acidotic groups of the latter study[6] had plasma HCO_3^- values of 35 and 17 mEq/l and plasma CO_2 tension of 43 and 36 mm Hg, respectively.

Discussion. Although there are good indications that the CSF possesses effective homeostatic mechanisms for keeping the pH constant, the present experiments have shown that factors such as the plasma pH and the plasma bicarbonate influence the resulting acid-base changes in the CSF. Apparently, the regulation of CSF pH towards normal values in hypercapnia will be less effective in the absence of a chemical diffusion gradient for bicarbonate.

There is a clear difference in the effectiveness of the CSF pH regulation in respiratory and non-respiratory acid-base shifts. In non-respiratory acid-base shifts this regulation appears almost perfect (maximal CSF pH change 0.05 pH units, see [6]). Since this difference was observed in the same species exposed to the acid-base shifts for an identical period it would appear that the mechanisms behind the acid-base changes in the CSF were different in respiratory and in non-respiratory conditions[7].

Zusammenfassung. Es wurde nachgewiesen, dass die Zunahme des HCO_3^--Gehaltes in der Cerebrospinalflüssigkeit bei konstantem Plasma-pH unter Hyperkapnie grösser als bei konstantem Plasmabikarbonatgehalt und wesentlich anders als bei der nicht respiratorisch bedingten Säurebaseänderung ist.

B. K. SIESJÖ and U. PONTÉN

Department of Neurosurgery, University of Lund and the Neurosurgical Research Laboratory, the Hospital of Lund (Sweden), May 18, 1966.

[7] Acknowledgment: Supported by the Swedish Medical Research Council (Project No. 14X-263-02), and by contract No. N62558-4288 between the Office of Naval Research, Department of the U.S. Navy, and the University of Lund, Sweden.

Gustatory Responses to Anomeric Sugars

Differences in taste between D- and L-enantiomorphs of amino acids[1] and between α- and β-configurations of carbohydrates have interested investigators from many disciplines. CAMERON[2] was among the first to report that a freshly prepared solution of 10% α-D-glucose was noticeably sweeter than a solution at equilibrium. This was later confirmed by others[3,4]. TSUZUKI and YAMAZAKI[5] observed that the linear relationship of the sweetness of fructose with temperature corresponded to the linear variation of its specific rotation with temperature. The sweetness intensity of α-fructose was estimated to be one-third of that of β-fructose. With L-rhamnose, the sweetness of the α-form was less than $^2/_5$ that of the β-form[6]. As early as 1939, BLAKESLEE[7] collected 25 different combinations of taste responses to mannose tablets from 3121 untrained volunteers. More recently, D-mannose was again referred to as an ambiguous taste stimulator[8,9] as the α-anomer is sweet and the β-anomer is bitter. BOYD and MATSUBARA[10] prepared the 'unnatural' L-forms of glucose and mannose; the former was slightly salty and the latter gave inconclusive results due to lack of agreement among the subjects.

Attempts have been made to correlate the taste of anomers with their chemical configuration, but few reliable generalizations have evolved. TSUZUKI[11] stated that sweeter sugar anomers had *cis*-hydroxyl groups on the carbonyl and adjacent carbon atom, while in the less sweet isomer the hydroxyls on the two carbon atoms were in the *trans* position. This relationship was confirmed for fructose[5] and rhamnose[6], but not for lactose, as the β-form is sweeter[4], yet has the *trans* configuration[12]. Measuring hydrogen-bonding, molecular models, and tasting of crystals of 7 sugars, SHALLENBERGER[13] concluded that sweetness varied with hydrogen bonding of hydroxyl groups. It is difficult to assume that a single hydroxy group – as in ordinary alcohol – can 'cause' sweetness. As pointed out by NICOL[14], the gustatory function of a single group can no more be solely responsible for evoking sweetness, than can hydrogen ion content be the sole cause of sourness. Relative to sugar alcohols, CARR et al.[15] found no relation between the number of carbon atoms, hydroxyl groups, molecular arrangement or spatial configuration and sweetness.

