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by M. Sakaguchi

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Occurrence of Histidine in the Muscle Extractives and its Metabolism in Fish Tissues

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1. Introduction

Tissue extractives from fish contain numerous components. Physically and bio-chemically these extractives are believed to comprise various compounds, which are largely present in a free state in the tissues or cells. As for the muscle extractives, they were studied mainly from the food chemistry point of view--the taste of fish meat[1-3] or its putrefaction[4]--since muscle is the main edible part of fish.

According to previous studies, most of the muscle extractives of fish generally comprises nigrogenous components.[5] Moreover, amino acids are always found in all these components in fairly large[5] proportion. Many of the analyses[6-10] in the past showed unusually high amounts of L-histidine (henceforth abbreviated as histidine) in the extractive amino acids in surface swimming fish, the so called red-meat fish.

Histidine is recognized as an essential amino acid[11,12] for some fish, not only from the viewpoint of food chemistry but also from that of the nutritional value for the fish itself. In spite of this fact, however, very few studies have been conducted on the metabolism of this amino acid in fish tissues.

The author has analyzed the muscle extractives of various fish from comparative bio-chemical points of view. This paper deals, first, with the occurrence of histidine in those extractives along with some reports prepared by others; and then explains the studies by the author and others, on the metabolism of this amino acid in fish tissues.

2. Occurrence of Free Histidine in Fish

The composition of the muscle extractives differs from fish to fish. Even in the same species, it is affected by age (the size of fish) and other physiological conditions. The histidine level in the extractives also fluctuates quantitatively.

Differences in Free Histidine Content According to Fish Species

Shewan[13] stated long ago in his general treatise that many researchers had reported the existence of histidine in the muscle extractives of dogfish shark, mackerel, sardine and tuna. Of these, tuna was reported to contain 470mg%. Shimizu[14] conducted detailed studies on extractive nitrogen in many teleost and selachian fish. He stated that the selachian and red-meat fish contained higher amounts of histidine than the white-meat fish. He clarified that this quantity in the case

of the teleost fish had its origin in the diamino group nitrogen, particularly the arginine and histidine group nitrogen. He then showed, by measuring histidine directly, that the quantity contained in the red-meat fish was 10 to 200 times more than in the white-meat fish (Table 1).

The author and others[15] have also measured 18 kinds of amino acids, taurine and urea of two species of red-meat fish (yellowfin tuna & mackerel), two species of white-meat fish (grey rock cod & flathead sole), the fish of light-colored meat (horse mackerel) and spotted shark which belongs to elasmobranch of selachian. As a result, it was confirmed that histidine in the red-meat fish was present in fairly large quantities, while it was about the same as other amino acids in the white-meat fish (Table 2).

Among imidazole compounds found in the muscle carnosine (β-alanyl-histidine), anserine (β-alanyl-1-methyl-histidine), valenine(?) (β-alanyl-3-methylhistidine) and methyl-histidine (1-methylhistidine and 3-methylhistidine) are known. Lukton and Olcott[16] measured the contents of histidine, methylhistidine, carnosine and anserine in 31 species of fish (Table 3). This table shows that, on the whole, the red-meat fish such as tuna, skipjack, mackerel and sardine contain histidine or histidine and anserine while only anserine is marked in black cod, swordfish and marine salmon. It is worth noting that the total amounts of these imidazole compounds make up more than 50% of the extractive nitrogen.

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On the whole, presence of methylhistidine and carnosine is not significant in fish muscle. But there is an exception with the latter in the case of eels. This was reported years ago by Ackerman and Hoppe-Seyler[17], and more recently by Konosu et al.[18] and Suyama et al.[19]. There are only a few reports[10,15,19] on the histidine content of the extractives from the elasmobranch, and they indicate the histidine content to be about the same as, or a little more than, that of the white-meat fish. Suyama[19] reports that many species of the elasmobranch contain substantial amounts of anserine.

An analysis of the histidine content in the extractives from various parts of the same fish throughout an experiment is seldom available. The only data available is a comparison of the contents of dark meat[10,16,20], liver[21] and blood[22] with that of ordinary meat from several fish. Also available are the results obtained with heart[21] and kidney[23] (Table 4). Histidine is found plentiful in the ordinary meat of yellowtail and mackerel; but in their livers, kidneys and hearts, the amount was no more than that in the muscle of the white-meat fish. Its content is relatively high in the dark meat, which is well developed in the red-meat fish. However, it never seemed to exceed that of the ordinary meat. Thus, it may be concluded that this situation--extremely high free histidine content--exists only in the ordinary meat of the red-meat fish.

Changes during Growth

The environmental conditions under which fish live and the changes in physiological conditions of fish themselves are coordinated intricately in nature, and affect the growth of the fish. It can be assumed that the composition of the extractives in growing fish changes gradually.

Data concerning free histidine in the muscle of growing fish is very scarce[10,24,25]. Only a comparison between big and small fish is found. The red-meat fish such as yellowtail, mackerel and horse mackerel, as well as fish of light-colored meat, seem to have a tendency to accumulate it as they grow bigger (Table 5). The author and others[26] studied the amount of free amino acids in carp. These carp were taken from the same hatchery and observed from the egg stage just before hatching and until growth into adulthood (Tables 5 & 6). Histidine is at its maximum level in young fish of about 3.6g in weight, and decreases afterwards. Moreover, this amino acid is found in greater quantity than any other of the 17 amino acids in all fish--from small fry of 0.3g in weight to adult fish. Generally speaking, the free histidine content of the muscle of crucian carp or adult carp is relatively high[22,27] and is more or less at the same level as that of fish of light-colored meat. The fact that it reaches its maximum level in still growing fish like young carp, may indicate some physiological demand for histidine at that stage in the life of the fish.

Others

Changes in environmental and physiological conditions of fish affect the composition of extractives either directly or indirectly. These changes may be observed as seasonal changes or changes related to spawning. Concerning these changes, Shimizu et al.[28,29] reported that mackerel caught in the Sea of Japan in spring contained less histidine than that caught in either summer or winter. Hughes[30] analyzed the muscle extractives of herring caught off England. He stated that although histidine seemed to decrease from fall towards winter, it is difficult to observe meaningful seasonal changes in the histidine content because of the differences in size and the degree of sexual maturity of the samples. Generally, uniform samples are hard to obtain for the study of seasonal changes, hence no definite conclusions can be drawn from the results of the analyses.

Wood[31,32] obtained very interesting results by analyzing extractives from the tissues of various organs of sockeye salmon journeying upstream to spawn.(Tables 7 & 8). During this journey there was a rapid decrease not only in the concentration of free histidine in the muscle but also in its level in whole tissue as well as in whole body. It is thought that because salmon do not feed during migration, its free histidine may be used up as a source of energy; or it may be changed to substances that are required for spawning.

Furthermore, he stated that during this period the anserine level did not register any change. At present the

physiological role of these substances in fish, that contain a large amount of histidine and its related compounds in the muscle (red-meat fish is a typical example), is not at all clear.

3. Histidine Metabolism Process in Fish Tissue

Whether it is a white-meat fish or a red-meat fish, histidine from food serves inside the fish as a synthetic substance for body protein. Other than that, it is thought to be metabolized and used as a source of energy or for other purposes. The metabolism of this amino acid is generally known to follow a histamine pathway with decarboxylation, and an urocanic acid pathway with deamination[33]. It is well known that among mammals, under normal physiological conditions, histidine metabolism mainly follows the latter pathway[34].

This pathway was studied with bacteria[36] besides the livers of mammals, and very interesting results were obtained. However, practically no studies were conducted with fish. In this paper reference is made only to the results obtained from the studies of mammals, and those of bacteria will be reported at some other time.

Occurrence of Histidine Metabolic Activity in Several Species of Fish

When a fish is under certain normal physiological conditions, histidine brought into the body pool is believed to degrade at a fixed rate. Therefore, the author et al.

studied the intensity of metabolic activity in various organs of several species of fish in order to study the tissue of the organs involved. The method used may be described briefly as follows: after the tissue was homogenized in five volumes of a cold 1% KCl solution, it was centrifuged at 10,000×g

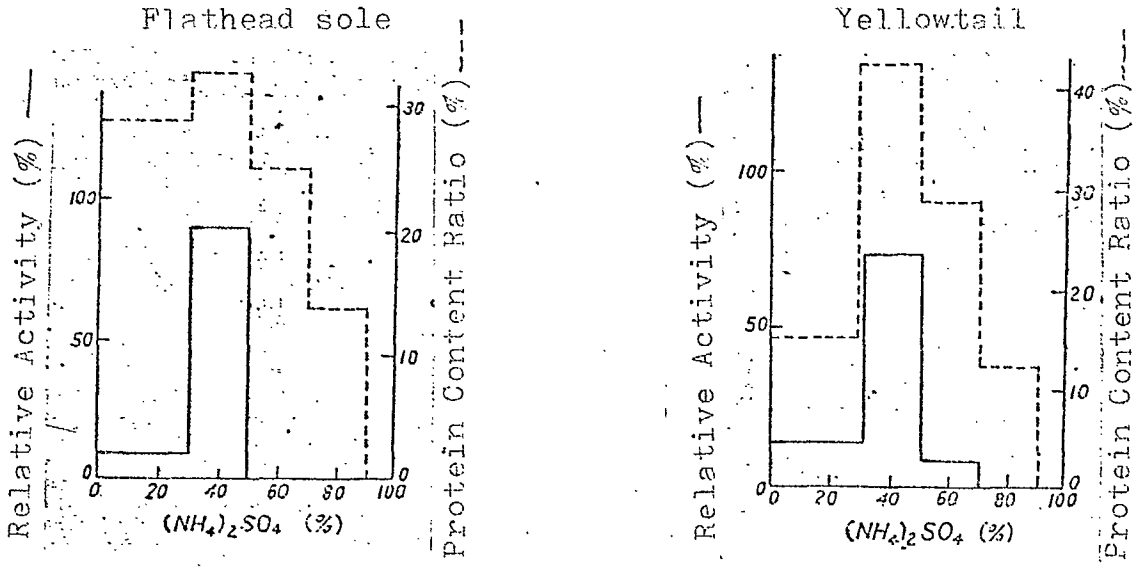

Fig. 1 Occurrence of Histidine Metabolic Activity in Ammonium Sulfate Precipitation Zone of the Liver Extractives of Flathead Sole and Yellowtail.

for 20 minutes. Ammonium sulfate was then added to its supernate to obtain 70% saturation. Precipitation zone was then collected. When this was dialyzed for 12 hours in 0.05M phosphoric acid buffered solution (pH 8.0) we found that this zone could contain almost the whole of histidine metabolic activity (Fig. 1). From similar samples, we compared the histidine metabolic activities in mackerel, yellowtail, grey rock cod, flathead sole and carp (Table ○).

