

## CHEMICALS

# HISTORY & DEVELOPMENT OF FLAVOR NUCLEOTIDES

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■ MSG AND IMP BEGINNING AS FLAVOR ENHANCERS. Since ancient times both sea tangle, *Laminaria* sp., and dried bonito have been generally used for seasoning Japanese meals, because they can be well preserved for a long time and their extracts have characteristic flavors.

• See Cover

• "FLAVOR THROUGH NUCLEOTIDES"

Ikeda (1908, 1909) reported that the main flavor component of sea tangle extract was MSG (the abbreviations used herein are explained in a note following the article). It was possible to start industrial production of MSG as a chemical seasoning almost simultaneously because it had been known that L-glutamate was present in protein hydrolysate. At present, MSG is produced not only by protein hydrolysis but also by direct fermentation or chemical synthesis, and it is used as an excellent flavor enhancer not only in Japan but also in all the world.

On the other hand, Kodama (1913) reported that the principal flavor component of dried bonito was the histidine salt of IMP. However, IMP has not been produced as a chemical seasoning on a large scale until quite recently, because both the biochemical background and the flavor property of IMP are more complicated than those of MSG.

For example, there are two types of glutamate—L- and D-isomers—whereas there are three types of IMP: 2', 3', and 5'-isomers. L-Glutamate is a natural component of protein, but D-glutamate is not. Flavoring action is recognized only in L-glutamate, and not in D-glutamate. The relationship between isomerism and flavoring action of IMP had never been reported, nor had experiments been made that would allow one to define the interaction between IMP and histidine. Furthermore, the biochemistry of nucleic acid is a more modern field than that of protein or carbohydrate, so the biochemical position of IMP was clarified much later than that of L-glutamic acid.

### Discovery of Flavor of 5'-IMP and 5'-GMP, and their Production from RNA.

In 1951, the authors started to study both the enzymic degradation of nucleic acid by microorganisms and the taste of the degradation products. Several *Aspergillus* enzymes involved in the RNA degradation were found, and the conditions for their activities were clarified [Kuninaka (1954, 1955, 1956, 1957, 1959)]. Using the enzyme system of *Aspergillus oryzae*, 3'-IMP could be obtained from RNA. However, the 3'-IMP obtained had little or no flavor. The mixture of 2'- and 3'-IMP derived from alkaline hydrolysis of RNA

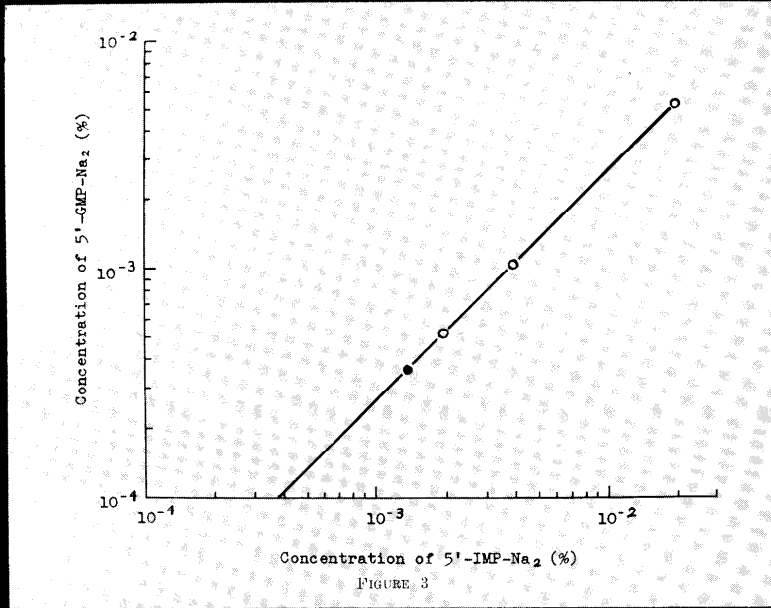
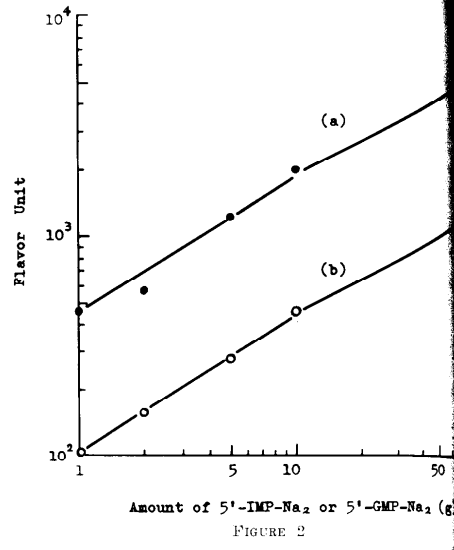
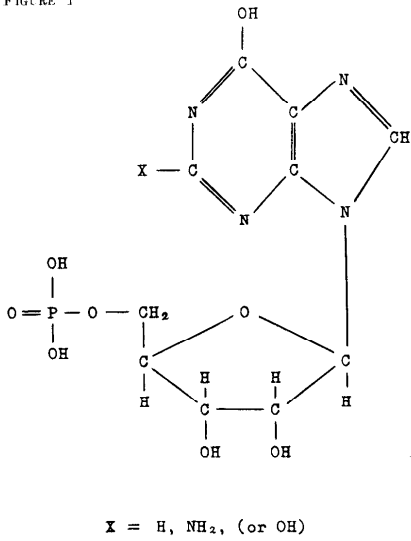
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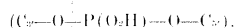
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was also found not to be flavorful. On the other hand, 5'-IMP free from histidine, which was prepared from muscle of rabbit or bonito, was confirmed to have a specific flavor, especially together with MSG. Thus, it was demonstrated that, among three isomers of IMP, only 5'-IMP was flavorful.

