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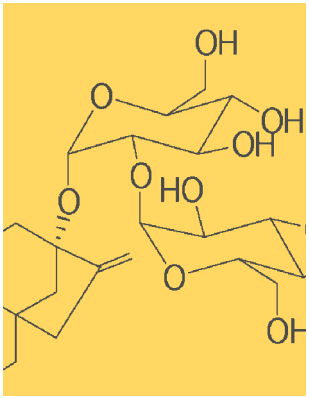
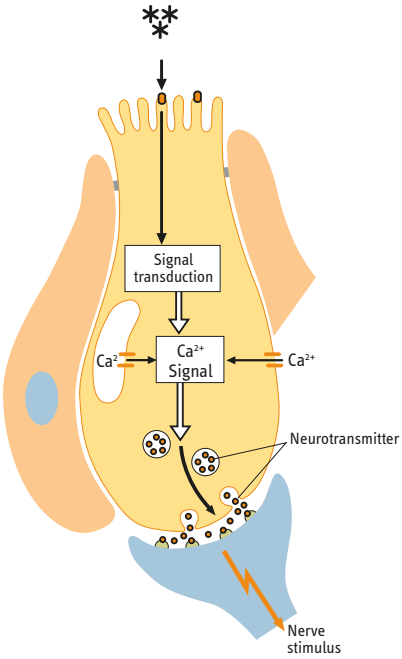
Molecular Aspects of Taste



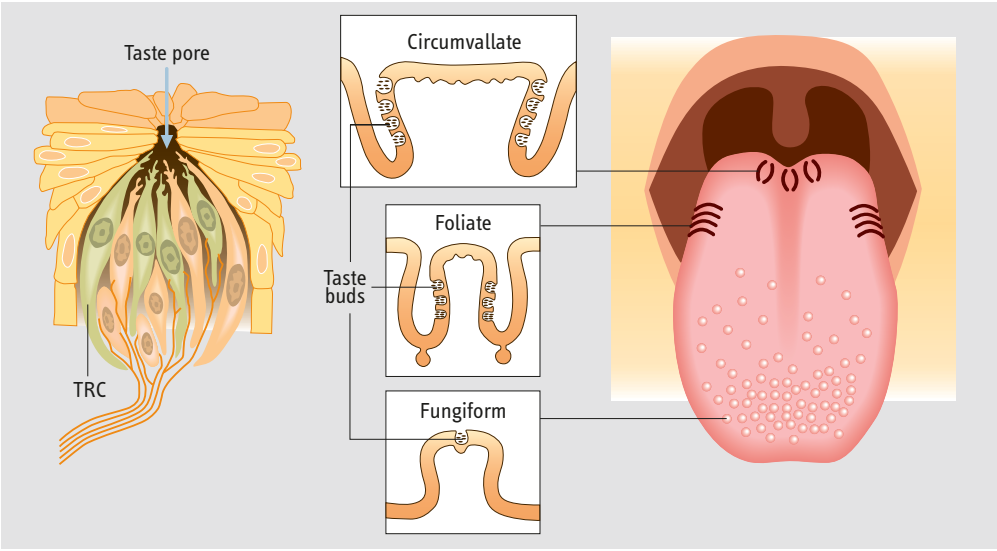
Eating is a pleasure and a need. In nature, gratified needs generate pleasure because they have to persuade us to meet those needs, whereas anything that can harm us causes pain and aversion.
In the case of food, the pleasure lies in the complex sensations generated by the 'good flavor' of a certain food that we like and we therefore introduce into our organism, while aversion lies in the 'bad flavor' of another food which we have tasted but do not like, and which we therefore refuse to eat (Saper *et al.*, 2002).
What is concentrated in the word 'flavor' is the result of a combination of connected sensations which food induces and which we can divide schematically into physical sensations (temperature, consistency, humidity, friction (1), chemical sensations (taste and smell), and chemesthetic sensations (2).
Each of these sensations is detected by specific receptors, complex protein molecules — or groups of molecules — that act as biological sensors, whose function lies at the basis of the system of chemical communications that coordinates the functions of all the cell groups that we are made of, as well as generating and transmitting to us a representation of the external world that we live in, through our senses: physical properties (hearing, sight, touch) and chemical composition (taste and smell).
Even if 'taste' is commonly used as a synonym of 'flavor', in the strictest sense of the term, it should only be used for the chemical sensations detected by specialized cells present in our oral cavity — the taste receptor cells — on which specific receptors are present that are sensitive to the molecules in food.
Therefore our sense of taste has to assess the content of a certain food, recognize the chemical substances it is made of (which is why we talk about chemoreception), and allow us to distinguish foods rich in the nutrients that are indispensable for our nourishment (and therefore accepted), from those potentially toxic or bad (and therefore rejected).
Basing ourselves on a simple analysis, we can say that sweet substances are generally pleasant and that they include sugars, which are an important source of energy; that the taste of meat is generally appreciated and that the amino acids that make up proteins are essential for our metabolism (they are the only source of nitrogen, which is not present in carbohydrates or fats); that we like the taste of fat and that fats are a significant source of energy.
On the other hand, bitter substances may be accepted, but only at very low concentrations. Most bitter substances are produced by plants, which have evolved the strategy of accumulating bitter secondary metabolites as a defense against herbivores and pathogens, even though many organisms, including man, have evolved by learning to detoxify them (3).
And finally, sour taste, which may be a sign of spoiled food, may be tolerated and appreciated only up to a certain level.
Every taste sensation entails three steps: the generation of the stimulus in the oral