Its activity was the strongest, for all fish, in the liver followed by kidney. It was observed that mackerel and

yellowtail had the activity concentrated in the tissues of their well-developed dark meat, and mackerel also had a little in its ordinary meat. Furthermore, we compared the livers with ~~with~~ strong activities, but it was difficult to detect any distinct differences between them except in the case of flathead sole. Also compared was the metabolic activity per unit weight of fish. Histidine amounts that were metabolized are as follows: mackerel 0.24μmole/hr., yellowtail 0.26, 36
grey rock cod 0.05, flathead sole 0.02 and carp 0.61. This result is quite interesting because it indicates that the metabolic activity is higher in species, including carp, that accumulate a comparatively large amount of free histidine in the muscle. The ordinary meat of mackerel had a little activity but yellowtail, which is also a red-meat fish, did not. When histidine occurrence was studied in the ordinary meat of several species, it was found to exist only in two of the red-meat fish; sardine and mackerel (Table 10). All of them had this activity in their livers.

Formation of Urocanic Acid from the Extractive Supernate of Muscle and Liver

We found that the sample from ammonium sulfate precipitation of the ordinary meat of mackerel had weak histidine metabolic activity. We also found that when the previously obtained supernate was incubated under identical conditions in the presence of toluol, free histidine in the muscle decreased gradually and at the same time its absorption in the ultraviolet region increased (277mu)(Fig. 2). Next, this supernate

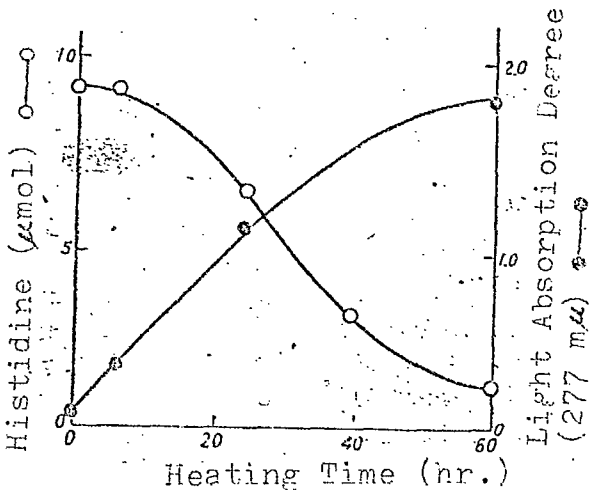

Fig. 2 Decrease of Free Histidine in Muscle Extractives of Mackerel & Increase in Light Absorption at 277mμ

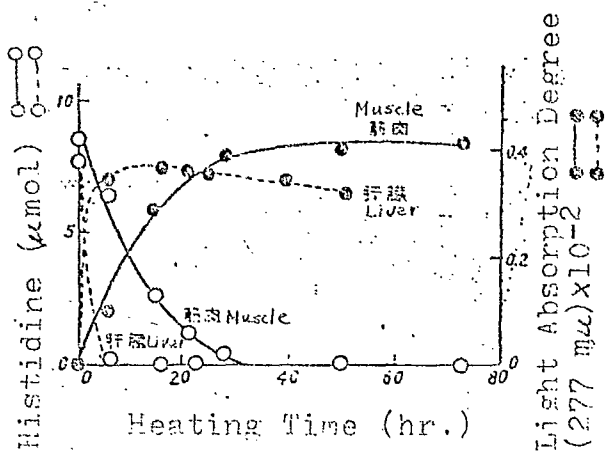

Fig. 3 Decrease of Histidine by the Liver Extractives Supernate (Dialyzed) and Increase in Light Absorption at 277mμ

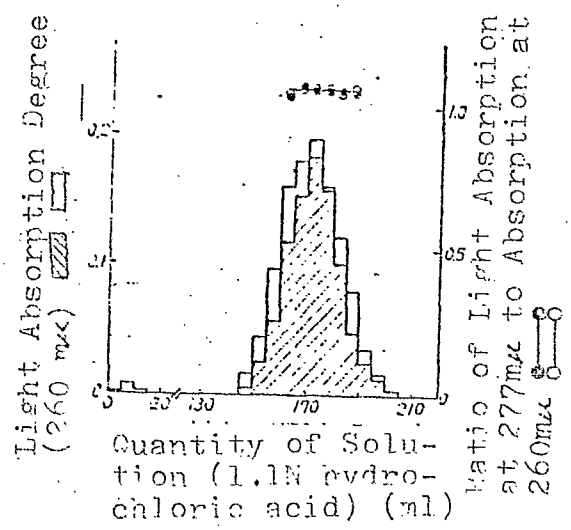

Fig. 4 Metabolic Product of Histidine in Muscle Extractives Supernate of Mackerel (Dialyzed) and Ion Exchange Chromatogram (Dowex 50-x column 1x20cm)[38] of Urocanic Acid

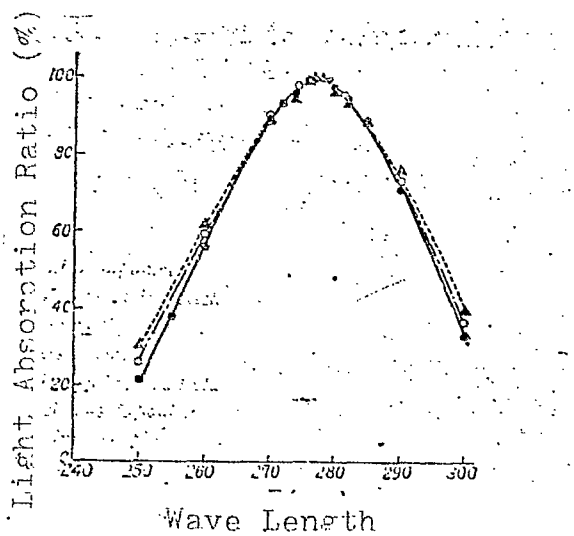

Fig. 5 Histidine Metabolic Product by Liver and Muscle Extractives Supernate (Dialyzed) and Ultraviolet Absorption Spectrum[38] of Urocanic Acid
- ●—● Pure Urocanic Acid
- o--o Product by Liver Extractive Supernate
- ▲---▲ Product by Muscle Extractive Supernate

was dialyzed and free histidine removed. A specific amount of histidine was then added and its degradation followed. It became clear that its absorption in the ultraviolet region increased along with its degradation (Fig. 3). When the liver was centrifuged at 10,000×g for 20 minutes, the amount of histidine that had been added degraded rapidly and its absorption in the ultraviolet region increased. However, it was also observed that with a longer incubation, this absorption decreased slightly (Fig. 3). 37

The substance which is produced by the absorption in the ultraviolet region, is believed to be L-urocanic acid (hereafter referred as urocanic acid), which is considered to be the first product of histidine metabolism. It was identified by the following three methods[38]; the dialyzed supernate mentioned above was incubated for one hour with histidine at 37° pH 8.6, and subjected to ion exchange chromatography using Dowex 50×8. This was later compared with a pure sample. The behaviour of both was exactly the same (Fig. 4).

The absorption spectrum of this substance in the ultraviolet region coincided with that of the pure product, and also with the spectrum of the substance produced from the centrifugal supernate of liver (Fig. 5). It was found out that in both the maximum absorption, at pH 7.3, was around 276 to 278 mu.

Also, when paper chromatography was developed (20°C) with Toyo filter paper No. 51 and n-butanol-acetic acid-water (4:1:1), both spots coincided precisely with Rf value of 0.59.

Thus the substance obtained from histidine by the muscle supernate was identified as urocanic acid. The substance obtained from the liver supernate can also be considered the same since it yielded the same spectrum.

Generally the enzyme related to the production of urocanic acid from histidine is known as histidineaminase or histidase or, more properly, histidine ammonia-lyase (E. C. 4.3.1.3)[39] and catalyzes the following reaction:

Histidine $\rightleftharpoons$ urocanic acid + ammonia

The ammonium sulfate precipitate of the mackerel muscle also yields equal moles of urocanic acid and ammonia along with the degradation of histidine, forming a chemical quantitative relationship (Table 11). From these results it can be said that the histidine metabolic activity found in the muscle of mackerel is based on histidine deaminase activity. Also, since urocanic acid is produced the histidine activity in the liver must be due to this enzyme action.

There has so far been no report dealing with the existence of this enzyme in the muscle of animals. This report must be the first one. The characteristics of this muscle enzyme are described later.

