with the exception that most members of the carp family possess it. The largest quantities are to be found in the vis­cera, although it is also distributed widely throughout the body of the fish. The function of thiaminase in metabolism is not known.

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FREEZING FISH AT SEA

At intervals during the past several years, plans have been prepared for a complete factory ship—that is, a vessel of sufficient size to contain freezing, canning, and byproducts equipment. A number of such vessels were in operation by the Japanese prior to the war and at present the Soviet Government is known to operate a fleet of such vessels.

The American counterpart of these vessels is the factory ship Pacific Explorer which recently returned from her maiden voyage in the South Pacific with a cargo of 2,300 tons of frozen tuna. This vessel was authorized by the Defense Plants Corporation, a subsidiary of the Reconstruction Finance Corporation, in the last months of the war.

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