A COMPREHENSIVE THEORY ON THE ETIOLOGY OF BURNT TUNA

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ABSTRACT

Over the past 14 years, the Hawaii handline fishery has experienced phenomenal growth in the catch for large yellowfin tuna, *Thunnus albacares*, and bigeye tuna, *Thunnus obesus*. These fish are primarily caught for the sashimi (raw consumption) market but have been continually plagued with a product quality problem known as "burnt tuna" or, in Japanese, yake niku. Not only does this problem significantly reduce the value of the catch, it also limits export markets and expansion of this low-capital, high-return fishery to other areas of the Pacific. Previous research and suggestions for mitigating burnt tuna have centered on the hypothesis that it is caused by high muscle temperature and low pH, which is the result of a violent struggle during capture.

A new, more comprehensive hypothesis is presented: Burnt tuna is actually caused by the postmortem activation of enzymes known as calcium-activated proteases and by the enhancement of the effect of these enzymes by high blood catecholamine levels. Previously unexplainable observations, such as the propensity of female fish to become burnt more often during the summer months, the efficacy of brain destruction in preventing burnt tuna, and the lack of effect of cooling on the incidence of burnt tuna, are explainable in light of this new hypothesis.

One of the largest fisheries in Hawaii is the handline fishery for large (>50 kg) yellowfin tuna, *Thunnus albacares*, and bigeye tuna, *T. obesus*, caught primarily for raw consumption as sashimi. Yearly landings increased from 89 short tons (ex-vessel value, $131,000) in 1973 to 615 short tons (ex-vessel value, $2.1 million) in 1984 (Yuen 1979; Hudgins and Pooley 1987). The total economic value of the fishery has been estimated as high as $5 million yearly (Ikehara[4]). In Hawaii, the night handline fishery is known as ika shibi from the Japanese words for squid and tuna, and the daytime fishery is known as palu ahi from the Hawaiian words for chum and yellowfin tuna. There is also growing international interest in this type of fishing because of its low initial capital investment, low operating and fixed expenses, strong export markets, and high profitability (Strong 1979; Gibson 1981; Jerrett 1984). Boats can be as small as 6 m and require only one- or two-man crew. Catch rates in Hawaii have ranged from two fish per hook per night (Yuen 1979) to one-half fish per hook per night (Bourke[5]), illustrating the profitability for the individual fisherman.

Unfortunately, handline (as well as primarily recreational trolling) fishermen are plagued by a product quality problem known as "burnt tuna" or, in Japanese, as yake niku (literally translated as "cooked meat"). When fish are intended for raw consumption, product quality is of utmost importance. Prime quality tuna flesh should be red, translucent, and firm and have a delicate flavor. Burnt tuna is pale, exudes a clear fluid, and has a soft texture and a slightly sour taste. Although perfectly palatable when cooked or canned, burnt tuna is considered unsuitable for raw consumption and commands only a fraction of the price of prime quality fish. Fish are usually exported whole to preserve freshness, so burnt tuna often is not detected until shipping costs have been incurred. This discourages exports of tuna caught from areas or via fishing techniques with high incidences of the problem.

Burnt tuna affects from 5 to 100% of the tissue from an individual fish and ranges from mild to severe. Approximately 25% of the fish caught by the Hawaii handline fishery are burnt, as are 50% of the large yellowfin tuna caught by commercial and recreational trollers (Bourke fn. 5).

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[4]Ikehara, W. N. 1981. A survey of the ika-shibi fishery in the State of Hawaii, 1980. Southwest Fish. Cent. Adm. Rep. H-82-4C, 12 p. Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, NOAA, Honolulu, HI 96822-2396.

[5]Bourke, R. E. 1985. Hilo ikashibi fishery 1984 survey: Problems of a maturing fishery and a potential solution to the burnt tuna problem. Poster presentation at 1985 Tuna Conference.

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Annual losses to the handline fishery are an estimated 16% of the value of the total catch (Cramer et al. 1981). The problem has also been reported in fish intended for raw consumption caught by purse seine (Nakamura et al. 1977). Surprisingly, the problem rarely occurs in fish caught by longlining (Williams 1986).

PAST RESEARCH ON BURNT TUNA

The Japanese were the first to investigate the causes of burnt tuna and possible mitigating strategies (Itokawa 1968, 1969). The first controlled laboratory investigations were those of Nakamura et al. (1977) and Konagaya and Konagaya (1978, 1979). Nakamura et al. (1977) concluded that high muscle temperature and low muscle pH caused myofibrillar protein denaturation and also noticed that, once denaturation began, it continued even if the tissue was kept at 0°C. Because of the relatively high thermostability of tuna myofibrillar protein and because yake niku occurs in species (e.g., frigate mackerel and sardine) that do not generate high muscle temperatures during struggling, Konagaya and Konagaya (1978) concluded that acid denaturation of myofibrillar proteins at moderate temperatures was the underlying cause.