cavity, its translation into a nervous stimulus that goes to the brain, and there its interpretation and processing into sensory and hedonic terms.
So the already complex picture of the various gustative sensations is further complicated by the fact that all this information is then transmitted and processed by the brain — phenomena that are studied by neurophysicists and psychologists. This article deals primarily with the first step: the interaction between a tastant (a molecule which has taste) and the structures that detect it.



Anatomy of taste

The taste receptors are positioned at the apex of the taste receptor cells, which are structured to form the taste buds, distributed in the various papillae of the tongue and the soft palate.
The circumvallate papillae are found at the bottom of the tongue and in humans they contain about one thousand taste buds. The foliate papillae are present at the posterior lateral edge of the tongue and contain a dozen taste buds, while the fungiform papillae contain only a few taste buds and are found primarily on the tip and, to a lesser degree, up to 2/3 of the tongue. The filiform papillae (the most numerous) do not have taste buds, but are involved in tactile perceptions. Recent molecular and functional data have shown that the various papillae are not selective for a certain taste and therefore the old map of fundamental tastes must be abandoned.



How many tastes do we have?

If we asked readers to list the principal tastes, all (or nearly all) of them would probably say sweet, bitter, salty and sour. Many more were identified in the past, as reported by Yann Grappe in another article in this issue. Why has this changed? Apart from the important cultural component of taste, taste related to habits and customs that change in space and time, this difference is due primarily to the fact that it is now generally accepted (and supported by scientific evidence) that, although we are able to perceive a wide range of chemical entities, in qualitative terms they provoke a limited number of gustative sensations, the fundamental tastes, activating specific receptors. Today, there are five so-called fundamental tastes: sweet, umami, salty, bitter and sour (4).

‘Our sense of taste has to assess the content of a certain food, recognize the chemical substances it is made of’

Sweet, salty, bitter and sour do not need any particular description because they are familiar to all of us. Umami, on the other hand, is probably unfamiliar to most people. It comes from a Japanese word, ‘umai’, which means delicious, and it is the taste associated with the amino acid L-glutamate, which is contained in meat extracts and in soy sauce, conferring them their peculiar taste. Because of its origin and the spread of fermented foods with this characteristic taste, this term has become a common descriptor used in eastern Asia, but it is rarely used in the West, in spite of the fact that foods such as mature cheese, tomato and derivatives are rich in L-glutamate and therefore umami. The best example of this taste in western cooking is probably meat stock.

