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Free and Bound Glutamate in Natural Products

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> For almost 90 years, glutamic acid, a ubiquitous component of protein of well defined chemical composition and a nonessential amino acid which the body can synthesize, has attracted relatively little attention. Suddenly, through the remarkable coincidence of many unexpected findings it has been brought into the bright light of an interest which goes well beyond the limits of science. It has now become the object of most vivacious discussions in front of public opinion. (7)

The state of affairs alluded to in the above statement from 1955 by Kuhnau in Klingmüller's *Biochemie, Physiologie und Klinik der Glutaminsaure* (7), a no longer readily available compendium of the scientific knowledge of this important amino acid, should certainly be applicable to our present situation; however, it is not. The opinions in 1955 concerning the dispute on whether glutamate (Glu) could enhance intellectual level could very well apply to the situation in 1978, but Kuhnau would be very surprised to learn the present meaning of public opinion: a heterogeneous mass of listeners and readers who can be easily reached and impressed.

For the vast majority of all known proteins, Glu is the major amino acid. As an essential link in intermediary metabolism, it is also largely present in its free form in animal and plant tissues. Table 1 shows the levels of Glu that have been reported in various human tissues. The small amount in plasma is remarkable, and it

TABLE 1. *Free Glu in the organs of a normal adult*

Tissue	Free Glu (mg)
Muscles	6,000
Brain	2,250
Kidneys	680
Liver	670
Blood plasma[a]	40
Total	9,640

[a] Glu in plasma—total free Glu: 0.41%.
From K. Lang, *unpublished*.

TABLE 2. *Free amino acids in the muscles of a man of 70 kg*

Muscle mass	28 kg
Intracellular muscular water	18.2 liters
Total amino acid intracellular pool consisting of	86.5 g
Glutamine	61%
Glu	13.5%
Alanine	4.4%
8 Essential amino acids	8.4%

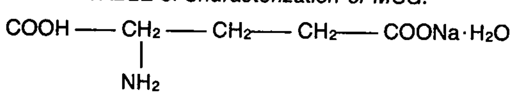
does not increase to more than 120 mg even after ingestion in excess of current average dietary amounts of the free form. This underscores the very high metabolic rate (5 to 10 g/hr). According to Bergstrom (1), the concentration in human muscle could be greater than the 6,000 mg shown in Table 1 (Table 2). It is remarkable that 79% of the free amino acids in the muscle consists of three closely related compounds: glutamine, Glu, and alanine.

As the sodium salt, Glu has become a widely used food ingredient. There is hardly a product that could more justifiably be called a food ingredient. Unfortunately, monosodium glutamate (MSG) is considered to be a "synthetic food additive" or a "chemical seasoning." Brillat-Savarin, who 150 years ago, claimed that the future of gastronomy belonged to chemistry, would have liked the definition. Today, "chemical" seems to imply danger and may elicit an emotional response to a given issue.

It is useful to remember the formula of MSG because the water of crystallization tends to be ignored in toxicological evaluations, and this makes a 10% difference in weight (Table 3). For current use, the sodium is insignificant (not more than in a glass of milk), unless total Na restriction is recommended. However, in the case of high-dose experiments, the risk of a concomitant hypernatremia should not be overlooked. Klingmüller (7) felt that it might be misleading to equate Na in MSG to the equimolecular amount in NaCl because Na and Cl follow a very close metabolic path, whereas Na and Glu widely diverge.

The Glu properties of improving the palatability of processed and preserved foods

TABLE 3. *Characterization of MSG:*



Constituent	Percent
Glu	78.2
Na	12.2
H_2O	9.6
Molecular weight	187.3
1 g Glu	1.27 g MSG
1 g MSG	0.122 g Na

TABLE 4. *Annual industrial production of Glu*

Country	Production (tons)
Japan	65,000
Europe	40,000
Korea	40,000
Taiwan	25,000
U.S.	20,000
Others	10,000
World production	200,000

and of giving the last touch to high gastronomy were known before its discovery. The origin of soy sauce (containing about 1% Glu) is lost to us. However, the actual beginning of industrial production of acidic protein hydrolysates could have been inspired by Justus von Liebig (1803 to 1873), who remarked that they tasted pleasant and meatlike. An enterprising young Swiss of Italian extraction, Julius Maggi, picked up the idea and developed the industrial production of protein hydrolysate, which became a striking commercial success. He thought, at first, that he had hit on an authentic reproduction of meat extract. He soon found that the composition was very different and that the quality of his product depended on the choice of protein, the best being wheat gluten and casein. He had actually discovered the two essential elements of what we today call a hydrolyzed vegetable protein (HVP): the reacted flavors and Glu. Maggi learned this before his death in 1912. In fact, Ritthausen had isolated Glu in 1866 without reporting its organoleptic properties. The properties of Glu were detected in a natural product where it is present in its free form: in 1908, Ikeda extracted Glu from a seaweed widely used in ancient Japanese cooking (*Dashikombu* or *Laminaria japonica*), which is one of the natural substances with the highest content of free Glu (0.42%). This discovery, coupled with the finding by Ritthausen that wheat gluten contained 25 to 30% of Glu, was the starting point for the industrial production of MSG.