The authors therefore undertook to prepare 5'-IMP from RNA. Recently, it has become evident that the RNA is a polynucleotide in which individual nucleoside residues are joined, one to the other, by phosphodiester linkages between the 3'- and 5'-positions. When RNA is degraded, cleavage must be considered to occur at either the 3'-phosphodiester linkages



or the 5'-phosphodiester linkages



Most of RNA-degrading enzymes split 3'-phosphodiester linkages in RNA, giving rise to nucleoside-2',3'-cyclic phosphates or 3'-nucleotides. Alkali splits the same linkages, giving rise to nucleoside-2',3'-cyclic phosphates, which are further cleaved to form 2'- and 3'-nucleotides.

Only so-called unspecific phosphodiesterases of snake venom and intestinal mucosa had been demonstrated

to split 5'-phosphodiester linkages in RNA, giving rise to 5'-nucleotides. However, it is very difficult to obtain large amounts of these enzymes free of phosphomonoesterase activity. Thus, chemical or enzymic production of 5'-nucleotides from RNA was very difficult, and economical industrial production thereof was quite impossible.

Kuinaka *et al.* (1957a,c) demonstrated that 5'-phosphodiesterases, which split 5'-phosphodiester linkages in RNA, giving rise to 5'-nucleotides, were formed by several microorganisms such as *Penicillium citrinum*. Using the microbial 5'-phosphodiesterase, which can be obtained in large amounts, 5'-AMP, 5'-GMP, 5'-CMP, and 5'-UMP could be produced first industrially from yeast RNA. 5'-IMP could be easily obtained by deminimizing 5'-AMP. Omura *et al.* (1958) also demonstrated similar enzymes independently in several microorganisms such as *Streptomyces*.

Thus, 5'-IMP has been produced microbiologically and used as a new seasoning. Furthermore, establishment of this RNA degradation process also led to recognition of the flavor of 5'-GMP, one of the RNA degradation products. For the first time, 5'-GMP was found to have stronger flavor than 5'-IMP (Sakaguchi *et al.*, 1958; Kuinaka, 1960).

Japanese Food Additive Petitions for 5'-IMP·Na<sub>2</sub> and 5'-GMP·Na<sub>2</sub> were respectively approved on Sep-

tember 10th, 1960, and on June 1st, 1961. The specifications of the Japanese Official Standardizations are summarized in Tables 1 and 2. Note that 5'-IMP·Na<sub>2</sub> usually contains 7.5 molecules of crystallization water, and 5'-GMP·Na<sub>2</sub> does not contain crystallization water. Used in the experiments reported here were standardized 5'-IMP·Na<sub>2</sub> and 5'-GMP·Na<sub>2</sub>.

