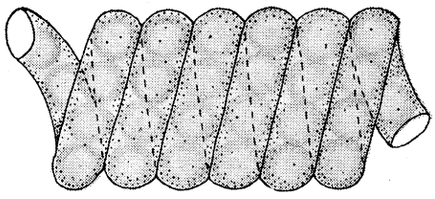

Fig. 9. Diagrammatic representation of cytoplasmic microtubule of lung-fluke sperm showing proposed structure. Pitch of the helix is about 15 degrees.

repeating at intervals of about 70 Å (Fig. 7). The pitch of the helix varies from 10 to 25 degrees. Sodium phosphotungstate does not appear to fill the lumen of the microtubule, even when broken so that "open" ends are exposed, and the appearance of the helical pattern is apparently due to the surface deposition of sodium phosphotungstate in the grooves between contiguous gyres. Thus, the helical structure suggested by longitudinal sections of microtubules is confirmed by negatively-stained preparations, even though negatively-stained microtubules show little evidence of the 65-Å subunits seen in transverse sections. In many broken microtubules, however, there appears to have been a disorientation of the helical structure at the broken ends. The result is a mass of particulate material at the free end, the mass being continuous with the microtubule proper (Fig. 7). The particles forming the mass appear to be roughly spherical, and they have a diameter of about 50 Å. It is probable that they represent the subunits seen in transverse sections of microtubules.

Axial units were easily recognized in negatively stained preparations. The nine peripheral doublet tubules of an axial unit do not show helical structure and, indeed, are quite different in appearance from the cytoplasmic microtubules (Fig. 8). They measure about 450 Å in width, and, unlike the microtubules, their lumina usually appear to be filled with sodium phosphotungstate. Negatively stained doublet tubules of sperm tails reportedly (*8*) have walls composed of 10 or 11 longitudinally oriented filaments, which are regularly beaded. Such well-defined filaments were not seen in doublets of axial units of lung-fluke sperm, although longitudinally oriented elements appear to be present. In Fig. 8, several such elements can be seen; the lumina of the two tubules are opaque, being filled with sodium phosphotungstate, and the longitudinal conection between the tubules is electron-lucent.

Cytoplasmic microtubules of lung-fluke sperm appear to be quite structurally stable, for with increased periods of sonic disruption they are broken into shorter and shorter fragments, which retain their basic helical structure after most other cell organelles have disintegrated. For example, after 4 minutes of treatment under the conditions designated, only short pieces of microtubules and pieces of the unique central core of axial units were recognized (doublet tubules of axial units were not seen).

Subunits forming the wall of these microtubules appear to be larger in diameter and fewer in number than those described for other such microtubular elements (*8, 9*). Assuming the subunits are globular, one can imagine them arranged in a helical pattern (Fig. 9), and I suggest that the microtubules I have described are so structured. Sandborn *et al.* (*10*) suggest that microtubules and microfilaments may be derived from one another, with globular units measuring 50 to 60 Å serving as building-blocks. While the helical structure of a lung-fluke sperm microtubule may result from the coiling and cross-bonding of a single microfilament, it appears that such is not the case for doublet tubules of axial units.

At least one function of the cytoplasmic microtubules of lung-fluke sperm is support, for living sperm show restricted flexing movements and give the appearance of being somewhat rigid or stiff. These qualities could also be used to describe their cytoplasmic microtubules.

