Technical Papers

Role of Histamine in Poisoning with Spoiled Fish

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It has been generally assumed that during "spoilage" of fish bacterial toxins accumulate in the otherwise edible fish meat and that the pathological symptoms observed after consumption of spoiled unprocessed or canned fish are produced by the action of bacterial toxins. Since 1946, however, three papers have been published in which it was suggested that in cases observed in Indonesia, France, and the Philippines, histamine was responsible for poisoning after consumption of spoiled fish (*1*). This article (*2*) reports experiments performed to investigate the role of histamine in fish spoilage.

About 14 years ago, we found that the skin irritation observed after handling of spoiled fish is similar to that produced by histamine. Following this lead, we established that the concentration of histamine, which is present only in traces in fresh sardines, mackerel, tuna, and salmon, increases rapidly during spoilage up to values of 120 mg/100 g fresh meat within 24 hr (*3*). As a result of this observation, pure histamine is now being produced in commercial quantities from fish waste that has been spoiled under controlled conditions. Consequently, on the basis of the increment of histamine during spoilage, a bioassay method for quantitative determination of spoilage has been described (*4*). The practical value of such a method has recently been further investigated in the laboratories of the U.S. Food and Drug Administration (*5*).

A review of the literature revealed that Japanese authors had already established, several years earlier, the presence of histamine in fish, but no mention is made in the available abstracts of any quantitative relationship between spoilage and histamine concentration, and no suggestion is made on how histamine is formed in fish. We therefore investigated the mechanism of histamine formation during spoilage and found that no histamine is produced during *aseptic* autolysis of fish; we also found that histamine is a product of bacterial decarboxylation (*6*). This point, however, will require further investigation, for I have found (*7*) that—in contradistinction to the round fishes, such as tuna, salmon, mackerel, and sardine—only small amounts of histamine are produced during spoilage of flat fish, such as sole and halibut. The total histidine content of all these various fish proteins seems to be identical, and therefore structural differences of the proteins may be responsible for the difference in the amounts of histamine produced during spoilage. Because of such structural differences, the histidine present in round-fish protein seems to be more susceptible to bacterial action.

These investigations, which indicate that considerable amounts of histamine are produced during spoilage of round fish, seem to support the assumption that histamine is the toxic ingredient in fish poisoning. However, the authors (*1*) who propose this theory do not discuss earlier (*8*) investigations according to which oral or enteral administration of large amounts of histamine did not prove to be toxic, probably because histamine is detoxified during its passage through the intestinal wall. Despite these findings, we must consider the possibility that in the cases observed by the authors cited (*1*), either the detoxification mechanism was disturbed, or the conditions of the intestinal tract were such that histamine was absorbed at an increased speed so that detoxification could not keep up with the entry of histamine into the circulation. Such a possibility is actually suggested by some earlier experiments on dogs in which it was found that, after introduction of dilute chloroform, ethyl alcohol, or HCl into the gut, the absorption of intraduodenally administered histamine produced a blood pressure depression characteristic of histamine action within a short time (*8*, p. 88).

We investigated this problem further, using guinea pigs, animals that are particularly sensitive to histamine. We found that 100 mg/kg (body weight) of histamine base dissolved in 5 ml of water and neutralized with HCl to *p*H 7.5 does not kill the guinea pig when it is given by stomach tube. However, when the intestinal mucosa was damaged beforehand by administration of saponin (*9*) for 2 consecutive days, the same dose of histamine killed six of 10 animals within 60 to 100 min.

In further experiments we tried to influence the intestinal absorption of histamine by producing intestinal irritation through *gavage* of 2 drops of Oleoresin Capsicum (N.F.) suspended in 3 ml of water. In these experiments, however, in spite of intestinal hyperemia, only one of five guinea pigs showed shock after *gavage* of histamine at a rate of 100 mg/kg (body weight).

In all these experiments the histamine solution was introduced through a rubber catheter attached to a 10-ml syringe. The catheter had to be filled with distilled water, because we found that when the histamine solution was spilled in the mouth, severe symptoms and even lethal shock followed. This observation confirms the reports of earlier authors who found that histamine produces shock when it is absorbed from the oral mucosa, the tongue, or the trachea (*8*, p. 78).