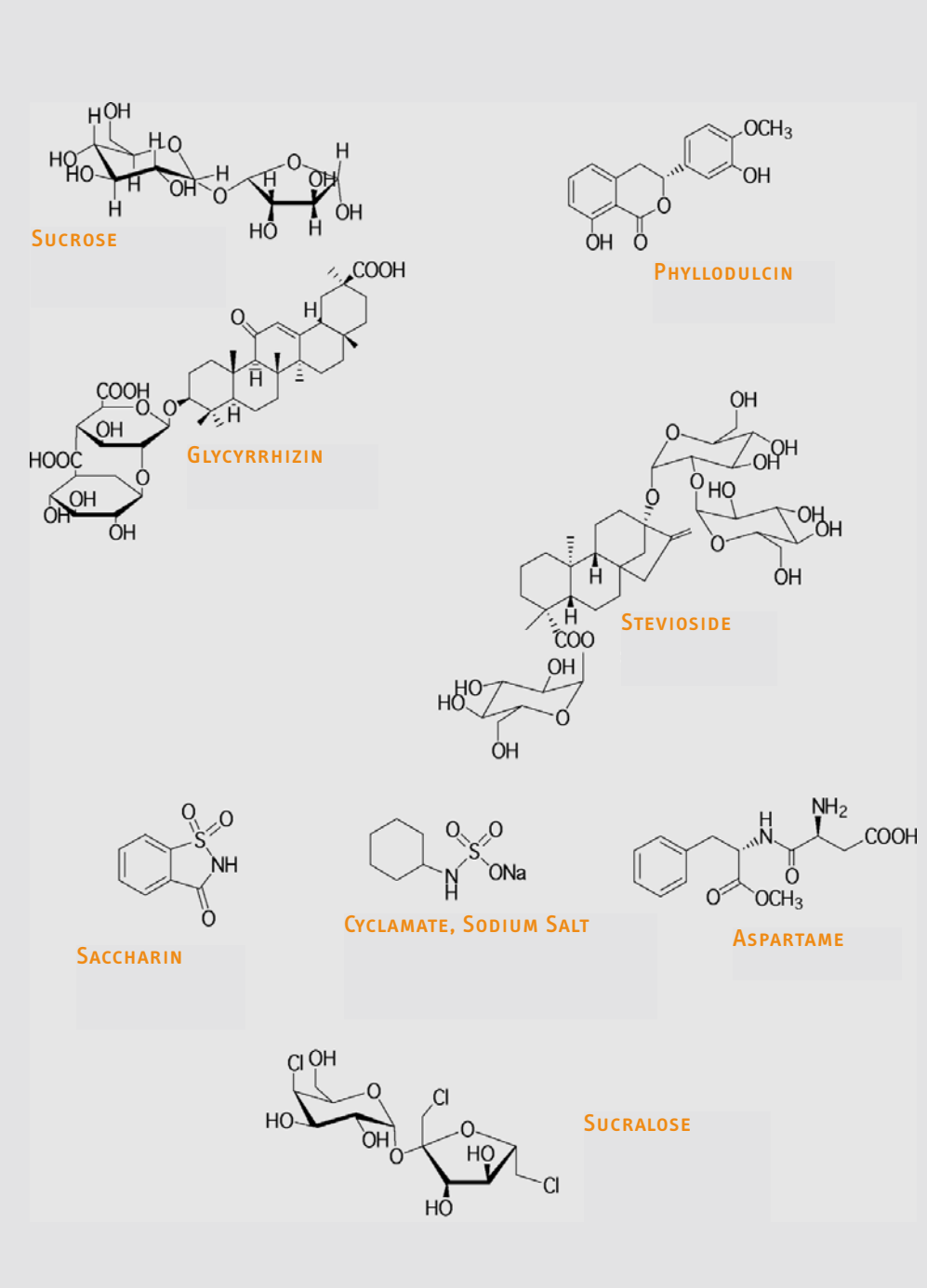
We should also note that, although the receptor that responds to the umami stimuli has been identified, cloned and functionalized, some authors have suggested that it should be considered more a taste enhancer than a taste per se, and that the sensation it provokes is mainly due to the taste and olfactory pathways convergence in the brain (McCabe *et al.*, 2007).