Today, MSG is produced primarily by a fermentation process. Some figures about Glu production are shown in Tables 4 and 5, the daily world metabolic turnover being 450,000 to 900,000 tons per day. Without Ikeda or Admiral Perry, a Frenchman pondering the ideal association of wine and cheese could have found

TABLE 5. *Some natural sources of Glu* (as *MSG*)

Food source (tons)	MSG contents (tons)
Tomatoes	
U.S. (6,500,000)	11,600
Western Europe (8,000,000)	14,300
Parmesan-type cheese	
Italy (150,000)	2,286

TABLE 6. *MSG consumption in selected countries*

Country	Total tons/yr	g/day
Taiwan	18,000	3
Korea	30,000	2.3
Japan	65,000	1.6
Italy	8,000	0.4
U.S.	28,000	0.35

TABLE 7. *Natural free Glu (as MSG) from other sources in Italy*

Food source (tons)	MSG contents (tons)	g/day
Tomatoes (3,000,000)	5,350	0.26
Parmesan cheese (150,000)	2,286	0.11

that the velvety smoothness was conferred to wine by the high content of free Glu in the cheese. Furthermore, an Italian could have asked why Parmesan cheese was such a popular all-purpose seasoning, why tomatoes are so essential in a sauce, and why they blend so well with Parmesan cheese. In fact, the consistency of many traditional foods seems to be related to the presence of free Glu.

Taiwan has the highest per capita consumption of MSG (3 g/day) (Tables 6 and 7). The consumption of Glu in the United States is marginal.

When speaking of the percentage of bound Glu in the food protein, it is most

TABLE 8. *Current food items for which Glu is not the leading amino acid on total nitrogen*

Food item	Glu (mg/g N)	Asp (mg/g N)
Potato	639	775
Sweet potato	541	825
Beet	946	1,131
Apple	700	1,300
Apricot	372	1,300
Avocado	769	1,413
Banana	575	656
Fig	600	1,500
Orange	760	880
Pear, Japanese	540	2,800
Strawberry	920	1,400
Brewer's yeast	669	678
Candida krusei	675	800
Ansenula anomala	775	779

TABLE 9. *Glu in animal proteins*

Protein	Glu (g/100 g)
Albumin (human serum)	17.0
Fibrinogen (human serum)	14.5
γ-Globulin (human serum)	11.8
Albumin (egg white)	16.5
α-Casein (milk)	22.5
β-Lactoglobulin (milk)	20.0
Actin (muscle)	14.8
Myosin (muscle)	21.0
Insulin	18.6
Pepsin	11.9
Keratin (human hair)	14.4
Keratin (wool)	11.9
Collagen (tendon)	11.3

practical to start by mentioning the few proteins in which Glu is not the major amino acid. The relevant FAO data do not concern unique proteins. In fruits and leaves, the nitrogen compounds include free amino acids and amides. If we disregard exotic roots and beggarstick and scratchbush leaves, we have the list of the more common items shown in Table 8. Glu and the leading amino acid, which is invariably aspartic acid (Asp), are given as milligrams per gram of nitrogen (4). Glu is also the most abundant amino acid in the proteins that have been isolated by scientific research (Table 9) (2). Various techniques have been used for the separation of proteins, as well as for their classification (Table 10) (2). Finally, for most food products it is possible to indicate their amino acid composition as a percentage of total nitrogen, since this has been done in the FAO tables, with the reservation, already mentioned, that some of the Glu and Asp are actually present in their free form. This figure is again a confirmation of the importance of Glu as a source of nonessential nitrogen (Table 11) (4).