Cramer et al. (1981) studied handline-caught yellowfin tuna in Hawaii and found that the occurrence of burnt tuna did not correlate with muscle temperature at time of landing and correlated only loosely with extracellular muscle pH. Ikehara[6] conducted an engineering study to develop methods to cool large yellowfin tuna more rapidly, the presumption being that rapid cooling would prevent muscle degradation. Although successful in developing a technique to increase cooling of deep muscle temperature, as shown in Figure 1, there was no apparent correlation between rate of cooling and incidence of burnt flesh. In spite of this lack of directly observed correlation, some publications designed for fishermen still stress that high muscle temperatures and low muscle pH are the prime causes of burnt tuna (Gibson 1981). Others have expressed doubt as to the validity of this hypothesis (Jerrett 1984).

The high muscle temperature-low pH hypothesis appears to fit with what is known about tuna physiology, in that these fishes are capable of producing muscle temperatures significantly above ambient (Carey et al. 1971; Carey 1973) and exhibit some of the highest rates of muscle glycolysis (production of muscle lactate and concomitant production of acidity) observed in nature (Hochachka et al. 1978; Hochachka and Mommsen 1983). Yet some observations do not fit this hypothesis. For example, burnt tuna occurs more frequently in summer, more frequently in female fish, and more frequently in fish fought for short periods of time than in fish fought for long periods (>7 minutes or <2 hours) (Davie and Sparksman 1986; Nakamura[7]). Furthermore, burnt tuna occurs rarely in longline-caught fish and in fish subjected to brain or spinal column destruction immediately following capture (Nakamura et al. 1977; [Suisan Sekai] 1977; Cramer et al. 1981; Davie and Sparksman 1986; Nakamura fn. 7). The hypothesis that burnt tuna is caused by high muscle temperature and low muscle pH does not seem to directly fit with any of these observations.

A NEW ANALYSIS OF THE BURNT TUNA PROBLEM

At the biochemical level, the high muscle temperature-low pH hypothesis would predict that the observed drop in extracellular pH would be accompanied by a similar drop in intracellular pH and activation of lysosomal proteases. These proteases would then degrade actin and myosin, the dominant muscular proteins, resulting in the

[6]Ikehara, W. N. 1981. Development of a small-boat chilling system for the reduction of burnt tuna. Final Report for a Pacific Tuna Development Foundation contract, var. pag.

[7]R. Nakamura, Department of Animal Sciences, University of Hawaii, Honolulu, HI 96822, pers. commun. October 1987.

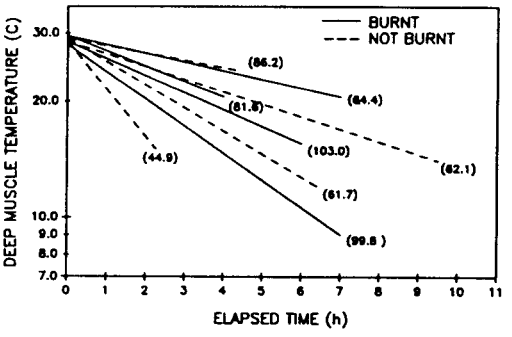

FIGURE 1.—Data are from Ikehara (text fn. 6). Initial and final deep muscle temperatures are plotted on semilogarithmic axes to linearize the rate of temperature change. Numbers in parentheses are the body weight (kg) for each fish.

undesirable changes seen in burnt tuna. The lysosomal proteases, specifically cathepsins B, D, and L, have an optimum pH of about 5 (Dahlmann et al. 1984). However, recent work by Abe et al. (1985, 1986) has shown that, intracellularly, tuna muscle is well buffered and that a fall in extracellular pH is not necessarily accompanied by an equivalent fall in intracellular pH. Using ischemic rat gastrocnemius muscle, Hagberg (1985) found that a 1 pH unit drop (7.30–6.36) in extracellular pH was accompanied by a drop in intracellular pH of only 0.4 unit (7.00–6.60). Thus, the acidic intracellular environment that would favor the action of the lysosomal proteases probably is not present in burnt tuna. Also, with the exception of calcium-activated neutral protease, all other muscle proteases (cathepsins B, D, L; alkaline serine protease; neutral trypsin-like protease; and alkaline cysteinyl protease) degrade myosin (Dahlmann et al. 1984). Yet Hochachka and Brill (1987) found burnt muscle had no increase in 3-methyl-histidine, a specific marker for myosin breakdown. Decomposition of other myofibrillar proteins is, therefore, implicated.