We noted that most investigators tasted the sugars in the crystalline form. STEINHARDT et al.[9] administered samples in the solid state due to the mechanical difficulties of comparing equilibrated solutions with freshly-prepared samples which are rapidly undergoing mutarotation. Their preliminary experiments with mannose had indicated little effect of concentration. Since our previous work had shown marked influence of concentration[4,16], we undertook the present study to determine the effect of concentration on both taste quality and intensity of the α- and β-configurations of fructose, mannose,

[1] J. SOLMS, L. VUATAZ, and R. H. EGLI, Experientia *21*, 692 (1965).
[2] A. T. CAMERON, in *The Taste Sense and the Relative Sweetness of Sugars and Other Sweet Substances* (Sugar Res. Found., Inc., New York 1947), Rept. No. 9, p. 44.
[3] H. G. SCHUTZ and F. J. PILGRIM, Food Res. *22*, 206 (1957).
[4] R. M. PANGBORN and S. C. GEE, Nature *191*, 810 (1961).
[5] Y. TSUZUKI and J. YAMAZAKI, Biochem. Z. *323*, 525 (1953).
[6] Y. TSUZUKI and N. MORI, Nature *174*, 458 (1954).
[7] A. F. BLAKESLEE, Sci. News Lett. *35*, 51 (1939).
[8] R. J. WILLIAMS, in *Biochemical Individuality* (Wiley, New York 1956), p. 128.
[9] R. G. STEINHARDT JR., A. D. CALVIN, and E. A. DODD, Science *135*, 367 (1962).
[10] W. C. BOYD and S. MATSUBARA, Science *137*, 669 (1962).
[11] Y. TSUZUKI, Science (Japan) *17*, 342 (1947).
[12] J. BÖESEKEN, Adv. Carbohyd. Chem. *4*, 189 (1949).
[13] R. S. SHALLENBERGER, J. Food Sci. *28*, 584 (1963). – R. S. SHALLENBERGER, T. E. ACREE, and W. E. GUILD, J. Food Sci. *30*, 560 (1965).
[14] H. NICOL, Brewers' Guild J. *46*, 588 (1960).
[15] C. J. CARR, F. F. BECK, and J. C. KRANTZ JR., J. Am. chem. Soc. *58*, 1394 (1936).
[16] R. M. PANGBORN, J. Food Sci. *28*, 726 (1963).

maltose, xylose, and rhamnose[17]. It is our contention that it is necessary to develop reliable techniques for reproducible measurement of human taste responses before a theory on the causative biophysical factors can be formulated.

Materials and methods. For each sugar, we screened a series of concentrations and selected final levels to range from slightly above threshold to intense. Subjects were 3 men and 3 women with extensive experience in judging the intensity of sweet stimuli. Testing sessions were conducted daily, in individual partitioned booths maintained at 21 °C. Compounds were dissolved in freshly-distilled water and served at 21 °C in 50 ml beakers. Red illumination in the test booths masked visual clues, e.g. bubbles in freshly prepared solutions of maltose. Subjects tasted 25 ml of solution according to a strict time schedule, did not swallow samples, and used distilled water for oral rinsing.

Differences in the taste of each sugar necessitated modification of a standard paired-comparison psychophysical method. Prior to initiating each method, 2 sessions were allocated for orienting subjects to the scaling and timing procedures, using an intermediate sugar concentration. Except for mannose solutions a paired presentation was used throughout. One solution had been prepared at least 6 h in advance to assure equilibrium[18] while the corresponding sample had been placed in solution 3–10 min prior to tasting. The time interval between preparation of the fresh sample and tasting was minimized by adding freshly-distilled water to the weighed compound in individual volumetric flasks as each subject entered the test booth. A stopwatch was activated at the time the water was added to the compound, then presented to the subject along with his samples, to time the tasting. Samples were served in randomized order, and subjects were requested to circle the number of the sample within each pair with the greater sweetness. With maltose and xylose, a labeled standard consisting of an equilibrium solution was presented for comparison. The subject indicated which member of the pair was identical to the standard, then scored sweetness intensity on a 7-point scale 0 = none; 6 = extreme).

[17] Reagent grade samples of β-D-fructose and α-L-rhamnose were purchased from Eastman Organic Chemicals, and α-D-mannose and β-D-maltose from Nutritional Biochemical Corp.; α-D-xylose was a gift of Sterwin Chemicals.

[18] The length of time required for complete equilibration of fructose, rhamnose, mannose, maltose, and xylose was, respectively, 1800, 1800, 3100, 15,750, and 7600 sec.

Table I. Polarimetric readings for xylose and maltose corresponding to the timed sensory comparison of freshly-prepared vs. equilibrated solutions. Ratio of α to β calculated from literature values and determined by extrapolation[19]. Sweetness intensity scores were made on a 7-point scale where 0 = none and 6 = extreme. Sensory responses did not differ significantly as a result of time of tasting.