#### Relations between Flavor and Chemical Structure of RNA Derivatives

The tastes of various RNA derivatives were summarized by Kuinaka (1960) as follows: Purine and pyrimidine bases, nucleosides, and polynucleotides had little recognizable taste. On the other hand, mononucleotides had good taste. The taste of 5'-nucleotides was much stronger than that of 2'- or 3'-nucleotides. Especially, 6-hydroxypurine ribonucleoside 5'-monophosphates (5'-GMP, 5'-IMP, and 5'-XMP) had very agreeable good tastes (5'-GMP > 5'-IMP > 5'-XMP). Furthermore, the equimolar mixture of inorganic phosphate and inosine (or guanosine) or the equimolar mixture of ribose 5-phosphate and hypoxanthine (or guanine) had no flavor. Thus, both riboside and phosphate ester linkages were considered to be essential for flavoring the action.

From these findings, the chemical structure necessary for the flavoring action may be summarized as follows:

Fig. 1. General constitutional formula for the flavor nucleotides.

Fig. 2. Difference of flavor unit of nucleotide-MSG-composition and that of MSG.

- (a): {Flavor unit of composition of each amount of 5'-GMP·Na<sub>2</sub> and 100 g of MSG} - {Flavor unit of 100 g of MSG}  
 (b): {Flavor unit of composition of each amount of 5'-IMP·Na<sub>2</sub> and 100 g of MSG} - {Flavor unit of 100 g of MSG}

1 Flavor unit was defined as flavor strength of 1 g of MSG.

The following relationship is suggested to exist between (a) and (b):

$$\log(a) - \log(b) = \log \frac{(a)}{(b)} = \log(3.57 \text{ to } 4.68)$$

$$(a) = (3.57 \sim 4.68)(b)$$

Fig. 3. Comparison of flavor activity of 5'-GMP·Na<sub>2</sub> and that of 5'-IMP·Na<sub>2</sub> in their synergistic action with MSG.

[5'-GMP·Na<sub>2</sub>] corresponding to [5'-IMP·Na<sub>2</sub>] were determined in the MSG-sodium chloride solution by means of paired comparison tests.  
 [5'-GMP·Na<sub>2</sub>]: concentrations of 5'-GMP·Na<sub>2</sub>  
 [5'-IMP·Na<sub>2</sub>]: concentrations of 5'-IMP·Na<sub>2</sub>  
 MSG concentration: 0.2% (○) or 0.07% (●)  
 Sodium chloride concentration: 1.2%

The following relationship was suggested to exist between [5'-GMP·Na<sub>2</sub>] and [5'-IMP·Na<sub>2</sub>]:

$$\log [5'-GMP \cdot Na_2] = \log \frac{0.00105}{0.0040} + \log [5'-IMP \cdot Na_2]$$

$$3.8 [5'-GMP \cdot Na_2] = [5'-IMP \cdot Na_2]$$

Therefore, flavor activity of 5'-GMP·Na<sub>2</sub> is 3.8 times as much as that of 5'-IMP·Na<sub>2</sub> in the synergistic action with MSG.

Table 1. Japanese official standardization.

Disodium 5'-inosinate	
Colorless or white crystals that have a specific taste.	
Nitrogen (dried) <sup>a</sup>	14.0-14.6%
Phosphorus (dried) <sup>a</sup>	7.6- 8.2%
Max. U.V. absorption (in 0.01N HCl)	250 ± 2 mg
Ribose (orcinol reaction)	positive
Organic phosphate	positive
Paper chromatography <sup>b</sup>	U.V. absorbing one spot
Sodium Solution (0.5 g→10 ml)	positive colorless, little or no turbidity
pH (1 g→20 ml)	7.0-8.5
Ammonium	negative
Arsenic	Max. 2 ppm
Heavy metal	Max. 20 ppm
Amino acid	negative
Loss on drying <sup>a</sup>	Max. 26.5%

<sup>a</sup> 120°C for 4 hr.

<sup>b</sup> A mixture of saturated ammonium sulfate, tert-butanol, and 0.025N ammonia solution (160:3:40) is employed as the solvent.

Table 2. Japanese official standardization.