Since the earliest times, cereals have been submitted to fermentation processes

TABLE 10. *Glu in isolated plant proteins*

Protein	Glu (g/100 g)
Gliadin (wheat)	45.7
Zein (maize)	26.9
Edestin (flax)	20.7
Hordenin (barley)	38.4
Globulin (coconut)	21.8
Arachin (peanut)	20.8
Globulin (cotton seed)	23.6
Glycinin (soybean)	20.5
Glutenin (wheat)	24.7
Lupin (lupine bean)	27.2

TABLE 11. *Glu as percentage of 16 g of total nitrogen in various foods*

Food item	Glu (%)	Food item	Glu (%)
Wheat gluten	37.4	Pea	14.6
Wheat flour (70–80%)	34.2	Lentil	16.7
Barley	23.6	Beet	14.9
Rye, whole meal	24.2	Asparagus	20.8
Rice, milled, polished	19.2	Carrot	19.4
Oats, meal	20.9	Potato	10.2
Maize, whole meal	19.0	Tomato	40.6
		Apple	11.4
		Apricot	6.1
		Grape	20.8
Hazelnut	20.6		
Brazil nut	18.6	Anchovy	15.1
Sunflower seeds	21.8	Haddock	15.8
Soya milk	17.4	Salmon	13.2

involving enzymatic hydrolysis of their proteins. Thus, free Glu became a taste component in human food. This is also evident for milk protein, and free Glu is an important flavor component of cheese (Table 12).

The levels of natural or added free Glu in the diet must be assessed within the framework of metabolic capacity by the normal activation of the digestive system. The latter is best observed in the course of feeding of a 4- to 6-month-old baby. There are basically two possibilities to consider when feeding, for instance, a jar of baby food to an infant. The infant either may accept the food or may react with rebellious sputtering until something is worked up. The digestive secretion is actually being worked up in the process. Figure 1 gives Nasset's sketch (9) of the mechanism of concurring actions that are set off by digestion. The gastrointestinal tract can deliver into its lumen a substantial amount of endogenous nitrogen (including amino acid), diluting the exogenous nitrogen in the small intestine by sevenfold in response to the ingestion of any type of meal. If the ingested protein has an unusual amino acid composition, as in zein, it is effectively obscured after

TABLE 12. *Free Glu in cheese*

Cheese	Glu (g/100 g)	MSG equiv.[a] (g/100 g)
Parmesan	1.2	1.52
Stilton	0.82	0.94
Roquefort	1.28	1.62
Gruyere de Comte	1.05	1.33
Saint Paulin	0.21	0.27
Camembert	0.39	0.49
Danish Blue	0.67	0.85
Gouda	0.46	0.58

[a] Glu × 1.27.

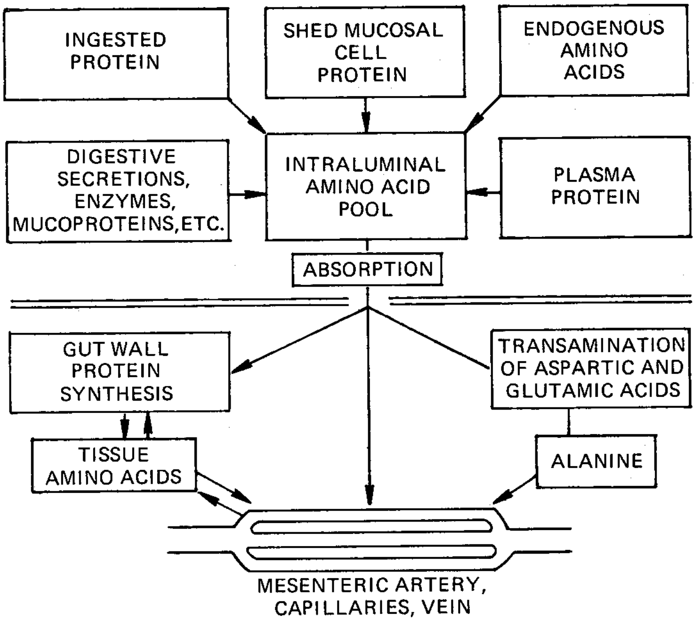


FIG. 1. Amino acid pool in small gut.

being exposed to the normal process of digestion. The amounts of amino acid delivered into the lumen depend on the chemical composition of the lumen. If several amino acids are present together, there is competition among them for sites in the transport system. In other words, there seems to be a mechanism that prevents acute, large fluctuations in the amino acid mixture that is available for absorption (a mechanism that is bypassed by intragastric alimentation). This may also be true for the ingestion of concentrated water solutions of Glu by human volunteers.