α-D-xylose $\begin{bmatrix}\alpha\text{-D-xylose } [\alpha]_D^{20} + 92.0 \rightarrow +19.0 \\ \beta\text{-D-xylose } [\alpha]_D^{20} - 20.0 \rightarrow +19.0\end{bmatrix}$ [19]

Polarimetric readings				Sensory response			
Time (min)	$[\alpha]_D^{22}$	% α	% β	Concentration	Correct separation (n = 24)	Sweetness score α	Equilibrated solution
3.5	(+ 86.70)	95	5	0.133 M	75.0% [a]	2.46	1.58 [b]
5.0	(+ 77.30)	87	13	0.333	66.7	2.29	1.83 [a]
6.0	+ 74.78	85	15	0.666	75.0 [a]	3.39	3.38
7.0	+ 71.77	82	18				
Equilibrated solution	+ 19.63	35	65				

β-D-maltose $\begin{bmatrix}\alpha\text{-D-maltose } [\alpha]_D^{20} + 163.6 \rightarrow +130.4 \\ \beta\text{-D-maltose } [\alpha]_D^{20} + 111.7 \rightarrow +130.4\end{bmatrix}$ [19]

Polarimetric readings				Sensory response			
Time (min)	$[\alpha]_D^{22}$	% α	% β	Concentration	Correct separation (n = 48)	Sweetness score β	Equilibrated solution
10.0	+ 118.09	12.3	87.7	0.083 M	41.7	1.15	0.96
12.5	+ 118.20	12.5	87.5	0.194	56.3	1.73	1.63
14.5	+ 118.64	13.4	86.6	0.416	62.5	3.23	3.60 [a]
16.5	+ 118.94	13.9	86.1	0.555	62.5	3.75	4.42 [b]
Equilibrated solution	+ 130.40	36.0	64.0				

[a,b] Significant differences between pairs at $P < 0.05$ and 0.001, respectively.

Solutions of mannose varied in both sweetness and bitterness, precluding the use of a paired test. Consequently, single samples of aged and of freshly prepared solutions were presented and the intensity of sweetness and of bitterness evaluated separately on a 9-point intensity scale (0 = none; 8 = extremely sweet or bitter).

Between 24 and 96 replications were collected per concentration for each compound. The same number of replications were collected for each subject, so that all subjects contributed equally to the observed results. Timed polarimetric readings were made on each sugar, and the ratio of α to β was calculated[19].

Results. As indicated in Tables I and II, the observed polarimetric readings corresponded closely with the values in the literature[19]. Concentrations of the compounds influenced taste differences to varying degrees. With xylose, the α-form was sweeter than the equilibrium solution at 0.133 and 0.333 M, whereas they were equally sweet at 0.666 M (Table I), despite the fact that $^3/_4$ of the responses showed correct separation at the latter concentration. This indicates that at equivalent sweetness intensities other sensory criteria assisted in distinguishing between the 2 forms. Correct separation of the 2 forms of maltose approached only $^2/_3$, less than that required for statistical significance (Table I). Sweetness intensity scores showed the equilibrium solution to be generally sweeter than the β-configuration at higher concentrations.

It was confirmed that the β-anomer of fructose is sweeter than an equilibrium mixture (Table II). The influence of concentration can be noted, as it was for rhamnose where the sweetness of the equilibrated solution exceeded that of the α-form. The latter compound mutarotated rapidly (30 min) and the subjects noted significant differences in sweetness intensity during the $7^1/_2$ min of testing. For mannose the α-form and the equilibrium mixture elicited both sweetness and bitterness. The exact relationship of the taste properties can be readily seen in the Figure. The α-configuration is sweeter and less bitter than the equilibrium mixture. Analyses of variance of these data showed significant differences in bitterness attributable to configuration, and subjects, but not to

[19] Literature values upon which ratio of α to β was calculated for fructose, rhamnose, mannose, maltose, and xylose, respectively, obtained from C. S. HUDSON and E. YANOVSKY, J. Am. chem. Soc. *39*, 1013 (1917). – E. L. JACKSON and C. S. HUDSON, J. Am. chem. Soc. *59*, 1076 (1937). – J. S. FRUTON and S. SIMMONDS, in *General Biochemistry* (Wiley, New York 1959), p. 406; J. Res. natn. Bur. Stand. *18*, 152 (1937); Int. crit. Tabl. *2*, 352 (1927).