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Sweet-Sensitive Protein from Bovine Taste Buds: Isolation and Assay

Abstract. *Using refractometry and ultraviolet-difference spectroscopy to indicate interaction between proteins and compounds of low molecular weight, we found a protein fraction in bovine tongue extracts that complexes sugars and saccharin. The strengths of the complexes parallel the degrees of sweetness of the compounds, and the effects of* p*H upon formation of complexes parallel the effects of* p*H upon sensitivity of taste buds to sweet compounds in vivo.*

In higher animals, a chemoceptor system is responsible for the sense of taste (gustation). While electrophysiological studies have firmly established the localization on the tongue of taste buds responding to sweet, sour, salty, and bitter substances, our understanding of the biochemistry of taste is rudimentary. Attempts to correlate the tastes of various compounds with their chemical structures or physical properties have met with only limited success (*1*). The nature of the initial interaction between taste stimuli and taste receptor has been the subject of considerable speculation (*2*). Attempts to correlate taste sensitivity with enzyme activities have been unsuccessful, and there are rather convincing arguments against taste being primarily enzymic in nature (*2, 3*). Indeed, available evidence strongly suggests that the initial interaction is formation of a weak complex between the stimulus compound and some receptor molecule at or near the surface of the taste bud (*2*).

Thus we tried to extract and purify "receptor molecules" from taste buds. In principle, the problems should be exactly analogous to those involved in extraction and purification of an enzyme, except that with enzymes one must seek fractions catalyzing a given reaction, while we had to seek fractions forming complexes with compounds of a given taste. Following the

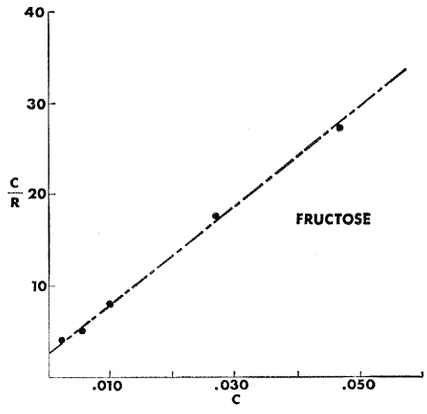

Fig. 1. Typical plot of data observed for fructose, according to theoretical treatment. C, Molar concentration of fructose; R, change in refractive index upon interaction of active fraction with fructose; slope is $1/R_m$, R_m being maximal response; $(1/R_m)$ $(1/K)$ is y intercept, where K is the equilibrium constant; ΔF is $-RT$ ln K.

enzyme analogy further, we reasoned that the receptor molecules would probably be proteinaceous, although other classes of molecules could conceivably possess the potential for the required degrees of specificity. Our assay method was based on the fact that proteins generally undergo conformational changes when implicated in formation of complexes; the result is alterations in their ultraviolet spectra and refractive indexes. Since salts and acids are known to cause conformational changes in virtually any protein, our procedure clearly could not be applied to search for molecules interacting with salty or sour compounds. Bitter substances absorb strongly in the ultraviolet region of the spectrum, with complication of an assay based on altered ultraviolet absorption. Accordingly, our first studies were directed toward sweet-taste receptors.

From Beidler's *(2)* suggestion that stimuli interact reversibly with receptor molecules, following the law of mass action, the following expression is readily derived:

$$n/(S - n) = KC \qquad (1)$$

in which n is the total number of ions or molecules that react with the receptors at any given concentration (C) of the chemical stimulus, S is the maximum number of ions or molecules that can react, and K is equilibrium constant.

It is assumed that the magnitude of response is directly related to the number of ions or molecules that have reacted with the receptors; therefore R is the magnitude of response and a is a constant. For a maximum response to a given substance, $R_m = a_S$.

By substitution in Eq. 1,

$$KC = R/(R_m - R)$$
$$C/R = (C/R_m) + (1/KR_m) \qquad (2)$$

This is Beidler's fundamental equation relating the magnitude of the taste response to the concentration of the stimulus compound. The parameters R_m and K can be determined graphically, since a plot of C/R versus C results in a straight line of slope $1/R_m$ and intercept $1/KR_m$. The free energy (ΔF) associated with the interaction can be calculated from the equation $\Delta F = -RT$ ln K.

Our first studies were performed by use of ultraviolet-difference spectroscopy *(4)* for assays. The reference cell contained extract; the sample cell contained extract at the same dilution, plus a known concentration of a sugar. Transmittance spectra were determined, at fivefold scale expansion, over the range 400 to 230 mμ; corrections for absorption by the sugars were made when necessary. The difference in transmittance was converted to absorbance and used as R in Eq. 2.