The concentration of MSG in baby food, before its voluntary removal by the manufacturers, was reported to be as high as 0.6% by the National Academy of Sciences in July 1970; (but no source of this information was given) (11). In my experience in this field, the addition was never more than 0.3%. Both 0.3 and 0.6% are generally within individual metabolic capacity; however, more than 0.3% in baby food is technologically excessive. The question, then, is whether MSG was a desirable ingredient. It has been said that its addition should have pleased only mothers, since infants are not taste conscious. This opinion is based on the assumption of the so-called cosmetic action of MSG, which should improve the quality of any food—the more the better, thus allowing for a reduction in the use of expensive ingredients. This is totally misleading. MSG cannot make a delicacy of meat and vegetable baby food. It can only make it more acceptable by reducing the occasional coarseness or astringency caused by processing. The favorable influence of Glu is primarily related to its taste-lingering effect, which, along with its stimulation of the salivary glands, is its most easily measurable action. MSG is not a flavor enhancer in the sense that has been purported. For instance, MSG makes saltiness milder, but does not increase the demand for salt. On the contrary, it tends to reduce the consumption of salt because it creates a new, more analytical perception of taste, as observations of patients deprived of salt have shown. This finding can also be applicable to infants. Supplemental baby foods have the advantage of assisting the cultivation of a taste for a variety of foods that, later on, are even more important in the diet. The parents' attitude toward food is unquestionably recognized by infants at an early age.

Protein-rich processed food may contain 0.3% MSG or slightly more. The clear broths (beef or chicken flavored)—which, combined with noodles, rice, or vegetables, constitute a wholesome and inexpensive staple food, not only in the Far East, but in all the Mediterranean countries—may contain 0.5 to 0.8%. It has been suggested that the occasional jar of baby food as well as the current processed food may expose the growing child to an excess of free Glu. From the still incomplete data, the ample supply of free Glu and Asp appears to come from fresh unprocessed foods and traditional foods (3,5,6,11). In Table 13 the contents of free Glu and Asp have been added together and transformed into their MSG equivalent.

Finally, it is noteworthy that a nutriment that nature has conceived for the requirements of the newborn—mother's milk—contains small but significant amounts of free Glu. The highest concentration is measured in the very first days after birth when the infant is supposed to be more sensitive. This may be nutritionally meaningless, although this was not the opinion given in 1971 by Montreuil, a pediatrician who felt that the existing knowledge justified the systematic and

TABLE 13. *Free Glu and Asp in natural foods*

Food item	Glu (mg/100 g)	Asp (mg/100 g)	MSG equiv.[a] (mg/100 g)
Tomato	140	35	221
Fresh tomato juice	260	60	406
Processed tomato juice	230	60	370
Grapefruit, white meat	11.5	87.1	125
Grapefruit juice	18.6	130	190
Orange juice	21	89	140
Nectarine, fruit	9.6	200	269
Peach, juice	32	212	274
Plum, yellow fruit	7.9	185	243
Prunes (California)	14.4	185.5	254
Prune dry	18.6	518.4	684
Grape, red Malaga	184	12	250
Grape juice	258	16.8	350
Strawberry	44.4	60.1	128
Potato	102	—	129
Broccoli	176	40	274
Parmesan	1,200	—	1,524
Gruyere de Comte	1,050	60	1,460
Mushroom (*Psalliota campestris*)	180	30	267

[a] Glu + Asp.

TABLE 14. *Free amino acid concentration in human and cow's milk*

Amino acid	Human milk (mg/100 ml)			Cow's milk (mg/100 ml)[a]
	2nd day	3 weeks	2 months	2 months
Glu	12.88	7.66	4.20	0.64
Glutamine	9.48	1.79	1.75	0.41
Asp	2.92	1.40	0.53	0.08

[a] At 2 months.

immediate supplementation of cow's milk with the free amino acid contained in human milk (8). At any rate, we can be sure that the wisdom of maternal physiology is not taking an unnecessary risk when the newborn has a plasma level above average, whereas the mother has only 50% of her basal level. According to the data in Table 14, the 3-kg newborn with a daily intake of 480 g of breast milk is exposed to 20.6 mg/kg body weight Glu and 4.7 mg/kg Asp. The total of the two dicarboxylic amino acids expressed as MSG is 32 mg/kg body weight.

CONCLUSIONS

A vast and long-standing literature indicates the overwhelming presence of bound and free Glu in the food supply. Some of the older figures are probably 25 to 50% too low. Similarly for Asp, some of the values may be only 50% of what would be obtained by the best modern methods.

The free dicarboxylic amino acids are quantitatively important constituents of tomatoes, fruit, cheese, and mushrooms. The relevant data are still incomplete, fragmentary and diverge widely according to variety, ripeness, fertilizing with respect to fruit and tomatoes, etc.

Glu is an important element in natural and traditional ripening processes that achieve fullness of taste. Its industrial use is a logical consequence.

Food preservation and processing tend to decrease free and bound Glu. The industrial use of MSG tends to compensate for this loss.

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