Disodium 5'-guanylate	
Colorless or white crystals or powder that has a specific taste.	
Nitrogen (dried) <sup>a</sup>	16.2-17.2%
Phosphorus (dried) <sup>a</sup>	7.3- 7.9%
Max. U.V. absorption (in 0.01N HCl)	256.12 mg
Specific U.V. absorption (in 0.01N HCl)	270-280 mg
Ribose (orcinol reaction)	positive
Organic phosphate	positive
Paper chromatography <sup>b</sup>	U.V. absorbing one spot
Sodium Solution (0.1 g→10 ml)	positive colorless and almost clear
pH (1 g→20 ml)	7.0-8.5
Ammonium	negative
Arsenic	Max. 2 ppm
Heavy metal	Max. 20 ppm
Amino acid	negative
Loss on drying <sup>a</sup>	Max. 8%

<sup>a</sup> 120°C for 4 hr.

<sup>b</sup> A mixture of saturated ammonium sulfate, tert-butanol, and 0.025N ammonia solution (160:3:40) is employed as the solvent.

1) The base moiety is to be a purine nucleus containing a hydroxy group in the 6-position. (An amino group in the 6-position reduces the flavoring action. The influence of the kind of the group in the position 2 on the taste is not so much. The tastes of pyrimidine nucleotides are inferior to those of purine nucleotides.)

2) The 5'-position of the ribose moiety is to be esterified with phosphoric acid.

The general constitutional formula for the flavor nucleotides may be shown in Fig. 1. In brief, both the 6-hydroxypurine moiety and ribose-5-phosphate moiety are essential to flavoring action of RNA derivatives.<sup>2</sup>

It is very interesting that the conditions necessary for the structure of nucleotides that are substrates of *Aspergillus ribosidase*<sup>3</sup> are the same as those for the flavor nucleotides.

It may be suggested that there is a kind of analogy between the specific relation of a flavor nucleotide to the human gustatory bud and that of a substrate for the enzyme. Therefore, it seems possible to select unknown flavor substances by means of enzyme action.

By the way, mosquitoes were demonstrated by Hosoi (1961) to be specifically fond of 5'-AMP or its derivatives but not fond of the other nucleotides, nucleosides, bases, ribose,

<sup>2</sup> Lately 5'-deoxy inosinate and 5'-deoxy guanylate were also reported to have flavorful taste (Nakao *et al.*, 1960). Furthermore, 2' and 3'-hydroxy groups seem not to be essential for the flavoring action (Honjo *et al.*, 1963).

<sup>3</sup> According to the prevailing theory, the phosphoric acid group in the nucleotides has to be removed before the ribosidic linkage can be resolved by enzymes. Kuninaka (1956, 1957, 1959) furnished the first evidence for the conclusion that hydrolytic enzyme (ribosidase) for the cleavage of the bond between ribose-5-phosphate and hypoxanthine or guanine does exist in *Aspergillus oryzae*. This enzyme cleaves specifically the ribosidic linkages of 6-hydroxypurine ribonucleosides (inosine and guanosine) or their 5'-phosphates, 5'-AMP, 5'-CMP, 5'-UMP, adenosine, cytidine, uridine, and various 3' or 2'-mononucleotides are not cleaved by the enzyme. No evidence has been found for the requirement of ATP or inorganic ortho or pyrophosphate. Hurwitz *et al.* (1957) also independently demonstrated the enzymic cleavage of ribosidic linkage in 5'-AMP, using *Azotobacter vinelandii*. Both *Aspergillus* and *Azotobacter* ribosidases are nonphospholytic hydrolases, and act on 5'-nucleotides but do not act on 2' or 3'-isomers. However, these enzymes are distinguishable from each other in regard to detailed substrate specificity and relation to ATP. While 5'-AMP-ribosidase of *Azotobacter* requires ATP, *Aspergillus* ribosidase does not.

and amino acids such as glycine, glutamic acid, cysteine, arginine, tyrosine and histidine. Thus, the human and mosquito palates are similar to each other in the selectivity of 5'-nucleotides. However, there are differences between their detailed specificities: the human palate is an *Aspergillus* ribosidase type, whereas the mosquito palate is an *Azotobacter* ribosidase type.

The taste of 6-hydroxypurine ribonucleoside 5'-monophosphates (5'-IMP, 5'-GMP, and 5'-XMP) was milder and softer than that of MSG, while the aftertaste of the former was much more remarkable than that of the latter. 5'-GMP has the strongest taste in these three 5'-nucleotides, and 5'-XMP has the weakest taste. The threshold level for recognition of these 5'-nucleotides was suggested to be about 0.01%. Toi *et al.* (1960) and Wagner *et al.* (1962) later respectively reported that the threshold level for recognition of 5'-AMP·Na<sub>2</sub> was 0.025% and 0.02%. Fujita *et al.* (1961) reported that the threshold levels for recognition of 5'-IMP·Na<sub>2</sub> and 5'-GMP·Na<sub>2</sub> were respectively 0.012% and 0.0035%.