In another group of experiments we incubated fresh tuna meat after inoculation with an unidentified strain of marine bacteria for 56 hr at 37°C. In preliminary experiments this strain of bacteria produced

large amounts of histamine from a histidine solution. The samples contained, at the end of incubation, 190 to 210 mg of histamine per 100 g of fresh substance. This spoiled fish meat was fed to dogs, cats, rats, and mice. Guinea pigs do not spontaneously consume fish tissue. These animals received, by stomach tube, 10 ml of a concentrated aqueous extract containing 80 to 100 mg of histamine made from spoiled fish. No symptoms of poisoning could be observed in these experiments, and all the animals survived.

These experiments do not support the contention that histamine present in spoiled fish is the cause of fish poisoning. Instead, they indicate that other toxins produced by certain toxin-forming microorganisms in the course of spoilage may be mainly responsible for toxic symptoms. The possibility that histamine may participate in cases of human fish poisoning cannot be entirely eliminated, because highly seasoned hot dishes prepared from spoiled fish, or simultaneous consumption of alcoholic beverages, may possibly facilitate the intestinal passage of histamine.

References and Notes

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5 January 1955.

On the Mechanism of an Anaerobic Exchange Reaction Catalyzed by Succinic Dehydrogenase Preparations

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In the course of studying the mechanism of enzymatic hydrogen transfer reactions, it became necessary to investigate further (*1*) an exchange reaction reported by Weinmann *et al.* in which dideuteriosuccinic acid was converted to normal succinic acid when it was incubated anaerobically with a rabbit kidney succinic dehydrogenase preparation (*2*).

When normal succinic acid was incubated anaerobically in a medium containing D_2O and a Keilin-Hartree heart-particle preparation made as is described by Slater (*3*), the exchange reaction was extremely slow compared with the possible rate of aerobic succinic acid oxidation (Table 1, experiment 1). However, it can be seen that initially added fumaric acid greatly stimulated the incorporation of deuterium into succinic (Table 1, experiment 2).

Table 1. Influence of fumarate on deuterium incorporation into succinate. The reaction mixture contained 2.44 mM of potassium phosphate buffer, *p*H 7.29; 11.0 mg of cytochrome *c*; 1.0 ml of an enzyme capable of oxidizing, under similar conditions, 418 μM of succine acid per hour with succinate as the sole substrate or 319 μM of succinic acid per hour when succinate plus fumarate were present; the fumarase equilibrium was established about the fourth hour. The total volume was 16.5 ml, having D_2O concentration by volume of 88.2 and 86.3 percent for experiments 1 and 2, respectively. Succinate was tipped into the main body of the Thunberg tube after the reaction mixture had been flushed with purified nitrogen gas and the tubes had been evacuated with an oil pump. Incubation time, 8 hr; temperature, 37°C.

Expt.	Acids added initially	Acids isolated at end of reaction	Deuterium (atoms/molecule)
1	1 mM succinic	Succinic	0.0310
2	1 mM succinic	Succinic	0.460
	+	Fumaric	0.0188
	1 mM fumaric	Malic	0.779

These results are most readily explained by the following equations.

$$^{-}OOC—CH_2—CH_2—COO^{-} + 2X \rightarrow {}^{-}OOC—CH{=}CH—COO^{-} + 2X^{-} + 2H^{+} \quad (1)$$

$$^{-}OOC—CH{=}CH—COO^{-} + 2X^{-} + 2D^{+} \rightarrow {}^{-}OOC—CHD—CHD—COO^{-} + 2X \quad (2)$$

Equation 1 represents a reduction of a natural carrier X by succinate, and Eq. 2 represents the reversal of this reaction in heavy water. Alternatively, the reduction of X would yield XH_2, the hydrogens of which would be rapidly exchangeable with the medium D_2O. The resulting XD_2 would subsequently be reoxidized by fumarate, yielding X and deuteriosuccinate. Spectrophotometric evidence for the reversal of reaction 1 with a partially purified enzyme preparation has recently been presented by Green *et al.* (*4*). According to this scheme, the addition of fumarate would promote the incorporation of deuterium into succinate by establishing a new equilibrium position in which the rates of both reactions 1 and 2 would be increased. The deuterium appearing in the succinate should, therefore, arise directly from the medium and not from a reduction of labeled fumarate.

The enzyme preparation used in these experiments contained sufficient fumarase to establish the fumarate-malate equilibrium. Hence, the finding of deuterium in succinate and the stimulation of this deuterium incorporation by fumarate may have reflected an initial incorporation of deuterium into fumarate via the fumarase equilibrium, provided that this enzyme behaved in a nonstereospecific manner with respect to the hydrogen atoms on the methylene carbon of malate. To test this possibility, all three acids from the reaction mixture, in which both succinate and fumarate were incubated anaerobically