Degradation of Urocanic Acid in the Extractive (Supernate) of the Muscle and Liver of Carp and Mackerel 38

In the case of mackerel liver, it was observed that urocanic acid yield decreased a little with longer incubation as seen in Fig. 3. This may be regarded as due to the existence of urocanase activity in the liver extractive (centrifuged supernate). Hence, the existence of urocanase activity

was studied in the muscle and liver samples of carp, besides mackerel (Table 12 and Fig. 6). In the case of muscle samples the urocanic acid degradation did not occur in either species. Degradation occurred in the liver extractive/supernate but the degree of activity was far weaker in the liver of mackerel than that of carp[40]. Already it has been reported[41~44] that this enzymic activity definitely exists in the livers of land mammals such as cats, cows and sheep; and the formation of imidazolonpropionic acid accompanying urocanic acid degradation has also been recognized[41~43].

Urocanic acid + H_2O ⇌ imidazolonpropionic acid

Rao and Greenberg[44] and, more recently Swaine[45] have taken this enzyme from the livers of cows and cats, refined it to a fairly high degree and studied a few of its characteristics. In the case of carp, comparatively strong activity was found not only in the liver but also in the kidney[79].

^{14}C-Histidine Metabolism in the Extractive/Supernate of the Livers of Carp and Mackerel

In order to learn the process of histidine metabolism in fish, it is necessary to study not only the step by step reaction mentioned above, but also the amount of histidine that can be metabolized in the tissue extractive/supernate. Therefore, ^{14}C-histidine (U) was used for the study to determine the metabolic medium in which this radioactivity penetrates the least. The centrifuged supernates of the livers of carp and mackerel, obtained in the manner described earlier, were incubated at pH 7.2 and 37°C. After that the metabolic product

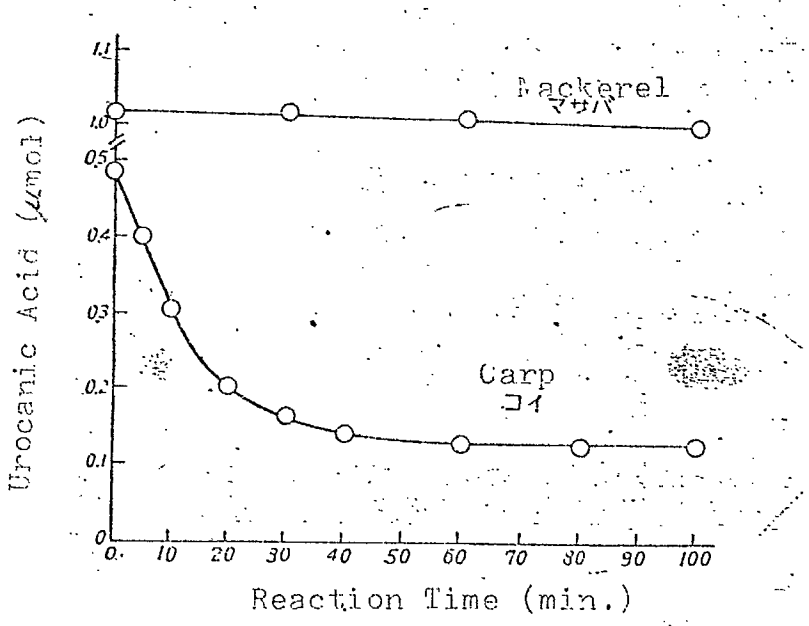

Fig. 6 Degradation[40] of Urocanic Acid by Liver Extractive Supernate of Mackerel and Carp

of histidine was put through an ion-exchange column of Dowex 1[40]. In the case of carp (Fig. 7), the peak of ^{14}C-histidine gradually decreased after 25 minutes and after 120 minutes; and the peaks of metabolic products such as ^{14}C-urocanic acid, ^{14}C-formiminoglutamic acid and ^{14}C-glutamic acid, increased. Among them the accumulation of formiminoglutamic acid was significant (Table 13). The formation of this substance means that imidazolonepropionic acid, formed by urocanase action on urocanic acid route (deamino route) that has so far been reported, is transformed into formiminoglutamic acid by the action of imidazolonepropionase (imidazolone-propionate amidohydrolase, E. C. 3.5.2.7). Also, the formation of glutamic acid suggests that this substance is then formed from formiminoglutamic acid by the action of glutamic acid formiminotransferase (N-formiminoglutamate: tetrahydrofalate-formiminotransferase, E. C. 2.1.2.5). 40

It has been reported that in the case of mammals the activity of imidazolonepropionase was found in the livers[46,47] and that of transferase was found not only in the livers[48,50] but also in the kidneys[50] and the spleens[50].

In the case of mackerel (Fig. 8), the peak of ^{14}C-histidine gradually decreased after 25 minutes and after 120 minutes, and the peak of ^{14}C-urocanic acid only increased. Radioactivity in other metabolic substances was almost nil. (Table 14). This, as stated before, coincides with the fact that urocanase activity is markedly weak in the liver of mackerel.

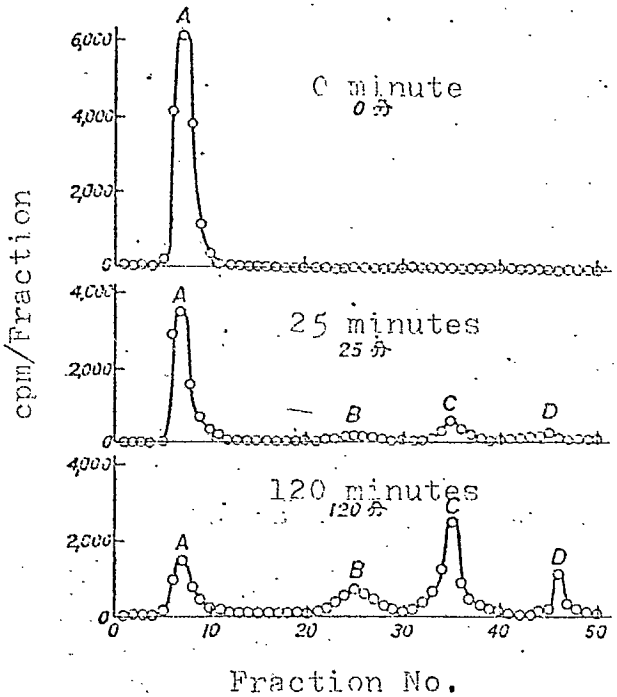

Fig. 7 Formation[40] of Intermediate Product Accompanying Catabolism of ^{14}C-Histidine (U) by Liver Extractive Supernate of Carp
A, Histidine
B, Urocanic Acid
C. Formiminoglutamic Acid
D. Glutamic Acid

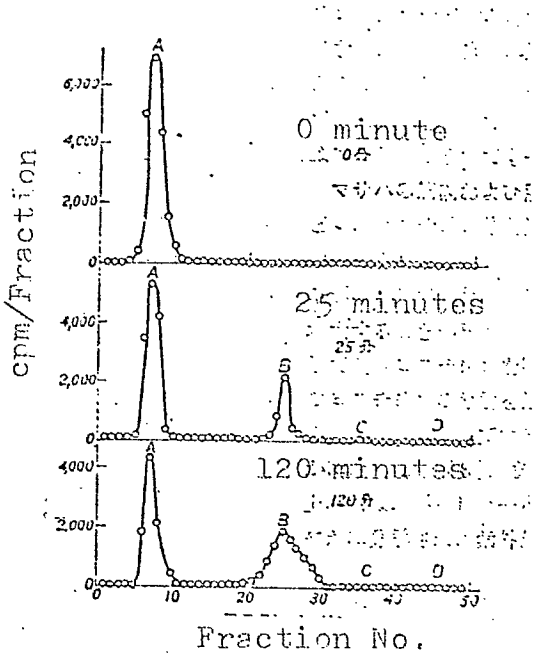

Fig. 8 Formation[40] of Intermediate Product Accompanying Catabolism of ^{14}C-Histidine (U) by Muscle Extractive Supernate of Mackerel
A. Histidine
B. Urocanic Acid
C. Formiminoglutamic Acid
D. Glutamic Acid

Studying the metabolic process of histidine in fish liver from these data, as seen in the case of carp, we find that histidine catabolizes rapidly to glutamic acid just as in the liver of mammals.

$$\text{Histidine} \xrightarrow{-NH_3} \text{urocanic acid} \xrightarrow{+H_2O} \text{imidazolonepropionic acid} \xrightarrow{+H_2O}$$

$$\text{formiminoglutamic acid} \xrightarrow{+\text{tetrahydrofolic acid}} \text{glutamic acid}$$

On the other hand, as seen in the case of mackerel liver, the degradation of urocanic acid does not occur further because of a weak urocanase activity. If this substance is not excreted from the body as it is, then it can be explained that it is carried from the liver to entirely different organs, and is catabolized there. Also, in the case of fish, urocanic acid, produced by histidineaminase in the muscle, does not degrade further since urocanase activity does not exist in the muscle and must be treated by tissue other than the liver. As to degradation of imidazolonepropionic acid, another route is known[34] for mammals but is omitted here.

4. Some Characteristics of Histidine Deaminase Obtained from the Muscle and Liver of Mackerel

It has been stated before that mackerel has histidine deaminase not only in its liver but also in its muscle (ordinary meat). This is an essential and very important enzyme for catalyzing the initial stage of the urocanic acid route. A study has been made on the enzyme samples obtained from bacteria Pseudomonas fluorescens and an explanation of its function was attempted[45]. Also recently Rechler[46] reported the biochemical and physicochemical nature of what are thought to be uniform samples obtained from Pseudomonas sp. There are many studies[53~60] related to metabolic control based on the quantitative changes of enzymes.

This enzyme, as has been known for long, occurs not only in the liver of many mammals and in various bacteria[33,61~63] but also in human epidermis[64]. The existence of this enzyme

in fish was known to be only in the liver[63]. The author et al.[65,71] found its activity in the liver, muscle (ordinary meat), dark meat and the kidney of mackerel. We have obtained a couple of interesting findings of the nature of histidine deaminase in muscle and liver.