#### Synergistic Action between MSG and 5'-Nucleotides

There are many kinds of interaction in taste among various components of foods or beverages. For example, an additive effect is recognized when two or more different acids or two or more different sugars are mixed; a contrastive effect is recognized when a small amount of salt is added to sugar; and a harmonization effect was recognized when MSG was added to saccharin (Maeda *et al.*, 1956) or salt.

The 5'-nucleotides were considered to have a same kind of taste, and an additive property was recognized between the taste of 5'-GMP and that of 5'-IMP. Succinate and basic amino acids, such as histidine, lysine, and arginine, were not recognized to enhance significantly the flavoring taste of 5'-GMP or 5'-IMP. On the contrary, MSG was recognized to enhance remarkably the flavoring taste of 5'-GMP or 5'-IMP, and vice versa.

For example, addition of 5'-IMP to 5'-GMP to MSG remarkably strengthened the taste of MSG, as follows: MSG and 5'-IMP·Na<sub>2</sub> or 5'-GMP·Na<sub>2</sub> were mixed in various ratios. Each mixture was dissolved in 1.2% sodium chloride solution at various concentrations. The tastes of the resulting solutions were compared with that of 1.2% sodium chloride solution

Table 3. Comparison of MSG-nucleotide-composition and MSG in flavor.

Ratio of components in composition	Concentration of the composition solution whose flavor strength corresponds to that of 0.3% MSG solution
MSG: { 5'-IMP·Na <sub>2</sub> } { 5'-GMP·Na <sub>2</sub> }	0.3%
1: 0	0.3%
1: 1	0.940% (0.010%)
10: 1	0.960% (0.016%)
20: 1	0.986% (0.024%)
50: 1	0.12% (0.047%)
100: 1	0.15% (0.055%)

Each solution contained 1.2% of sodium chloride.

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aining 0.3% MSG by *paired comparison tests*, respectively. Then the concentration of each mixture solution, that had a taste strength corresponding to that of 1.2% sodium chloride solution containing 0.3% MSG, was determined as shown in Table 3. The results show that a small amount of 5'-IMP or 5'-GMP can synergistically increase the strength of the taste of MSG.

As shown in Fig. 2, the synergistic action between MSG and 5'-GMP was about four times as much as that between MSG and 5'-IMP. Another experiment also indicated that 5'-GMP·Na<sub>2</sub> was as effective as about 3.8 times as much 5'-IMP·Na<sub>2</sub> when dissolved in 0.2% or 0.07% of MSG solution (Fig. 3).

Although there was a small harmonization effect between the taste of 5'-GMP or 5'-IMP and that of histidine or succinate, the synergistic action between MSG and 5'-nucleotides was much more remarkable than such harmonization effect. Furthermore, this synergistic action was recognized both in the presence and in the absence of the other components. Such remarkable synergistic action as mentioned above had been never conceived, at least in the field of taste chemistry.

By the way, the threshold level for recognition of 5'-IMP·Na<sub>2</sub> or 5'-GMP·Na<sub>2</sub> in 0.1% MSG solution was reported to be 1/120 or 1/117 that in water (Fujita *et al.*, 1961). Independently, the threshold levels for recognition of 5'-IMP·Na<sub>2</sub> in 0.1% sodium aspartate solution, MSG solution, sodium β-hydroxyglutamate solution, sodium α-amino-adipinate solution, and sodium homocysteinatate solution were also reported to be 1/10, about 1/100, 1/250, 1/10, and 1/100 of that in water, respectively, and the threshold levels for recognition of sodium aspartate, MSG, sodium β-oxoglutarate and sodium α-aminoadipinate in 0.01% 5'-IMP·Na<sub>2</sub> solution were confirmed to be 1/16, 1/15, 1/15, and

1/100 of those in water, respectively, too (Toi *et al.*, 1960).