In our later studies and for all data now reported we used a refractometric assay so that synthetic sweeteners, which absorb in the ultraviolet, could be included. The refractometric method of detecting complexes has been used with dilute aqueous solutions of sugars and proteins *(5)*. Results with sugars were essentially identical with those obtained spectrophotometrically. In the refractometric method, the refractive increments for the extracts and for the sweet compounds were determined individually *(6)*. Refractions were then determined of (i) mixtures of each extract with each test compound, and (ii) each extract and each test compound individually. Derivations from additivity of refractive increments were determined, and were used as R in Eq. 2; each point was determined in triplicate, deviations usually not exceeding 0.0002 refractive-index units.

Bovine tongues obtained from a local slaughterhouse were the source of taste buds; behavioral studies with cows *(7)* indicate definite sugar preferences. Papillae from the tip of the tongue were dissected free of underlying muscle, disintegrated in a Waring blender, and homogenized further in

Table 1. Complexing of sweet compounds by material extracted from bovine taste buds. R_m data are in refractive-index units. Relative sweetnesses were determined in vivo: columns A *(12)*, B *(13)*, and C *(14)* for humans; column D for dogs *(8)*.

Compound	R_m	K (1 mole^{-1})	ΔF (kcal mole^{-1})	Sweetness			
				A	B	C	D
Fructose	0.0016	1030	−4.15	1	1	1	1
Sucrose	.0008	85	−2.66	2	2	2	2–3
Glucose	.0017	27	−1.97	3	3	3	4
Galactose	.0007	10	−1.39	4			5
Mannose	.0009	35	−0.75				2–3
Saccharin	.0011	226	−3.25				

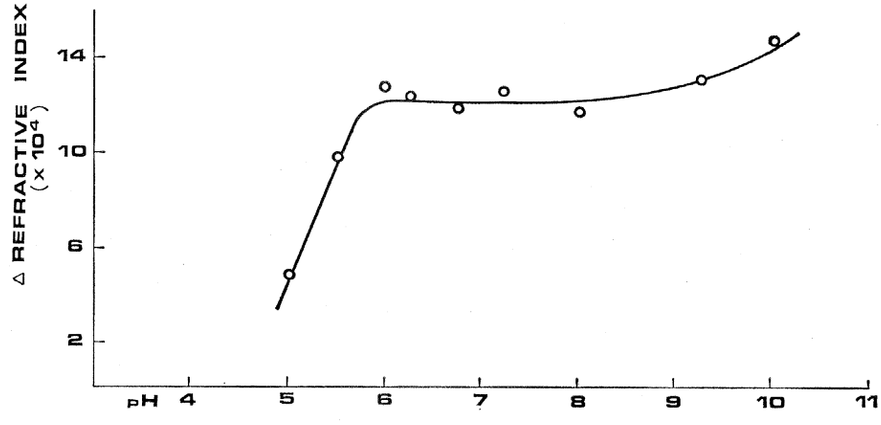

Fig. 2. Variation of complexing of 0.1M fructose by the 40-percent fraction with variation in pH.

a motor-driven glass homogenizer in four volumes of 0.1*M* phosphate buffer, *p*H 7.0; all operations were carried out at 4° ± 2°C.

The supernatant from centrifugation at 75,000*g* was subjected to successive ammonium sulfate fractionations. The only fraction showing significant response to sweetness was the one containing material soluble in 20-percent ammonium sulfate but insoluble in 40-percent ammonium sulfate (40-percent fraction), and the data now reported are results obtained with this material. In no instance was the response time-dependent.

Except for the study of effects of *p*H, all assays were carried out in 0.05*M* sodium phosphate buffer at *p*H 7.0. For the *p*H variations, the following buffers were used, all at 0.1 ionic strength: formate, *p*H 3.6; acetate, *p*H 4.4; formate, *p*H 4.9; acetate, *p*H 5.4; phosphate, *p*H 6.0; acetate, *p*H 6.1; phosphate, *p*H 6.7; tris, *p*H 7.1; phosphate, *p*H 7.6; tris, *p*H 8.6 and 9.6.