Characteristics of Enzyme Obtained from Muscle

There are many reports[66,67] on the nature of histidine deaminase originating in animals--all based on the utilization of partially purified samples. However, all of them were obtained from livers, and there has been no report on its nature, or even its existence, in muscles. Therefore, the author et al.[65] extracted this enzyme (henceforth referred as muscle histidine deaminase or simply muscle enzyme) from the muscle (ordinary meat) of mackerel. After it was purified as much as possible, some very general studies were made on its nature. As a sample, the ordinary meat of mackerel with rigor mortis, was separated carefully so as not to mix it with the dark meat. After it was homogenized with three volumes of a cold 1% calcium chloride solution, it was centrifuged at 10,000Xg for 20 minutes. In order to remove the abundant free histidine this supernate was dialyzed in 0.1M phosphoric acid buffered solution, and crude enzyme solution was obtained. This crude enzyme solution was purified in the following order: ammonium sulfate precipitation at 70% saturation, heat treatment, ammonium sulfate precipitation at 35 to 65% saturation, DEAE-Sephadex and hydroxylapatite chromatography

and gel filtration using Sephadex G-200. Since we obtained samples with relative activity of 300 to 400 times more than that of the crude enzyme solution, they were used for our experiments. The measurement of enzyme activity was based more or less on the method by Tabor et al.[70].

From the temperature stability point of view, this 41
enzyme is stable at below 55°C, but rapidly loses its activity above this (Fig. 9). A similar result was obtained using the

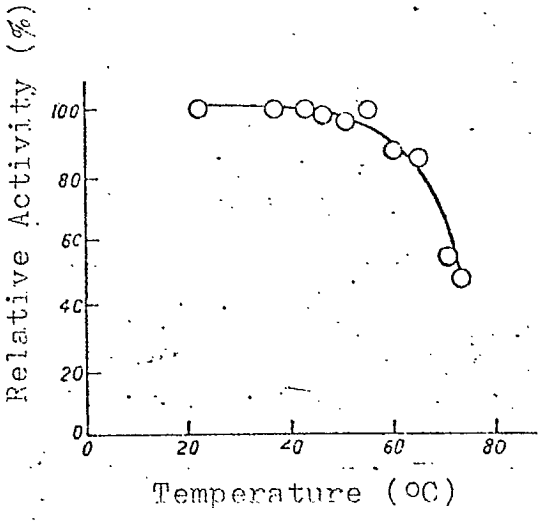

Fig. 9 Heat Stability[65] of Histidine Deaminase Obtained from Mackerel Muscle (pH 8.0)

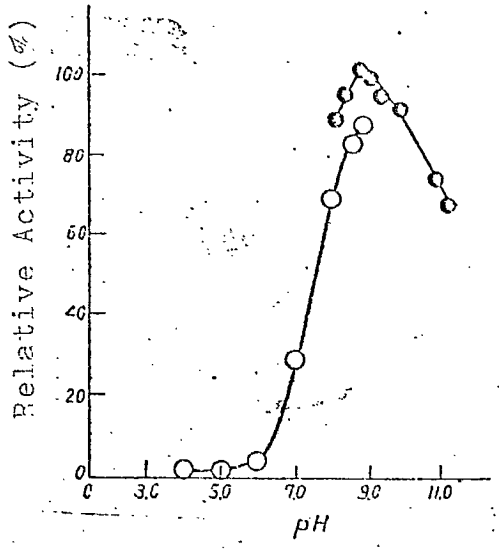

Fig. 10 pH--Activity Curve[65] of Histidine Deaminase Obtained from Mackerel Muscle o—o Phosphate Buffered Solution(0.1M); o—o Carbonate Buffered Solution (0.1M)

ammonium sulfate precipitated zone which is one step prior to the heat treatment. In general histidine deaminase seems to be a comparatively stable enzyme against heat. This was clear from the fact that heat treatment was effectively used for its purification[66,68]. Next, drawing a pH activity curve for this muscle enzyme (Fig. 10), we found that its optimum pH is

around 9.0. The optimum pH of histidine deaminase, collected by Cornell and Villee[69] appears to be around 8.5 to 9.2 (Table 20). Kato et al.[66] have also pointed out that it is at 8.7, using the enzyme from guinea pig liver. Generally, the substrate specificity of histidine deaminase has not been studied in detail but it is considered to be very high[61,70]. On this point, the author et al. examined its capability to produce ammonia using aspartic acid, tyrosine, phenylalanine, tryptophan, histamine and carnosine; and found that muscle histidine deaminase has no effect on them[71]. 42

Its behaviors against metal ions, EDTA, SH compounds and SH inhibitors, showed some very interesting results (Table 16). The enzymes obtained from the liver of mammals and microbes[66,67,72] are said to be activated by Zn^{++}, Co^{++} and Mn^{++} ($10^{-3}\sim10^{-4}$M). But the enzymes obtained from the muscle have no such effect and have not been inhibited even by EDTA. Thus it seems that it does not require metal ions as activators. According to Tabor and Mehler[70] and Peterkofsky[51] histidine deaminase of bacteria is activated by SH compounds such as GSH, thioglycol etc. Cornell and Villee et al.[67] also point out that a similar effect is obtained for the enzyme in rat liver by GSH. On the other hand, muscle enzyme has not been affected in that manner by these SH compounds. Instead there was a reverse effect and it was a little inhibited. However, since it was inhibited strongly by pCMB (1×10^{-4}M) and recovered by cysteine, it is assumed

that it belongs to a kind of SH type enzymes. Whatever it is, these two points--failure to be inhibited by EDTA and lack of activation by GSH etc.--do not coincide with any characteristic of histidine deaminase with a different origin.

Characteristics of Enzyme Obtained from Liver and Bacteria

It has been stated before that the liver of mackerel had a strong histidine metabolic activity based on histidine deaminase. Of the characteristics observed with muscle enzymes, the effect of EDTA and GSH are studied here. Furthermore, the enzyme was also taken from the muscle and bacteria Pseudomonas fluorescens mentioned before, and it was reciprocally compared as to Km value and molecular weight against substrate histidine.

The muscle enzyme of mackerel was extracted and purified according to the method described earlier. Crude enzyme solution extracted in the same way as from the liver, was purified[73] to about 100 times of crude enzyme stage by ammonium sulfate zoning, chromatography using DEAE Sephadex and hydroxylapatite and gel filtration, and was then used as an enzyme sample. Also, Pseudomonas fluorescens was cultivated in culture solution containing histidine, extracted and purified by Peterkofsky's method[51], was further purified to about 100 times of crude enzyme solution by gel filtration.

It was understood that muscle histidine deaminase was inhibited by SH compounds like GSH and thioglycol. Comparing this with other histidine deaminase(Table 17), however, it

became clear that liver enzyme got activated twice as much at $10^{-3}\sim10^{-4}$M GSH and that of bacteria got 1,000 to 2,000 times more at 10^{-3}M. The enzyme of bacteria is reported to be activated strongly by thioglycol[51,52] in addition to GSH[70], but Rechler[52] showed that in the case of thioglycol the effect was observed only with its optimum concentration, and it was gradually lost as the concentration became any higher. In the case of muscle enzyme it was expected that a similar phenomenon occurred with glutathione, but it was never activated even at relatively weak concentration of under 10^{-4}M. EDTA inhibited the functions of bacteria and liver enzymes with an increase in its concentration, but it never inhibited the liver enzyme as mentioned above (Table 18). According to Rechter[52], the enzyme obtained from bacteria is not inhibited even at 10mM EDTA concentration when thioglycol is not present, but a marked inhibition phenomenon is observed in the presence of this SH compound thereby suggesting that SH compounds have some kind of relationship with this chelation inhibition. It was attempted to determine whether a similar phenomenon also took place in liver enzyme in the presence of GSH, but it was not detected. 43

Obtaining the Km value by Lineweaver-Burk plot (Fig. 11 and Table 19), we find it to be in the following order: bacteria 2.7×10^{-2}M, liver 7.8×10^{-4}M and muscle 3.3×10^{-5}M. When GSH was present the values of the former two decreased slightly, but in the case of muscle it showed an increase.