Electronmicrographs of postmortem burnt tuna muscle (Davie and Sparksman 1986) showed a consistent, rapid disintegration of Z-discs and irregularities in the sarcoplasmic reticulum (SR). The changes in burnt muscle were not different in kind from postmortem changes seen in unburnt tissue, but were a result of a significant increase in the rate of disintegration. Selected destruction of the Z-discs, troponin and tropomyosin, and the SR is characteristic of a pair of proteases known as calcium-activated neutral proteases (CANP's) (Sugita et al. 1984; Suzuki et al. 1984). These proteases are cytoplasmic, ubiquitous, activated by increased intracellular calcium levels, and active at pH 5.5–8.0 (Sakamoto and Seki 1985; Koohmaraie et al. 1986; Seki and Kimura 1986; Zeece et al. 1986b). The intracellular pH most likely found in burnt tuna muscle is, therefore, more consistent with CANP action than with lysosomal proteases whose activity requires a pH closer to 4.5 (Hochachka and Brill 1987). In addition, while cathepsin D's activity is greatly restricted at 15°C, CANP is still active at 5°C (Koohmaraie et al. 1986; Zeece et al. 1986a).

A new etiology of burnt tuna proposed by Hochachka and Brill (1987) is summarized in Figure 2. Their hypothesis predicts that low intracellular ATP concentrations lead to the leaking of Ca^{++} into the cell and increases in intracellular

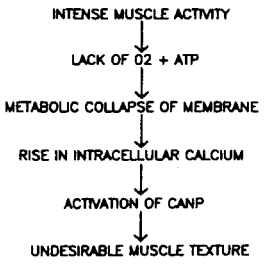

FIGURE 2.—Biochemical reactions involved in the development of burnt tuna as proposed by Hochachka and Brill (1987).

Ca^{++} concentrations. These increases, in turn, activate CANP, which specifically attacks troponin, tropomyosin, SR, and mitochondria. The breakdown of the latter two intracellular organelles releases more calcium into the cytoplasm, thus further increasing the activity of CANP.

The effect of brain and spinal cord destruction on reducing the incidence of burnt tuna and of similar muscle degradation seen in other fish species can be explained by this new hypothesis, which assumes the initial drop in ATP is the root cause of the elevated intracellular calcium (Amano et al. 1953; Fujimaki and Kojo 1953; Konagaya and Konagaya 1978; Ikehara fn. 6). Brain destruction maintains elevated muscle ATP levels after capture (Boyd et al. 1984). Data recently collected on the use of brain and spinal cord destruction in large yellowfin tuna (Fig. 3)

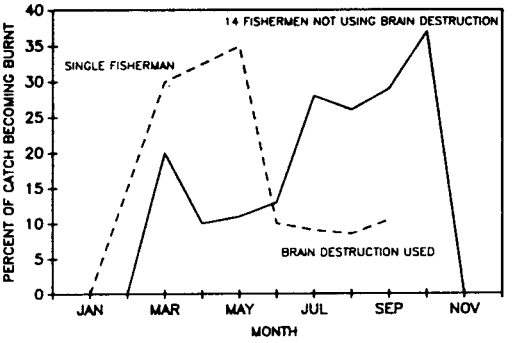

FIGURE 3.—Incidence of burnt tuna occurring in large yellowfin tuna caught by 14 commercial handline fishermen operating out of Hilo, HI, January–September 1984, and by 1 fisherman who began using brain destruction in May. His incidence of fish becoming burnt dropped dramatically in spite of the normal summer increased incidence seen in the catch of 14 other fishermen.

clearly show the effectiveness of this technique in preventing burnt tuna. A drop from 30 to <10% of the tuna becoming burnt was noted when the fisherman changed his killing technique from shooting to the use of a brain spike.

A MORE COMPREHENSIVE HYPOTHESIS ON THE ETIOLOGY OF BURNT TUNA

The hypothesis presented by Hochachka and Brill (1987) does not explain all the observed factors leading to tunas' different propensity to become burnt. It cannot explain why tunas struggling for <7 minutes rarely become burnt (Nakamura fn. 7), nor can it account for the observations of Davie and Sparksman (1986) and Bourke (unpubl. data), who found that tuna struggling for extended (>1 hour) periods of time have a lower probability of becoming burnt. It also cannot explain why female fish become burnt more often than males (Nakamura et al. 1977), or why fish caught in summer become burnt more often (Fig. 3).