Equation 2 proved to be adequate to describe the data obtained with all test compounds. Figure 1 shows data obtained with fructose, which are typical of the results obtained. Values of R_m and K, determined experimentally, are shown in Table 1, along with the values of $\triangle F$ calculated from the values of K; the table also includes the relative sweetnesses of the sugars, as determined in vivo by others for the dog *(8)* and for humans *(9)*.

The 40-percent fraction from bovine taste buds appears to interact with sugars, forming complexes whose strengths parallel the sweetnesses of the tastes of the sugars (see Table 1). Physicochemical studies, to be reported elsewhere, show this material to be proteinaceous and nearly homogeneous. Sugar-protein interactions have been previously observed, but only at sugar concentrations far higher than we used. Thus it appears unlikely that the interactions observed are nonspecific, a conclusion supported by our observation that most of the protein extracted from bovine taste buds, which appears in the other fractions in our procedure, shows no evidence of formation of complexes with comparable sugar concentrations.

Beidler's *(2)* "fundamental taste equation," originally used to describe interactions of salts with taste buds, described the interaction of sugars with the 40-percent fraction most adequately *(10)*. The values calculated for $\triangle F$ suggest weak interactions (the figures of 1 to 2 kcal/mole are generally accepted as being the approximate strength for a hydrogen bond) rather than chemical reactions; in accord with this is our failure to observe any time-dependency for the changes in spectrum or refractive index. Beidler *(2)* and Evans and Mellon *(11)*, who used mammalian material and blowfly salt receptors, respectively, concluded that the response of taste receptors to salts entailed weak interactions.

Since our material was obtained by pooling papillae from several tongues, our values represent averages from individuals and can probably be used for comparison with results of in vivo studies. Such a comparison (Table 1) shows that the strengths of the complexes parallel the relative sweetnesses of the sugars; the value for K is used in ranking, since it describes the relation between degree of binding and concentration.

Since the response to sweet-tasting compounds in vivo is virtually independent of *p*H above *p*H 4 to 5 *(3)*, it was of interest to compare the *p*H dependence of the interaction between a sugar and our protein fraction. Figure 2 shows that the interaction of fructose with our material is virtually independent of *p*H between *p*H 9.6 and about *p*H 5.5, and that it falls off dramatically at lower *p*H.

Although it is difficult to prove conclusively that the material prepared by our procedure is in fact the component of the taste bud that is normally responsible for the initial interaction with sweet compounds, the following points suggest that it is:

1) It is derived from bovine taste buds as a fraction having an extraordinary tendency to complex sweet-tasting compounds.

2) The relative strengths of the complexes between this fraction and sugars parallel the relative sweetnesses of the sugars.

3) The *p*H-dependence of formation of complexes between this fraction and sugars parallels the *p*H dependence of the sweet tastes of sugars.

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Parathyroid Hormone in Plasma in Adenomatous Hyperparathyroidism, Uremia, and Bronchogenic Carcinoma

Abstract. *The concentration of parathyroid hormone (measured by radioimmunoassay) in plasma of patients with severe chronic uremia is frequently much higher than it is in the majority of cases having adenomatous hyperparathyroidism. Higher-than-normal concentrations of parathyroid hormone in plasma are found in a significant percentage of unselected patients with bronchogenic carcinoma.*

Enlargement of the parathyroid glands is a frequent consequence of chronic renal disease associated with hypocalcemia. Primary hyperparathyroidism may be caused by functioning tumors of the parathyroid glands or by the ectopic production of hormone by tumors other than those of the parathyroid glands, particularly bronchogenic carcinoma *(1)*. Recently, Tashjian *et al.* *(2)* have extracted parathyroid hormone from such tumors. However,