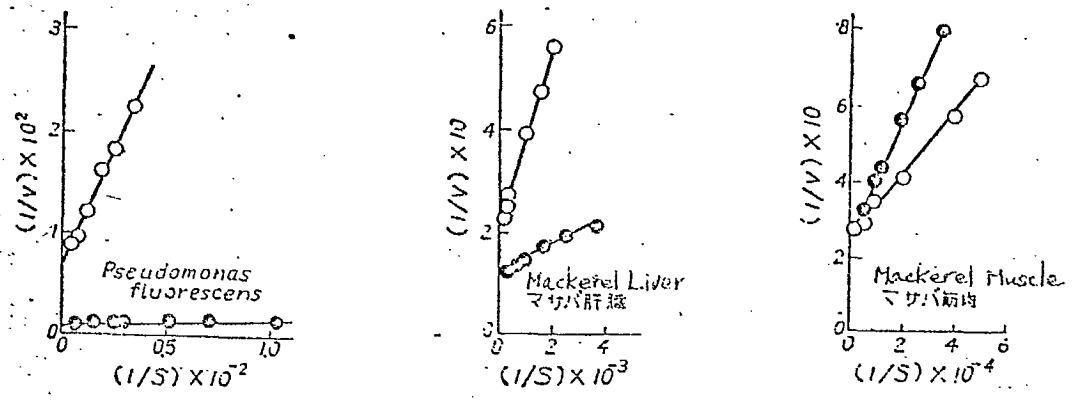

Fig. 11 Effect of Substrate Concentration on Histidine Deaminase Activity Obtained from Bacteria Pseudomonas Fluorescens, and the Liver and Muscle of Mackerel

●—● GSH is added (1.0×10^{-3}M)
○—○ GSH is not added

This implies that at least a part of the inhibition by GSH mentioned so far results in something of a rivalry. From the reports made so far, the Km value of histidine deaminase measured under similar conditions, is in the order of around 10^{-3}M[66,67,69,74]. The Km of the liver of mackerel in the presence of GSH (1.0×10^{-3}M) looks somewhat lower at 2.5×10^{-4}M. By the way, as stated before, the free histidine concentration in the muscle and liver tissues of mackerel differs greatly--the former 5.1×10^{-2}M, and the latter only 1.3×10^{-3}M. Moreover, the bacterial cells obtained by ^{14}C-histidine showed a much lower value of 3.5×10^{-4}M (initial concentration of culture solution 1.0×10^{-2}M). The order of the concentration of these substrates has a reverse relationship with that of Km values of their enzymes. That is, whether or not GSH is added, Km follows the order of bacteria, liver and muscle (Table 18). The reason is not clear and its explanation will require the

solution of problems such as those of the differences in the concentration of substrate histidine in both inside and outside of muscle and liver cells and the distribution of substrates and enzymes in the cells. The occurrence of histidine deaminase in the cells is, however, as has been known from a study[75] using floating cells in the blood serum of mammals, 44 in the supernate and not in nucleus, mitochondria or microsome. As in the case of enzymes related to metabolism of many amino acids, in the liver cells this enzyme also has a possibility of existing in the supernate.

A lot of data[68,69] has been available on the molecular weight of this enzyme (Table 20), which is around 200,000. The author et al. also estimated the molecular weight of the enzymes of muscle, liver and bacteria of mackerel by the sucrose gradient sedimentation method[76] (Table 19). Each was around 200,000 and there was not much difference between those three. Rechler[52] estimated the molecular weight of histidine deaminase of Pseudomonas sp. as 214,000 by the sedimentation method, and 211,000 by the sedimentation equillibrium method. He further devided it into subunits with 35,000 molecular weight, by treating it with 6M hydrochloric guanidine 0.1M thioglycol system. It seems that the enzymes of muscle and liver too are composed of some subunits. The sustenance of such subunit composition impart the allosteric nature to the enzyme protein[77]. However, only Tessie and Neidhardt[78] have suggested the possible appearance of allosteric effect on hirtidine deaminase of bacteria which has been studied

relatively well. Since histidine deaminase is an enzyme to catalyze an important reaction--the first step of main metabolic pathway of histidine--a study in this direction in the future is indicated.

Cornell and Villee[69] studied histidine deaminase of three kinds of bacteria, and that of the liver of four kinds of animals including a human being and obtained their optimum pH, Km values and molecular weights. They concluded that in general there was no difference in the nature of this enzyme between the various species. However, detailed studies in the nature of the enzymes taken from the muscle and the liver of mackerel, and bacteria pseudomonas fluorescens, showed that the enzyme in mackerel liver was similar to that in the liver of mammals[33,67] since it was inhibited by EDTA and was activated by GSH. It was also similar to that of bacteria. On the contrary, however, the author et al. are at present of the opinion that this histidine deaminase is somewhat of a special type, because that in the muscle is entirely different from the other two at two points stated above, and also because of the fact that the muscle enzyme does not adhere to DEAE-Sephadex column under the same conditions under which the enzyme of mackerel liver adhered.

5. Fluctuations in Activities of Histidine Deaminase and Urocanase in Carp Liver and Free Histidine Content in Carp Muscle

A significant amount of free histidine is believed to play a special role in the muscle of some fish as mentioned

before. It is, in general, used as merely a kind of amino acid to synthesize body protein. It may also, in certain cases, act as the source of energy as a result of catabolism or as a supplier of formyl radical. Because histidine has proven to be an essential amino acid in chinook salmon[20,21], there must be some mechanism to strictly control histidine metabolism in the body of fish.

Not only fish but all living beings react to the changes in environment external to the body, including food. They try to maintain the stability of their internal environment in a broader sense, and metabolic control in the body seems to be one step in this direction. The control of metabolism is often observed as the quantitative fluctuation of enzymes related to its metabolism. So far as fish is concerned the whole picture of histidine metabolism is not quite clear at present. It seems important to pursue the research on the enzyme of urocanic acid route--a main metabolic route especially for histidine deaminase and urocanase. 45

As a start a couple of experiments have been conducted on how free histidine concentration and enzyme activity in the muscle would be affected by treating carp with protein defficient feed and by starvation. This, along with a similar study using rat, is being introduced here.

Effect of Low Protein Feed on Histidine Deaminase and Urocanase Activities in the Liver of Carp

Young carp (30 to 50g in weight) were given feed equivalent of 5% of its weight (Table 21) twice a day (morning and

evening) for 14 days. After homogenizing its tissue in four volumes cold 1% potassium chloride, it was centrifuged for 15 minutes at 10,000xg, and its supernate was used as the enzyme solution for the measurement of the activity. The measurement was carried out using the method of Tabor and Mehler[70] with a slight modification[79].

Inside the carp histidine deaminase and urocanase were most active in the liver[79]. Therefore, only the liver was used for the measurement of enzyme activity. It was stated earlier that in the extracted supernate of the carp liver histidine was catabolized at least to glutamic acid. When the enzymes activity of the liver of carp fed on low protein food (including 5% casein), was compared with that of the carp with high protein feed (including 80% casein).both the enzymes were found to have clearly more activity in the latter. Further, when carp were given low protein feed to which histidine had been added to bring it to the level of the high protein feed, the activity of histidine deaminase increased slightly and the urocanase activity rose to a level fairly close to that of high protein feed. Comparing the low protein feed with medium protein or high protein feeds, the activity of amino acid metabolic enzymes in the livers was generally repressed. This phenomenon was observed with rats[89,91]. Similar reports have been made with histidine deaminase and urocanase in rat liver livers[80 82] (Table 23). The causes of this phenomenon are very complex. It is believed that one of the causes may be

the repression effect of glucose as suggested in an experiment by Sahib and Krishna Murti[81~83]. That is, when rats with low protein feed were given casein solution for a week, histidine deaminase activity of the liver increased. But if glucose was added at the same time, this increase was repressed (Table 24). This can be interpreted [81] to have a connection with catabolite repression[53,59,78] associated with microorganisms. The author et al.[95] also found the repression action of glucose and glutamic acid in a preliminary study using carp. 46

When histidine was added to low protein feed the activity of both enzymes in carp liver increased a little (Table 22). A similar effect was observed[95] when this was injected into the abdominal cavity. On the other hand, the experiment using rats showed no effect of histidine[81~86] (Table 26), when it was added to low protein and medium protein feeds. It is also reported that no effect is shown when histidine only is injected[87,88]. Even carp seems to show a considerable effect of the composition of food given as a treatment prior to injection and the length of period of starvation following after that[96]. Meanwhile, Sahib and Krishna Murti[81] observed that in rats fed on low protein diet histidine deaminase activity did not increase if only histidine was given. But by adding a small amount of casein solution a similar or better effect was observed (Table 25). This does not indicate that protein synthesis of net has occurred in the liver cells or inert protein molecule existing before were activated. It is interesting, however, to know that a small amount of casein solution

has some role, even though indirect, tp play in bringing about even this effect. More explanation is expected in the future.

Effect of Starvation on the Activities of Histidine Deaminase and Urocanase in the Carp Liver 47

It is thought that a very delicate change occurs in the metabolism of substances if the food stimulus--one of those affecting internal body environment--is removed by not feeding an animal. Both enzyme activities were compared[79] (Table 27), therefore, between the carp which were kept without food for 14 days and the previous carp fed on high protein diet. In both histidine deaminase and urocanase the activity per unit weight of the liver tissue was higher in the fish kept without food. On the other hand, when calculated as activity per whole liver there was not much difference between those two groups. The activities of both enzymes in the fish without food was about the same as that in the fish with food. But as the number of days without food increased, the liver weight decreased[71] and after 14 days the rate of decrease was 33.3%[79]. The increase in the activity per unit weight can be attributed to the concentration of the enzyme. It has been recognized that in the case of the liver of a rat with relatively short period of starvation, the activities of both enzymes did not decrease[82,89] (Table 28) either. Moreover, Kolenbrander[90] established the fact that the activity of this enzyme decreases after seven days of starvation, but does not decrease for two or three days and maintains its original level. Also, the activity of urocanase, though a bit faster

than the other, does not decrease easily. It is known that the activities of arginase and threonine dehydrase[91] of enzymes, related to amino acid metabolism, also increases through starvation. From the point of energy supply by catabolism, the existence of these enzymes in the liver tissue is important, and it can be considered that there is some kind of control mechanism that stops the metabolism of these enzymic proteins simply by starvation. Since a study has started on the effects[82,92~94] of feeding various hormones on histidine deaminase and urocanase activities in the liver of a rat, it is expected that it will gradually elucidate more details of the histidine metabolic control mechanism.

Effect of Low Protein Feed and Starvation on the Free Histidine Content in Carp Muscle.

It was stated before that the carp muscle contains relatively high concentration of free histidine. If this is to constantly carry on an important physiological mechanism in this tissue, it must not be affected by any sort of treatment that may possibly change the physiological conditions inside the body, such as low protein feed or starvation; and it may well be assumed that a certain concentration of free histidine is maintained. Under similar feeding conditions (Table 21) as mentioned above, low-protein-fed carp (including 5% casein), high-protein-fed carp (including 80% casein) and starved carp were studied for 14 days and measurements taken of free histidine in their muscle and, at the same time as a contrast, of free lysine whose content is relatively high (Table 29).