The specific synergistic action indicated above is to be the basis for application of 5'-IMP, 5'-GMP, or their mixtures as new chemical seasonings, because many foods and beverages contain a rather large amount of MSG in themselves whereas 5'-nucleotides are rather unstable intermediates in living tissue so that the distribution of 5'-nucleotides, especially 5'-GMP, in foods and beverages is much more limited than the distribution of MSG. Therefore, addition of 5'-IMP, 5'-GMP, or mixtures of them to those foods or beverages remarkably strengthens and improves the taste of those foods or beverages according to the synergistic action between 5'-nucleotides added and MSG present there originally. Furthermore, the mixture of MSG and 5'-nucleotide(s) is a very effective chemical seasoning for all kinds of dishes.

A composition of MSG with 5'-IMP·Na<sub>2</sub> was first sold in Japan as a new chemical seasoning in 1960. At present 5'-IMP·Na<sub>2</sub>, 5'-GMP·Na<sub>2</sub>, and mixtures of them are used mainly in food processing, whereas compositions of MSG with 5'-nucleotide(s) are mainly used in home life. The following ratios are generally being used: MSG:5'-IMP·Na<sub>2</sub> = 88:12, 92:8, or 96:4. MSG:5'-IMP·Na<sub>2</sub>:5'-GMP·Na<sub>2</sub> = 95:2.5:2.5.

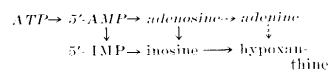
At any rate, it is interesting from the viewpoint of biochemistry that there is a synergistic action in taste between MSG derived from protein and 5'-nucleotides derived from nucleic acid, because the interaction between protein and nucleic acid is well known as one of the most important biochemical reactions.

#### Production of 5'-Nucleotides

There are several methods for production of 5'-nucleotides.

**Extraction from animal tissues.** Mammalian muscle has been well

known to contain 5'-IMP. The IMP in fish muscle was also confirmed to be 5'-isomer, which might be derived from ATP.



Several methods have been developed in Japan to get 5'-IMP from marine-animal muscle economically.

**Separation of 5'-IMP from fish muscle.** According to the author's experiments, fresh muscle of marinefish, such as mackerel-pike and sardine, contained a large amount of 5'-IMP and a very small amount of 5'-AMP. Probably the reactions forming 5'-IMP from ATP are carried out in a fishing-boat on the way to port. During the time muscle was stored at room temperature, 5'-IMP was observed to be degraded enzymically to inosine or hypoxanthine. To steam fish muscle was an effective way of stopping the enzymic degradation of 5'-IMP. Thus the intact waste juice or stick water liberated from fish muscle by steaming, the first general process of canning, was a good source. The juice was equal to about 20% by weight of the intact fish and contained about 0.1% of 5'-IMP.

The impurities that obstruct crystallization of the nucleotide could be removed from the waste juice by three steps (Kunimaka *et al.*, 1957b, 1960): 1) depolymerization of protein by bacterial protease; 2) exclusion of cationic impurities by treatment with cation exchange resin; and 3) precipitation of inorganic phosphate by addition of barium or calcium ion. After these treatments, 5'-IMP was directly crystallized as barium or calcium salt. To separate IMP from fish muscle extracts, electrolytic dialysis was also effective (Yoshida *et al.*, 1953).

**Deamination of 5'-AMP of cuttlefish muscle.** Saito (1960) reported that cuttlefish muscle lacked adenyldaminase and that 5'-AMP derived from ATP was accumulated in the muscle effectively without further degradation. Thus, 5'-IMP was produced by deaminating the 5'-AMP accumulated.

The method of preparing 5'-IMP from marine animals is not entirely satisfactory, because the raw material is not stable from either an economical or biochemical viewpoint. Furthermore, the nucleotide that can be separated from marine animals is only 5'-IMP, or 5'-AMP, and it is impossible to separate 5'-GMP economically, though a very small amount of the

latter was also observed to be present in usual fish muscle.

**Degradation of RNA by 5'-phosphodiesterase.** Using the microbial 5'-phosphodiesterase, four 5'-nucleotides—5'-AMP, 5'-GMP, 5'-CMP, and 5'-UMP—can be produced simultaneously from RNA (Kuninaka *et al.*, 1957*a,c*, 1959, 1961; Omura *et al.*, 1958; Ogata *et al.*, 1963). 5'-IMP can be produced enzymically or chemically from 5'-AMP. It is not necessary to purify RNA or the 5'-phosphodiesterase before the enzyme reaction.