But if taste serves to identify the nutrients in what we eat, how come there is no fundamental taste for the fatty flavor, if fats account for about 40% of the daily calorie intake in the West? For many years experts thought that the preference of fat was linked only to the texture that it gives food and the pleasant sensation generated by its consumption. The existence of a specific sensor, which was only hypothesized in the past, was demonstrated recently in the rat (Laugerette *et al.*, 2006). By combining genetic, morphological, behavioral and physiological approaches, the multifunction CD36 glycoprotein was

identified (also called FAT or 'fatty acid transporter') as a possible receptor for fat. The mechanism proposed is the following: the cells on the tongue produce an enzyme capable of hydrolyzing the triglyceride molecules, releasing fatty acids, which are the true activators of the receptor.
This is why we have specified that 'today' there are five fundamental tastes, because recent and future discoveries are almost certain to change the scenario in a sector which, although under investigation for many years, only accelerated drastically when the sequence of the genome (human and of other mammals) become common knowledge. This made it possible to identify and subsequently clone the genes that codify the taste receptors: those of bitterness in 2000 (Chandrashekar *et al.*, 2000; Adler *et al.*, 2000; Matsunami *et al.*, 2000) and those of sweetness and umami in 2001 (Kitagawa *et al.*, 2001; Li *et al.*, 2001; Max *et al.* 2001; Montmayeur *et al.*, 2001; Nelson *et al.*, 2001; Sainz *et al.*, 2001).
Taste receptors are transmembrane proteins able to connect the outside of the taste receptor cell in contact with the oral cavity (where the tastants are located) with the inside of the cell in which, following the activation of the receptor itself, other changes take place generating the nervous stimulus that is then transmitted to the brain.
There are two types of transmembrane receptors that are important for taste, which differ in the way the signal is transmitted inside the cell and translated into a nervous stimulus: the ionic channels and the G Protein Coupled Receptors, which are generally known as GPCRs (5).
An ionic channel may be seen as a gate that will open to let specific ions through on the basis of their concentration gradient

'Recent and future discoveries ...'

(i.e. from where they are more concentrated to where they are less concentrated). Following the accumulation of these ions inside the cell, there is a cascade of reactions that releases the neurotransmitters, which reach the neurons that finally transmit the signal to the brain.
This class includes the receptor for the salty taste (sensitive to the Na+ ion) and the receptor for the sour taste (sensitive to the H+ ion). In the case of saltiness, several possible receptors have been suggested, but to date the identity of the salty receptor is still speculative and controversial (Heck *et al.*, 1984; Avenet *et al.*, 1988; Lyall *et al.*, 2004).
The situation for the sour taste was no less complex, but recent studies have limited the search, indicating an ionic channel of the TRP (Transient Receptor Potential) type as a possible receptor for sourness (Ishimaru *et al.*, 2006; Huang *et al.*, 2006).
There is more information and proof surrounding the receptors for sweetness, bitterness and umami, which belong to the GPCR class. These proteins form complex aggregates in the cell: when it binds with a tastant, an enzyme becomes active, producing a second messenger inside the taste receptor cell. In this case it is the change in concentration of the second messenger that triggers the cascade reaction, which in turn causes the neurotransmitters to be released, with the generation of the nervous stimulus.
Where the sweet taste is concerned, we should point out that there are not many sweet compounds present in nature, and



that they are structurally very different, ranging from small molecules such as simple sugars, disaccharides, amino acids and peptides, to proteins (some sweet fruit contains proteins, therefore macromolecules, such as brazzein, thaumatin and monellin). Moreover, we must add a number of synthetic compounds and their derivates that belong to other classes of organic compounds that are also sweet: saccharin, cyclamate, and sucronic acid, to name just a few.