Low-protein-fed carp have markedly less lysine content compared to that among the high-protein-fed carp. Also a similar decrease was observed in starved carp. On the contrary, histidine content did not show much change and it became clear that, with this kind of treatment, this amino acid was maintained in a specific concentration in the muscles[96]. It is imagined that in the fish body free histidine is usually carried from the muscles to the liver through the blood stream, and its amount is mainly controlled in this tissue, and returned through the blood stream to the muscles. The control mechanism is also believed to exist in the cell membranes of the muscles. 48

The importance of free histidine in the muscle of carp is suggested here again. Lukton[97] fed chinook salmon on two kinds of food--one lacking histidine and the other lacking methionine--for a long time and compared the contents of four kinds of imidazole compounds (histidine, l-methyl histidine, carnosine and anserine) in muscle extractives with those of contrasting fish. He found out, as a result, that except for anserine other imidazole compounds decreased in content because of this treatment. As we know that in the muscle of sockeye salmon, in transit for spawning, free histidine content decreases during its progress but anserine content does not change[31], so in this kind of fish, that has little histidine in the muscles, anserine may play an important role.

6. Conclusion

The mechanism of free histidine accumulation in the muscle of dark meat fish and carp or its physiological meaning is not clear. There is no simple explanation for it. In order to accomplish this type of study, it is dangerous to apply deductively any knowledge or laws, obtained from studying land mammals, to the aquatic animals. Things basic with all living beings on the earth are also basic with the aquatic animals; but special physiological mechanisms or metabolism evolved to help them adapt to their external environment. These seem to be common to all animals in that particular group living in that particular environment.

As the analysis and observation techniques improve, we expect that new facts will be found from an unknown part of aquatic living beings and different law will be born based on that.

Table 1. Free Histidine Content in Fish Muscle[10)]

	Fish Species	Weight (g)	Histidine (mg%)
Red-meat fish	Frigate mackerel Auxis thazard	350	1,010
	Yellowtail Seriola quinqueradiata	1,020	1,035
	Mackerel Scomber japonicus	600	802
	Striped marlin Makaira mitsukuri	18,750	1,320
	Big-eyed tuna Parathunnas mebachi	15,290	1,010
	Mackerel pike Cololabis saira	75	1,610
	Dolphin Coryphaena hippurus	12,370	937
	Sardine Sardinea melanosticta	75	606
Light colored meat fish	Horse mackerel Truchurus japonicus	260	368
	Japan sea bass Lateolabrax japonicus	650	6.9
	Barracuda Sphyraena pinguis	64	170
White-meat fish	Nibbler Girella punctata	60	5.5
	Puffer Lagocephalus lunaris	355	4.7
	Dab Limanda herzensteini	520	16.7
	Triggerfish Stephanolepis cirrhifer	12	4.6
	Red sea bream Pagrosomus major	100	47.7
	Common flounder Paralichthys olivaceus	300	4.7
	Sillago Sillago sihama	67	40.3
	Gurnard Chelidonichthys kumu	154	7.4

Table 2. Free Amino Acids, Taurine and Urea Content in Fish Muscle[15)]

	Yellowfin tuna Neothunnus albacora	Mackerel Scomber japonicus	Horse mackerel Trachurus japonicus	Grey rock cod Sebastes inermis	Flathead sole Hippoglossoides dubius	Spotted shark Mustalus manazo
	(mg%)	(mg%)	(mg%)	(mg%)	(mg%)	(mg%)
Urea	-	-	-	-	-	1001.0
Taurine	13.3	(±)	18.5	29.8	64.6	28.0
Aspartic acid	-	2.3	8.8	4.8	15.3	5.9
Threonine	1.9	8.1	1.8	3.1	5.1	12.1
Serine	2.2	(±)	0.2	0.5	(±)	4.9
Glutamic acid	1.4	17.8	14.4	2.4	5.0	2.7
Proline	-	-	1.8	(±)	4.8	-
Glycine	6.5	15.8	12.8	6.5	7.1	16.8
Alanine	8.6	22.2	13.8	6.4	2.2	23.8
Cystine	-	-	-	-	-	29.9
Valine	-	1.4	5.4	(±)	9.7	(±)
Methionine	4.0	2.5	(±)	0.6	0.7	14.0
Isoleucine	0.1	0.9	0.8	1.0	1.8	2.8
Leucine	2.5	4.7	0.1	4.1	2.8	4.3
Tyrosine	3.2	5.5	1.9	3.5	(±)	1.2
Phenylalanine	1.5	3.0	9.7	3.9	(±)	2.1
Tryptophan	-	2.3	0.1	(±)	(±)	(±)
Histidine	832.0	781.2	174.1	2.8	9.8	63.6
Lysine	23.6	17.1	28.8	7.5	34.3	0.2
Arginine	32.6	(±)	14.1	7.6	(±)	9.5

(±) Trace

Table 3. Imidazole Compounds Present in the Muscle Extractives from Various Fish[16]

Fish species	Histidire	Methyl-histidine	Camosine	Anserine	Extractive nitrogen	Imidazole nitrogen
Marine fish	(mg%)	(mg%)	(mg%)	(mg%)	(mg%)	%*
Yellowfin tuna						
Neothunnus macropterus	550	0	7	718	500	63
"	735	0	0	737	620	60
"	570	0	0	998	650	60
"	412	0	2	612	1020	26
"	739	0	113	458	660	51
Albacore						
Thunnus germo	606	0	27	933	1070	36
"	775	0	99	254	370	80
Skipjack						
Katsuwomus pelamis	1190	-	43	588	-	-
"	403	-	36	475	-	-
"	888	0	181	124	740	43
Big-eyed tuna						
Parathunnus mebachi	473	0	0	670	510	55
"	488	0	0	761	640	48
North American Pacific mackerel						
Pneumatophorus diego	600	0	5	17	370	45
"	519	0	14	5	420	35
Black cod						
Anoplopoma fimbria	33	0	32	442	270	45
Swordfish						
Xiphius gladius	3	0	11	372	240	38
North American sardine	763	0	2	7	480	44
Sardinops caerulea						
Chinook salmon						
Oncorhynchus tshawytscha	22	29	18	278	290	29
"	37	0	27	478	300	43
"	25	0	0	420	490	22
Pink salmon						
Oncorhynchus gorbuscha	40	0	0	552	210	67
Coho salmon						
Onchrhynchus Kisutch	48	25	11	612	440	37
Atlantic menhaden						
Brevoortia tyrannus	233	5	7	0	380	17
Thread herring						
Alosa sapidissima	113	0	9	0	220	15
Cod species						
Gadus sp.	3	20	5	120	280	13
Barracuda species						
Sphyraena argentea	130	0	2	2	290	12
Halibut						
Hippoglossus stenolepsis	5	0	11	89	380	7
"	3	0	7	36	180	6

* Ratio to extractive nitrogen

Table 3. Con'd.

Fish species	Histidine	Methyl-histidine	Carnosine	Anserine	Extractive nitrogen	Imidazole nitrogen
Marine fish	(mg%)	(mg%)	(mg%)	(mg%)	(mg%)	%*
Rock cod species						
Sebastodes sp.	5	0	0	0	290	0.4
"	9	0	5	0	240	1.1
Atka mackerel species						
Oohiodon elongatus	3	0	0	0	310	0.3
"	5	0	5	7	250	1.6
Pacific Ocean perch						
Sebastodes alutus	8	0	7	5	290	1.7
Rainbow smelt						
Atherinopsis californiensis	2	0	5	36	250	0.4
Sole						
Glyptocephalus zachirus	12	0	7	7	310	2
Sea bass						
Stereolepsis gigas	5	0	0	0	160	0.6
Sole species (Californian)						
Isopsetta isolepsis	3	0	0	0	200	0.5
Ray species						
Raja inornata	0	2	7	0	1250	0.2
Shark species						
Triakis semifasciata	0	0	2	0	1110	0.1
Fresh water fish						
Croaker family						
Aploceinotus grunniens	50	0	20	0	230	8
Rainbow trout						
Salmo gairdneri	39	0	47	10	380	16
Buffalo fishes						
Ictiobus sp.	37	0	16	0	220	6
Carp						
Cyprinus carpio	60	0	2	0	350	5
"	157	0	7	0	290	15
Yellow perch						
Perca flavescens	47	0	9	17	270	7
Walleye						
Stizostedion vitreum	62	0	9	84	220	17

* Ratio to extractive nitrogen

Table 4. Free Histidine Content in Fish Organs

Fish species	Organs	Histidine (mg%)
Frigate mackerel[10] Auxis thazard	Ordinary meat	1010
	Dark meat	433
Yellowtail[10] Seriola quinqueradiata	Ordinary meat	972
	Dark meat	247
Mackerel[10] Scomber japonicus	Ordinary meat	802
	Dark meat	207
Yellowfin tuna[16] Neothunnus macropterus	Ordinary meat	740
	Dark meat	223
Albacore[16] Thunus germo	Ordinary meat	775
	Dark meat	272
Skipjack[16] Katsuwanus pelamis	Ordinary meat	889
	Dark meat	172
Barracuda[16] Sphyraena argentea	Ordinary meat	130
	Dark meat	51
Horse mackerel[20] Trachurus japonicus	Ordinary meat	163
	Dark meat	69
Mackerel[21] Scomber japonicus	Ordinary meat	656.1
	Liver	22.3
Yellowtail[23] Seriola quinqueradiata	Liver	1.1
	Kidney	0.4
Yellowtail[21] Seriola quinqueradiata	Liver	7.5
	Heart	15.1
Carp[22] Cyprinus carpio	Muscle	242
	Blood	3
Carp[21] Cyprinus carpio	Muscle	138.6
	Liver	20.2