RNA can be separated from any animal or plant tissues. It is convenient, however, to utilize microorganisms containing a large amount of nucleic acid. At present, yeast is the best source of RNA in Japan. It is expected that RNA, protein, and vitamin will be separated from yeast and utilized effectively in respective fields. Since yeast is rich in protein and vitamin as well as nucleic acid, nucleic acid might be regarded as a by-product of yeast protein or vitamin. In fact, removal of nucleic acid is considered to enhance the value of the yeast as a protein fodder. The 5'-phosphodiesterase is formed during culture of the screened microorganism. The cultured liquid or the cell extract, as is, can be employed as the enzyme solution.

The pharmaceutical value of each of 5'-nucleotides produced from RNA is being disclosed. (Now that 5'-nucleotides can be supplied economically in large amounts for their biological tests, their pharmaceutical uses are expected to develop rapidly. For example, Hirata *et al.* (1962) disclosed the role of 5'-nucleotides as *Bifidus* factor, and Kato *et al.* (1962, 1963) recognized the remarkable effects of 5'-IMP on recovery from the insulin-shock coma.) It appears that valuable products for food and/or pharmaceutical uses can be obtained simultaneously from the economical raw materials with this simple procedure. This is the reason why this RNA process has been regarded as the most effective for producing 5'-nucleotides. This process is expected to be further developed as pharmaceutical uses of 5'-nucleotides are developed.

**Direct fermentation (biosynthesis).** The direct fermentation process is now being studied most actively in Japan.

Several nucleosides have been observed to be accumulated in rather large amounts. For example, 1.55 g/L, more than 5 g/L, 2.7 g/L, or more than 7 g/L of inosine was accumulated

by an auxotroph of *Bacillus subtilis* (Aoki *et al.*, 1959; Uchida *et al.*, 1959, 1961; Nara *et al.*, 1963; Aoki *et al.*, 1963), and 4 to 5 g/L of xanthosine was also accumulated by a guanine-requiring auxotroph of *Aerobacter aerogenes* (Nakayama *et al.*, 1963).

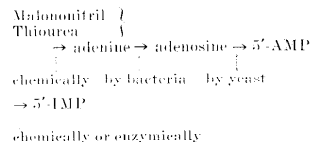
In these cases, the nucleosides might be synthesized *de novo*. On the other hand, adenosine was observed to be produced by a nonexacting purine-requiring auxotroph of *Bacillus subtilis* during its growth in a medium containing adenine (Hara *et al.*, 1962*a*), and inosine was also observed to be produced from hypoxanthine by an adenine-requiring auxotroph of *Bacillus subtilis* (Kuninaka *et al.*, 1963).

As for the nucleotide, 850 mg/L of 5'-AMP was accumulated by a wild strain (Furuya *et al.*, 1963). It seems more difficult to accumulate large amounts of 5'-nucleotides using microorganism than to accumulate the same amounts of nucleosides.

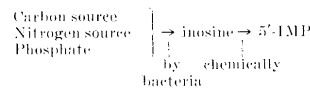
**Chemical synthesis.** In several Japanese laboratories chemical synthesis is being studied to produce 5'-nucleotides economically. For example, inosine was reported to have been economically phosphorylated to 5'-IMP (Kato *et al.*, 1963).

**Combination.** There are various possibilities to combine the above four processes. The following two methods are actually going to be developed in Japan.

*Method A* (Hara *et al.*, 1962*b*)



*Method B* (Akoki *et al.*, 1963; Kato *et al.*, 1963).



**Conclusions**

5'-Nucleotides and their compositions with MSG are now reforming Japanese food practices. As the processes for their production continue to be improved, they are expected to attain world-wide recognition as new, excellent flavor enhancers. It also appears that improved availability of these compounds will stimulate the development of many other uses of interest to food technologists, nutritionists, and physiologists.

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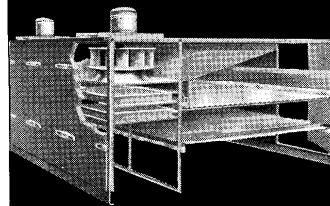
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THE ABBREVIATIONS used herein are as follows: adenosine monophosphate (AMP); inosine monophosphate (IMP); guanosine monophosphate (GMP); xanthosine monophosphate (XMP); uridine monophosphate (UMP); cytidine monophosphate (CMP); ribonucleic acid (RNA); deoxyribonucleic acid (DNA); monosodium L-glutamate (MSG); adenosine triphosphate (ATP).

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