Our revised hypothesis is presented in Figure 4. We have incorporated into the Hochachka and Brill (1987) hypothesis, the action of the neurotransmitters-hormones norepinephrine (NE) and epinephrine (E) (collectively referred to as catecholamines). Periods of intense physical activity or capture stress increase the levels of circulating catecholamines by about 10–200 times in fish other than tunas (Nakano and Tomlinson 1967; Ling and Wells 1985). Most likely a similar situation occurs in tuna during hooking, fighting, and capture. The importance of elevated circulating catecholamines in this particular schema comes from their potentiating effect on the action of CANP. Catecholamines cause the phosphorylation of troponin, resulting in a more rapid and prolonged proteolysis of this muscle structure (Toyo-oka 1982); in other words, high circulating levels of catecholamines greatly increase the effectiveness of the enzymes responsible for a tuna becoming burnt.[8]

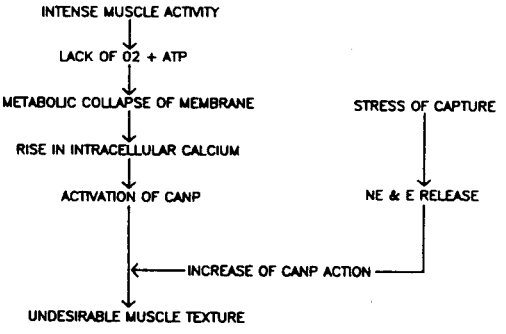

FIGURE 4.—Comprehensive theory of the etiology of burnt tuna proposed in this paper.

The gills are the organ primarily responsible for the degradation of catecholamines, and circulating catecholamines are rapidly cleared from the blood (Nekvasil and Olson 1986). We estimate circulating catecholamines in tuna have a half-life of 30 minutes or less. Therefore, we hypothesize that, in a longline-caught fish remaining in the water for several hours after hooking (Davie and Sparksman 1986), plasma catecholamines are reduced to low levels before the fish is landed and killed, thus resulting in a low percentage of burnt fish. Similarly, fish caught by rod and reel or by handline and fought for several hours would have the low circulating catecholamine levels and low propensity to become burnt. Indeed, Gibson (1981) recommends that handline-caught fish be attached to a buoy and left for an hour prior to being brought on board and killed, as a measure to prevent burnt tuna. That this technique presumably lowers blood catecholamine levels prior to death could explain its efficacy. On the other hand, we hypothesize that a fish landed during the peak of its blood catecholamine concentrations would have the greatest propensity for becoming burnt. This indeed appears to be the case.

The mechanism that ties catecholamines to the observed seasonality and increased number of female fish becoming burnt lies in the biochemical structural similarity of catecholamines and reproductive steroids, particularly estrogen. These steroids reach a peak during spawning season, which is May through October for yellowfin tuna in Hawaii (June 1960). A corresponding increase occurs in the percentage of the tuna catch that becomes burnt during this season (Fig. 3). In the presence of tyrosine hydroxylase, estrogen is converted to catecholestrogen and consequently competes for the same degradative enzymes in the gills, therefore slowing the clearance of catecholamines from the blood (Nekvasil and Olson 1986). Female fish, with high circulating estrogen levels, would then be expected either to reach

[8]Although cortisol is also known to be released during stress, its mechanism of action is much slower than NE or E, making it unlikely that it could exert catabolic effects within 15 minutes.

higher maximum circulating catecholamine levels or to maintain high levels for longer periods, thus explaining the greater number of female fish that become burnt and the seasonality of the occurrence of burnt tuna.

As pointed out by Davie and Sparksman (1986) and Hochachka and Brill (1987), burnt tuna is a quantitative change in tissue decomposition, not a qualitative one. High blood catecholamine levels at the time of death act as accelerators in this inevitable metabolic cascade. However, because we are dealing with a change in rate, the action of catalysts can make all the difference as to whether or not a fish becomes burnt due to CANP.

FUTURE RESEARCH

Under normal conditions, CANP is inhibited by calpastatins. This class of proteins is distinct from the other cysteine protease inhibitors, the cystatins. Although the cystatins can inhibit cathepsins B and H, they are unable to alter CANP activity. Conversely, calpastatin is only effective against calcium-activated neutral protease (Barrett et al. 1986; Parkes 1986). Unfortunately, almost nothing else is known about the relationship of CANP to its endogenous inhibitor, either structurally or physiologically. Certainly investigations into the role of calpastatin would prove valuable for this research.

Immediate future research will concentrate on tracking the specific action of CANP in burnt tuna and attempting to stop this action with Ca^{++} chelating agents such as EGTA (ethyleneglycol-bis-(aminoethyl ether)-N,N,N′,N′-tetraacetic acid) or by use of its intracellular inhibitor, calpastatin. Also, blood catecholamine levels of stressed and unstressed fish will be measured, along with metabolic clearance rates of norepinephrine and epinephrine.

When viewed as a process of metabolic deregulation of CANP, the rapid deterioration of tuna muscle ceases to be an isolated muscular phenomenon. The Z-disc disintegration characteristic of burnt tuna is also present in cardiac muscle injury due to ischemia and muscular dystrophy (Sugita et al. 1984). Given the highly conserved nature of muscle tissue, an understanding of burnt tuna may also provide insights into the metabolic processes of human disease.

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