Table 5. Free Histidine Content in the Muscle of Growing Fish

Fish species	Weight (g)	Histidine (mg%)
Yellowtail[10] Seriola quinqueradiata	750 1020	927 1035
Mackerel[10] Scomber japonicus	110 450 110	428 704 802
Horse mackerel[10] Truchurus japonicus	260 40 4	164 302 368
Mackerel[24] Scomber japonicus	30 650	656.1 914.9
Carp[26] Cyprinus carpio	* 0.3 3.6 52.5 870	(±) 23.0 318.4 165.5 126.6

* Fertilized eggs, (±) Trace

Table 6. Changes in Free Amino Acid Composition in the Muscle of Growing Carp[26]

Amino acids	Fertilized eggs (-)*	Fry (0.3g)*	Young fish-I (3.6g)*	Young fish-II (52.5g)*	Grown fish (870g)*
	(mg%)	(mg%)	(mg%)	(mg%)	(mg%)
Aspartic acid	(±)	0.1	1.3	1.9	1.7
Threonine	0.1	(±)	23.1	15.1	16.3
Serine	1.4	5.4	6.8	16.6	6.4
Glutamic acid	0.7	2.4	11.4	10.9	4.5
Proline	0.1	2.3	15.6	26.1	22.0
Glycine	0.2	4.8	60.1	96.9	62.9
Alanine	0.3	4.5	17.0	37.8	21.5
Cystine	0.0	(±)	4.0	2.5	2.2
Valine	0.3	2.1	4.5	3.0	2.6
Methionine	0.1	0.9	2.5	2.3	2.2
Isoleucine	0.1	1.7	3.4	2.1	2.0
Leucine	0.3	2.7	5.8	4.6	3.7
Tyrosine	0.1	1.4	3.4	2.1	13.1
Phenylalanine	0.3	2.0	3.7	2.7	0.3
Tryptophan	0.0	(±)	(±)	(±)	(±)
Lysine	0.2	21.9	43.3	35.8	75.7
Histidine	(±)	23.0	313.4	105.5	126.6
Arginine	33.0	16.2	13.6	6.7	17.3
Total	46.7	91.4	537.9	432.6	381.0

* Average weight; (±) Trace

Table 7. Free Histidine Concentration in the Tissue of Sockeye Salmon in Migration for Spawning[32]

Tissue	Sex	Lummi Island 64 Km*	Lillooet 400 Km*	Forfar Creek 1150 Km*
		(mg%)	(mg%)	(mg%)
Heart	♂	81.0±11.5	87.3±9.6	81.8±38.8
	♂	83.2±15.2	91.0±8.9	79.9± 5.9
Alimentary canal	♂	138.4±13.3	48.1±12.2	30.3±10.7
	♂	174.6±39.2	40.0± 7.0	20.3± 3.3
Spleen	♂	24.4± 3.7	24.4± 6.7	27.0± 5.9
	♂	30.0± 5.9	27.0± 4.8	20.0± 5.9
Liver	♂	21.1± 3.0	20.7± 1.8	16.3± 3.0
	♂	23.3± 3.7	12.2± 0.4	16.6± 0.7
Kidney	♂	37.0± 1.5	47.4± 5.5	46.6± 3.0
	♂	51.4± 6.7	47.0± 3.3	50.7±10.4
Blood	♂	--	12.6± 1.5	12.9± 5.5
	♀	--	15.5± 5.2	9.2± 0.7
Reproductive organ	♂	19.6± 2.6	15.2± 2.2	6.7± 3.3
	♂	6.7± 2.6	5.2±1.1	3.7± 0.7
Muscle	♂	40.0± 4.8	7.8± 0.7	4.8± 0.4
	♂	55.9± 3.0	10.0± 1.5	11.5± 0.4
Head-Skin-Bone-Tail	♂	22.6± 1.1	12.9± 1.1	9.2± 0.7
	♂	22.9± 3.0	10.7± 3.0	9.2± 0.7

* Distance from the mouth of the river

Table 8. Free Histidine Content in the Tissue of Sockeye Salmon in Migration for Spawning[32]

Tissue	Sex	Lummi Island 64 Km*	Lillooet 400 Km*	Forfar Creek 1150 Km*
		(mg/Tissue)	(mg/Tissue)	(mg/Tissue)
Heart	♂	4.9	5.1	4.7
	♀	4.2	4.5	3.7
Alimentary canal	♂	125.2	14.5	5.0
	♀	136.8	10.5	2.6
Spleen	♂	0.7	0.5	1.0
	♀	0.6	0.4	0.4
Liver	♂	6.7	5.3	6.1
	♀	9.4	5.4	6.7
Kidney	♂	9.6	11.0	13.1
	♀	11.1	9.4	10.0
Reproductive organ	♂	11.1	12.4	5.7
	♀	5.3	7.1	11.0
Muscle	♂	584.6	99.9	62.9
	♀	747.4	114.7	107.3
Head-Skin-Bone-Tail	♂	199.1	112.4	97.2
	♀	179.8	81.8	76.7
Total	♂	941.9	261.1	195.7
	♀	1094.6	233.8	218.4

* Distance from the mouth of the river

Table 9. Metabolic Activity of Histidine in the Tissue of Various Fish

Fish species	Weight (g)	Tissue	Whole tissue weight (g)	Metabolic activity (μmole/hr/g)
Mackerel *Scomber japonicus*	350	Liver	3.2	15.2
		Kidney	1.0	9.6
		Dark meat	14.1	0.6
		Ordinary meat	170	0.1
Yellowtail *Seriola quinqueradiata*	800	Liver	14.5	11.0
		Kidney	4.8	2.7
		Dark meat	48.8	0.8
		Ordinary meat	315	0
Grey Rock cod *Sebastes inermis*	290	Liver	9.5	14.2
		Kidney	2.7	0
		Muscle	180	0
Flathead sole *Hippoglossoides dubius*	300	Liver	5.5	5.3
		Kidney	0.7	0
		Muscle	105	0
Carp *Cyprinus Carpio*	240	Liver	8.5	16.7
		Kidney	1.6	3.3
		Dark meat	--	--
		Ordinary meat	--	0

The values are based on an average of five fish.

Table 10. Occurrence of Metabolic Activity of Histidine in the Muscles and Livers of Various Fish[38]

Fish species*	Muscle (Ordinary meat)	Liver
Mackerel Scomber japonicus	+	+
Sardine Sardinops melanosticta	+	+
Tuna Thynnus thynnus	-	+
Skipjack Auxis thazard	-	+
Yellowtail Seriola quinqueradiata	-	+
Horse mackerel Trachurus japonicus	-	+
Carp Cyprinus carpio	-	+
Surf perch Ditrema temmincki	-	+
Flathead fish Hippoglossoides dubius	-	+
Gray Rock cod Sebastes inermis	-	+
Sea bass Lateolabrax japonicus	-	+
Red sea bream Chrysophrys major	-	+
Spotted shark Mustelus manazo	-	+

*Red-meat fish; + Activity detected; - Activity not detected

Table 11. Chemical Quantitative Activity of Histidine

Reaction time (hr)	Amount of histidine dissociation (μmol)	Amount of urocanic acid formation (μmol)	Amount of ammonia formation (μmol)
1.5	0.6	0.6	--
3	1.3	1.3	1.6
5	1.9	1.9	--
7	3.0	3.0	3.1

Table 12. Dissociation of Urocanic Acid by Enzyme Samples Extracted from the Muscles and Livers of Mackerel and Carp (Dialyzed Sample, 70% Saturated Ammonium Sulfate Precipitation Sample and Crude Enzyme Sample)

Tissue	Sample	Protein amount	pH	Amount of urocanic acid dissociation (μmol)		
				1 hr	2 hr	3 hr
Mackerel Muscle Liver	Dialysis	7.6	7.3	--	0	0
			9.2	--	0	0
	Precipitation	9.5	7.3	--	0	0
			9.2	--	0	0
	Crude enzyme	9.5	7.3	28	59	117
			9.2	--	16	32
Carp Muscle Liver	Dialysis	7.5	7.3	--	0	0
			9.2	--	0	0
	Precipitation	9.0	7.3	--	0	0
			9.2	--	0	0
	Crude enzyme	9.1	7.3	350	--	--
			9.2	--	--	--

Table 13. Formation of Metabolic Substances from ^{14}C-Histidine(U) in the Extractive/from the Liver of Carp[40] Supernate

Reaction time	0 minute		25 minutes		120 minutes	
	(cpm)	(%)*	(cpm)	(%)*	(cpm)	(%)*
Histidine	15550	97.5	8240	51.6	3830	24.0
Urocanic acid	0	0	560	3.5	3170	19.9
Formiminoglutamic acid	0	0	1580	9.9	6440	40.4
Glutamic acid	0	0	510	3.2	1780	11.2
Total	15550	97.5	10890	68.2	15220	95.5

(%)*: (cpm of each compound/total cpm)x100

Table 14. Formation of Metabolic Substances from ^{14}C-Histidine(U) in the Extractive Supernate from the Liver of Mackerel[40]

Reaction time	0 minute		25 minutes		120 minutes	
	(cpm)	(%)*	(cpm)	(%)*	(cpm)	(%)*
Histidine	17960	97.9	9810	52.9	8650	46.7
Urocanic acid	0	0	2950	15.9	8320	44.9
Formiminoglutamic acid	0	0	170	0.9	20	0.1
Glutamic acid	0	0	0	0	0	0
Total	17960	97.9	12930	69.3	16990	91.7