This variety in the structure of sweet compounds, combined with the fact that we are able to distinguish between sweet substances (we can distinguish the sweetness of sucrose from that of a sweetener), has for years fueled the discussion as to whether there was a single receptor able to bind all these compounds, or a number of receptors. The question was only answered in 2001: today, in fact, a single receptor for sweetness has been identified, formed of two proteins (T1R2 and T1R3) which are able to respond to all the sweet substances for which the receptor has been tested **(6)** when they form a dimer (a supermolecule formed of the two subunits T1R2 and T1R3). The sweet taste receptor is able to do so because it has several binding sites that can host the various ligands (Xu *et al.*, 2004; Jiang *et al.*, 2005), but it has also been suggested that a single site could host structurally different molecules (Morini *et al.*, 2005).

It has also been demonstrated that it is again the T1R2-T1R3 receptor that is involved in the action of substances capable of inhibiting the perception of sweetness, such as lactisol, which seems to bind in the transmembranic part of the receptor itself, preventing it from functioning properly (Max *et al.*, 2005; Winning *et al.*, 2005). The picture outlined so far has also allowed us to speculate on another very important and puzzling phenomenon: the synergism exerted by certain sweet compounds when used in mixtures. We have, in fact, suggested (Morini *et al.*, 2005) that a compound is active if it is bound to its site and giving a certain response in terms of relative sweetness **(7)**. If another compound that can bind to another site is present in the oral cavity, the simultaneous occupation of the two sites in the receptor causes the response to be enhanced synergetically. This phenomenon is particularly important because it allows us to use smaller quantities of sweetening substances to obtain the same relative sweetness.

> ‘Our senses have evolved less rapidly than the way we procure food, eat and live’

The umami taste receptor is also a dimer, like the sweet taste receptor. They even share a subunit, because the umami receptor is made up of T1R1 and T1R3. There are a few compounds able to stimulate this receptor in man, among which L-glutamate and L-aspartate. Even purinic nucleotides such as IMP and GMP (inosine-5’-monophosphate and guanosine-5’-monophosphate) have a bland umami taste but above all they exert a considerable synergy with L-glutamate, a fact discovered and exploited by the food industry to formulate cubes well before the specific receptor was identified.

Unlike the sweet and umami tastes, which were evolved to positively select a limited number of molecules, the bitter taste performed the role of preventing the ingestion of a very large number of structurally different compounds. Another difference with respect to the sweet taste is that all these compounds evoke the same sensation that we describe simply as bitter. For the bitter taste, about 30 receptors belonging to the GPCR class have been identified, indicated as T2Rs. These

receptors are somewhat dissimilar, with a 10 to 75% variability in their amino acid composition. And it is this variability that allows only 30 receptors to respond to thousands of structurally different bitter compounds (each receptor responds to a certain number of compounds). The various receptors probably also use different systems to translate the sensory response into nervous stimuli, but these mechanisms have not yet been explained with certainty.

‘Are we born greedy?’

To each his own

It is common experience that we do not all perceive tastes in the same way. Some people are greedy and others are not; some people prefer sweets, and others savory foods; some like the bitterness of certain vegetables or of beer, and others only manage to drink coffee if they add a generous amount of sugar to hide its bitter taste. All this is due to the variability between individuals, to age and to environmental factors.

The variability between individuals (polymorphism) has been related to the sensitivity to the bitterness of certain substances such as phenylthiocarbamide (PTC) and 6-n-propyl thiouracil (PROP), due to the presence and functionality of a particular bitter receptor, TAS2R38. It has been discovered that someone who is sensitive to these two substances has a greater density of fungiform papillae (Duffy *et al.*, 2004) and therefore more sensitive to both bitter and sweet substances (Prutkin *et al.*, 2000).

