

Inactivation of Vitamin B₁ by Raw Fish.*

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Considerable work in connection with a deficiency disease in foxes occurring as a result of feeding raw fish has been reported in the literature.¹⁻⁷ More recent evidence^{8, 9} indicates that this deficiency disease is fundamentally a B₁ avitaminosis. Experiments were set up in this laboratory to confirm the above observations and to study in detail the action of fresh fish upon the vitamin B₁ both in a typical fox ration and in pure solutions.

During the course of confirmatory experiments with foxes, it was found possible not only to produce the deficiency by the feeding of raw carp, but also to reproduce the condition in the same animals after recovery from a previous attack. From recent reports in the literature,^{8, 9} and also from the results of our own curative therapy, it was possible to postulate that the fundamental condition observed when raw carp was fed was a B₁ avitaminosis. An experiment with chicks was designed in an attempt to confirm this theory. In this study, the basal ration used was identical with that in the fox experiments except that 2-3 drops of Haliver oil were fed to each chick weekly instead of including cod liver oil in the mixed ration. All diets were fed as a wet mash. Fourteen groups of day-old white leghorn chicks (10 per group) were used in these trials. A summary of each group and the results obtained are presented in Table I.

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TABLE I.
Effect of Raw Carp on Vitamin B₁ Content of a Typical Fox Ration.

Group No.	Ration	% polyneuritis
1	Basal fox ration	0
2	" + 25% raw whole carp	100
3	" + 25% cooked whole carp	0
4	" + 25% raw carp heads and tails	100
5	" + 25% raw carp muscle meat (skin included)	100
6	" + 25% raw carp entrails	100
7	" + water extract \approx 25% raw whole carp	0
8	" + ether extract \approx 25% raw whole carp	40
9	" + residue after water and ether extractions \approx 25% raw whole carp	100
10	" + 25% raw whole carp + 200 μ g B ₁ /100 g ration	100
11	" + 25% raw whole carp + 350 μ g B ₁ /100 g ration	100
12	" + 25% raw whole carp + 500 μ g B ₁ /100 g ration	80
13	" + 25% whole air-dried carp	50
14	" + 25% canned ocean fish	0

These data indicate that a typical B₁ avitaminosis can be produced in the chick by feeding 25% raw whole carp in a diet that is otherwise adequate in vitamin B₁. The above data also confirm the observations previously made with foxes that the factor (or factors) found in raw whole carp is heat labile, since cooking the fish rendered them safe. In an attempt to detect a specific portion of the fish which would show activity, raw heads and tails, muscle meat (skin included) and entrails were fed to individual groups. All chicks in Group 6 developed polyneuritis at an earlier date than those in Groups 4 and 5.

With the availability of the thiochrome method for the determination of thiamin,¹⁰ experiments were designed (1) to determine whether the actual inactivation of the vitamin B₁ took place in the digestive tract of the animals or within the feed mixture itself, (2) to confirm the observation that cooking renders carp safe, (3) to determine the actual level of inactivation offered by a given amount of carp, and (4) to study in more detail the biochemical phenomena involved.

Since the data obtained from our experiments with the chick (Table I) seemed to indicate that raw entrails were more potent than whole raw carp, the procedure involved the addition of known amounts of crystalline thiamin hydrochloride to a given weight of raw carp entrails. Then, by means of a modification of the thiochrome method, we attempted to recover the added vitamin B₁.

The data in Table II present evidence that the actual inactivation of vitamin B₁ by raw carp takes place within the feed mixture itself.

¹⁰ Hennessy, D. J., *Ind. and Eng. Chem. (Anal. Ed.)*, 1941, **13**, 216.

TABLE II.
In Vitro Studies of Effect of Raw and Cooked Carp Entrails on Crystalline Thiamin Hydrochloride (All determinations made at room temperature).

Wt entrails, g	Incubation time, min	μg vitamin B ₁ added	% vitamin B ₁ destroyed
2.5 raw	15	100	100
2.5 "	30	100	100
2.5 "	60	100	100
2.5 "	15	200	71
2.5 "	30	200	90
2.5 "	60	200	100
2.5 "	15	300	56
2.5 "	30	300	62.5
2.5 "	60	300	95
2.5 "	720	600	100
2.5 (cooked)	15	100	0
2.5 raw	15	400	45.5
2.5 "	30	400	50
2.5 "	60	400	70
2.5 "	15	500	31.5
2.5 "	30	500	35.5
2.5 "	60	500	48
2.5 "	15	600	25
2.5 "	30	600	25
2.5 "	60	600	37
5.0 "	60	500	100
2.5 (cooked)	120	100	0

These data also confirm the observations previously made *in vivo* that cooking the fish renders them safe. From Table II, there would seem to be a proportional inactivation depending upon the length of time that the fish is in actual contact with the feed mixture. Studies are now in progress to further standardize the conditions involved in the reaction. At the present time, it is impossible to conclude anything definite as to the nature of the reaction. However, from the data obtained, it is possible that the phenomenon is of enzymatic nature.

Summary. Previous observations that a dietary deficiency disease, produced in foxes by feeding raw fish, is fundamentally a B₁ avitaminosis, have been confirmed in the chick. The additional observation has been made that this condition can be reproduced in foxes which have recovered from a previous attack. Evidence obtained from experimentation *in vitro* confirms the observation previously made *in vivo* that the inactivation of vitamin B₁ by raw carp

occurs within the feed mixture itself. The level of inactivation depends upon the length of time that the fish is in contact with the feed mixture. Studies to standardize the conditions of the reaction are now in progress. Present evidence indicates that the reaction may be enzymatic in nature.