(%)*: (cpm of each compound/total cpm)x100

Table 15. Effect of Various Chemical Substances on the Activity of Histidine Deaminase Obtained from the Muscle of Mackerel[65]

Chemical substance	Concentration (M)	Activity ratio (%)
--	--	100
Cu^{++}	1×10^{-3}	86
Zn^{++}	1×10^{-4}	100
Cd^{++}	1×10^{-3}	1
Co^{++}	1×10^{-4}	89
Mn^{++}	9×10^{-4}	72
EDTA	1×10^{-3}	100
Cysteine	1×10^{-3}	66
Cysteine	1×10^{-5}	100
GSH	1×10^{-3}	94
GSH	1×10^{-4}	96
Thioglycol	1×10^{-3}	90
Iodacetamid	1×10^{-3}	90
Iodacetamid	1×10^{-4}	95
pCMB	1×10^{-4}	13
pCMB+Cysteine	$1\times10^{-4}+1\times10^{-3}$	66

Table 16. GSH Effect on the Activity of Histidine Deaminase Obtained from Bacteria Pseudomonas Fluorescens And the Liver and Muscle of Mackerel[73]

Pseudomonas fluorescens		Mackerel Liver		Mackerel Muscle	
GSH (M)	Activity ratio(%)	GSH (M)	Activity ratio(%)	GSH (M)	Activity ratio(%)
--	100	--	100	--	100
1.0×10^{-5}	186	2.3×10^{-5}	128	1.0×10^{-5}	100
2.0×10^{-4}	557	2.3×10^{-4}	223	1.0×10^{-4}	96
1.1×10^{-3}	915	2.3×10^{-3}	226	1.0×10^{-3}	90
2.6×10^{-3}	2500			5.0×10^{-3}	77

Table 17. EDTA Effect on the Activity of Histidine Deaminase Obtained from Bacteria Pseudomonas Fluorescens and the Liver and Muscle of Mackerel[73]

EDTA (M)	Relative activity (%)		
	Pseudomonas fluorescens	Mackerel liver	Mackerel muscle
--	100	100	100
1.0×10^{-4}	79	--	--
1.6×10^{-4}	--	53	97
5.0×10^{-4}	49	--	--
1.0×10^{-3}	--	49	100
1.6×10^{-3}	--	--	--
2.5×10^{-3}	--	--	97

Table 18. Km Values of Histidine Deaminase Obtained from Bacteria Pseudomonas Fluorescens and the Liver and Muscle of Mackerel[73]

Origin	Km (M) in presence of GSH*	Km (M) in absence of GSH
Pseudomonas fluorescens	5.6×10^{-3}	2.7×10^{-2}
Mackerel liver	2.5×10^{-4}	7.8×10^{-4}
Mackerel muscle	6.3×10^{-5}	3.3×10^{-5}

*1.0×10^{-3}M

Table 19. Molecular Weight of Histidine Deaminase Obtained from Bacteria Pseudomonas Fluorescens and the Liver and Muscle of Mackerel[73]

Origin	Molecular Weight
Pseudomonas fluorescens	193,000
Mackerel liver	198,000
Mackerel muscle	208,000

Table 20. Optimum pH, Km Value and Molecular Weight of Histidine Deaminase Obtained from Various Bacteria and the Livers of Animals[69]

Origin	Optimum pH	Km (M)	Molecular weight
Bacillus subtilis	8.5~9.1	2.8×10^{-3}	220,000
Pseudomonas aeruginosa	---	2×10^{-3}	210,000
Pseudomonas fluorescens	8.8	2.3×10^{-3}	218,000
Toad liver	9.1	3.6×10^{-3}	204,000
Rat liver	8.8~9.2	2.8×10^{-3}	226,000
Cat liver	8.5~9.0	1.2×10^{-3}	---
Human liver	9.1	2.1×10^{-3}	243,000

Table 21. Composition and Histidine Content in Each Feed[79]

Feed	Vitamin salts mixture (%)	Casein (%)	Potato starch (%)	Histidine content (%)
A High protein feed	15	80	5	2.5
B Low protein feed	15	5	80	0.1
C* Histidine-added-feed	15	5	80	2.8

* 2.7% histidine is added to the low protein feed

Table 22. Effect of Low Protein Feed and Histidine-Added-Feed on the Activities of Histidine Deaminase and Urocanase in the Liver of Carp[79]

Treatment	No. of fish	Weight (g)	Liver weight (g)	Histidine deaminase (μmole/hr/g)	Urocanase (μmole/hr/g)
A High protein feed	7	34.9±5.1	1.7±0.1	19.2±1.9	21.8±6.4
B Low protein feed	6	35.2±4.2	3.5±0.4	2.0±0.2	4.8±2.1
C Histidine-added-feed	6	34.5±3.8	2.7±0.3	4.6±0.6	12.9±4.9

Histidine deaminase A vs B, $P<0.01$; A vs C, $P<0.01$; B vs C, $P<0.01$
Urocanase A vs B, $P<0.01$; A vs C, $P<0.05$; B vs C, $P<0.05$

Table 23. Activities of Histidine Deaminase and Urocanase in the Livers of Rats That Were Given Feeds with Varying Protein Content[81]

Feed	No. of animals	Weight (g)	Liver weight (g)	Histidine deaminase (mμmol/min/mg protein)	Urocanase (mμmol/min/mg protein)
Feed with 6% casein	6	20.5±1.8	1.08±0.16	N	1.46±0.24
Feed with 18% casein	10	84.1±4.1	2.71±0.17	2.18±0.14	2.34±0.23
Feed with 40% casein	12	110.2±5.1	3.60±0.14	4.26±0.31	2.88±0.17

N: No activity is detected

Table 24. Effect of Casein Water and Glucose on the Activity of Histidine Deaminase in the Livers of Rats[81]

Treatment	No. of animals	Liver weight	Histidine deaminase activity
		(g)	(unit/g)
Contrast*	6	2.47±0.06	131.7±26.9
Casein water (1g) administered	5	2.91±0.01	377.0±82.4
Casein water (1g)+ glucose (1g) administered	5	3.87±0.18	144.3±21.6

*Glucose (1g) administered

Table 25. Effect of Casein Water and Histidine Administration on the Activity of Histidine Deaminase in the Liver of Rat[81]

Treatment	No. of animals	Liver weight	Histidine deaminase activity
(Female)		(g)	(unit/g)
Contrast	4	2.55±0.10	186.3± 6.0
Casein water(1g) administered	4	3.60±0.33	369.3±29.0
Casein water(50mg)+histidine (200mg) administered	4	3.47±0.13	320.3±27.3
(Male)			
Contrast	3	3.54±0.64	80.6±15.2
Casein water(1g) administered	3	3.61±0.20	212.0±28.2
Casein water(50mg)+histidine(200mg) administered	4	3.79±0.17	393.5±85.7
Histidine(200mg) only administered	4	3.39±0.36	111.3±32.3

Table 26. Effect of Histidine-Added-Feed on the Activities of Histidine Deaminase and Urocanase in the Liver of Rat[82]

Feed and period			Average liver weight (g)	Histidine deaminase		Urocanase	
Casein feed (%)	Histidine feed (%)	Days fed (day)		(μmol 10^{-1}/min/g)	(μmol 10^{-1}/min/whole liver tissue)	(μmol 10^{-1}/min/g)	(μmol 10^{-1}/min/whole liver tissue)
80		14	10.93 (9.17~12.23)	5.7 ±0.4	62 ±2	5.8±012	63 ±2
12	4	5	12.61 (12.21~13.00)	1.53±0.02	19.3±0.4	3.6±0.1	46 ±2
12	4	14	13.03 (10.47~16.08)	0.89±0.04	12 ±1	3.3±0.2	43 ±1
12		5	9.97 (8.43~12.09)	2.5 ±0.1	25.0±0.9	4.0±0.2	39.0±0.8
12		14	11.17 (10.28~12.05)	1.74±0.07	19.4±0.9	2.9±0.1	32 ±1

Table 27. Effect of Non-Feeding on the Activities of Histidine Deaminase and Urocanase in the Liver of Carp[79]

Treatment	No. fish	Weight (g)	Liver weight (g)	Histidine deaminase		Urocanase	
				(μmol/hr/g)*	(μmol/hr/whole liver tissue)**	(μmol/hr/g)*	(μmol/hr/whole liver tissue)**
D High protein feed	6	37.2±0.6	1.8±0.1	20.1±2.1	36.2±6.0	21.5±1.7	38.7±5.2
E Non-feeding	6	36.9±4.7	1.3±0.1	27.8±4.1	36.1±8.2	24.4±2.4	31.7±5.6

Histidine deaminase D vs E, * $P<0.01$; ** $P<0.05$
Urocanase D vs E, * $P<0.05$; ** $P<0.05$

Table 28. Effect of Non-Feeding on the Activities of Histidine Deaminase and Urocanase in the Liver of Rat[82]

Treatment	Histidine deaminase		Urocanase	
	(μmol 10^{-1}/min/g)	(μmol 10^{-1}/min/whole liver tissue)	(μmol 10^{-1}/min/g)	(μmol 10^{-1}/min/whole liver tissue)
Feed with 18% casein	1.0±0.1	8 ±1	2.3±0.2	18.2±0.4
Non-feeding 24 hrs	1.6±0.1	8.8±0.6	2.6±0.1	14.2±0.8
Non-feeding 48 hrs	1.9±0.1	10.2±0.6	3.6±0.1	18.7±0.9

Table 29. Effect of Low Protein Feed and Non-Feeding on Free Lysine and Histidine Content in the Muscle of Carp

Treatment	Lysine (mg%)	Histidine (mg%)
High protein feed administered	111.2	156.1
Low protein feed administered	1.3	139.9
Non-feeding	29.3	152.0

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