In the case of fatty taste, it has been seen (only in rats to date) that the lingual stimulation of CD36 by fatty acids impacts behavioral physiology, while the deactivation of the CD36 gene completely eliminates the spontaneous preference for fat and changes in the gastrointestinal secretions triggered by the oral administration of fats (Laugerette *et al.*, 2006). The data for rats suggest that an alteration of the fat perception system can increase the risk of obesity.

It may be a lean consolation, but ... are we born greedy?

It is not difficult to note that children have a particular sensitivity for sweet, fat and salty foods, with a strong repulsion for anything bitter or astringent, which makes it difficult to make them eat vegetables (polyphenols, flavonoids, isoflavones, terpines and glucosinolates, secondary metabolites of plants which are often bitter or tannic, as we said earlier). It has been demonstrated that the aversion to these tastes decreases with age and the education of one’s taste, as a result of exposure (environmental factors) to foods that contain them (Mennella *et al.*, 2005). Even traditional foods — in particular non-domesticated plants, which are often attributed medicinal properties — have particular tastes that make them unique, but are also enjoyed only by people who are accustomed to eating them.

This margin of possibility for educating taste is particularly important because it has been demonstrated (Dinehart *et al.*, 2006) that the individual’s perception of sweet and bitter associated with the vegetables is the main driving force in their consumption, encouraging or discouraging the consumption of foods rich in micronutrients and antioxidants which our organism needs as it ages, and in which science has recently shown

considerable interest (Drewnowski *et al.*, 2000).

And whatever we learn will be remembered, even in our genes, thus contributing to the evolution of the species.

The problem is that our senses have evolved less rapidly than the way we procure food, eat and live. We must understand this and invest in educating our taste as a result, so that we do not die of what has kept us alive until now.

Notes to the text

1) The presence of particular types of substances such as polyphenols, the class to which tannins belong, reduces the lubricating properties of saliva, and astringency is therefore perceived as friction between two non-lubricated surfaces.
2) Chemesthetic sensations are chemically induced gustative sensations, which do not imply the activation of the taste or odor receptors. In this case other receptors may be activated both by the temperature (physical stimulus) or by substances contained in certain foods: the TRPV1 vanilloid receptor is sensitive to temperatures above 43°C and to capsaicin, the compound responsible for the hot taste of chili peppers, while TRPM8 is activated by cold stimuli (temperatures between 8 and 28°C) and menthol. As a result, chemesthesis can be defined as the chemical activation of receptors for physical stimuli, which therefore indicate a non-existent increase (hot) or decrease (cool) in temperature.
3) Over 2,500 plants produce bitter cyanogenic glycosides. Some of those contained in cassava, for example, are toxic both for man and for malaria parasites. Man has learned to treat cassava before eating it in order to make it edible, but this detoxification is rarely total. The bitter receptor that recognizes these substances has mutated and is not very active (Soranzo *et al.*, 2005) in the populations of sub-Saharan Africa, where malaria is endemic, so that the bitterness of these plants is tolerated better, encouraging their consumption, which gives a certain resistance to malaria.
4) I use the expression 'fundamental taste' which is in frequent use, but I would like to see this term abandoned.
5) Once again, it was molecular cloning which, thanks to the amino acid sequence, made it possible to classify these proteins in greater detail, supplying the homology percentages as the unit of measurement for the resemblance between receptors, and to probe the human genome in search of hypothetical new receptors.
6) We should note that for this receptor, and also for the receptor of umami and those of bitterness (there are more than one as we will see soon), only their amino acid sequence is known and not their three-dimensional structure.
7) The relative sweetness of a particular compound is calculated by preparing solutions of the compound under examination in a known concentration and comparing them (through tasting) with a standard 3% solution of sucrose. The solution of the compound under examination is diluted until it is isosweet, like that of 3% sucrose. If, for example, to obtain the same sweetness as the standard, the solution is 100 times more diluted than the standard, the compound under examination will have a relative sweetness of 100.

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