Table II. Polarimetric readings for fructose and rhamnose corresponding to the timed sensory comparison of freshly-prepared vs. equilibrated solutions. Ratio of α to β calculated from literature values and determined by extrapolation[19]. Sensory responses are expressed in terms of selection of sweeter sample within each pair. Time of tasting had a significant influence on response to rhamnose ($P < 0.05$), but not fructose

β-D-fructose $\begin{bmatrix}\alpha\text{-D-fructose } [\alpha]_D^{20} - 21.0 \rightarrow -92.0\\ \beta\text{-D-fructose } [\alpha]_D^{20} - 133.5 \rightarrow -92.0\end{bmatrix}$ [19]

Polarimetric readings				Sensory response		
Time (min)	$[\alpha]_D^{22}$	% α	% β	Concentration	% sweeter ($n = 96$)	
					β	Equilibrated solution
3.3	(– 112.70)	18	82	0.061 M	60.4	39.6
5.0	– 106.50	24	76	0.244	70.8	29.2[b]
6.6	– 102.72	27	73	0.427	70.8	29.2[b]
3.3	– 99.90	30	70			
Equilibrated solution	– 91.38	37	63			

α-L-rhamnose $\begin{bmatrix}\alpha\text{-L-rhamnose } [\alpha]_D^{20} - 7.7 \rightarrow 8.9\\ \beta\text{-L-rhamnose } [\alpha]_D^{20} - 38.0 \rightarrow 8.9\end{bmatrix}$ [19]

Polarimetric readings				Sensory response		
Time (min)	$[\alpha]_D^{22}$	% α	% β	Concentration	% sweeter ($n = 48$)	
					α	Equilibrated solution
4.5	(– 1.71)	84.9	15.1	0.165 M	27.1	72.9[a]
5.5	(– 0.29)	81.3	18.7	0.274	20.8	79.2[b]
6.5	+ 0.98	78.1	21.9	0.494	18.8	81.3[b]
7.5	+ 1.82	76.0	24.0			
Equilibrated solution	+ 8.77	58.5	41.5			

[a, b] Significant differences between pairs at $P < 0.01$ and 0.001, respectively.

replication, indicating that despite wide variation among subjects, there is consistency from one replication to the next.

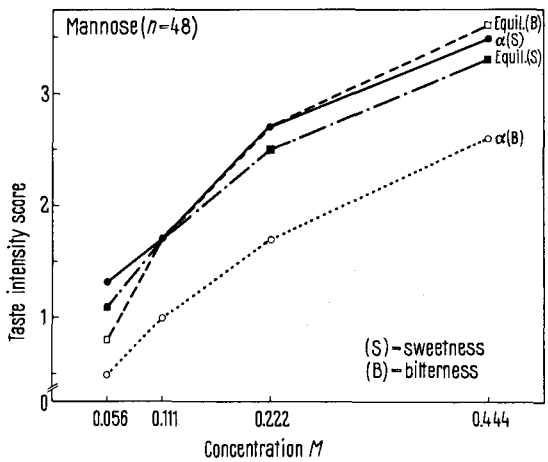
Several factors influence the variability of taste results and could contribute to the 'uncertainty' mentioned by SHALLENBERGER[13]. (a) Among several sugars, concentration has a marked effect on taste differences. SHALLENBERGER's conclusion that β-D-glucose was sweeter than the α-form may be attributable to tasting only crystals. (b) In compounds having multiple tastes it is difficult to assign intensity values to one sensation independent of the other. This was observed with mannose, as reported herein and by others[8–10], and was also observed with xylose which appeared to have several tastes. (c) The purity of the compound obviously could contribute to variability of response. The multiple tastes of xylose could have been due to impurities. Despite the fact that the maltose was the highest purity available, distinct differences in 'flavor' were observed between Lots No. 5319 and 2710. KARE and MEDWAY[20] also speculated that the inability to obtain maltose of absolute purity contributed to their results on taste discrimination by the fowl. In spite of being analytically pure, some compounds adsorb volatile, odorous constituents from the environment, e.g. from contact with paper, plastic or metal, which interfere with a true taste response. (d) It is recognized that subjects differ in sensitivity, as well as in their estimation of intensity and interpretation of quality. This can be minimized by careful selection and training.

Despite the variability mentioned above, this investigation reconfirms that there are distinct differences in taste between configurations of these anomeric carbohydrates. Both quantitative and qualitative gustatory properties can be measured reliably by trained human subjects, thus providing a basis upon which molecular biologists can elucidate the causative mechanisms.

Zusammenfassung. Es wurden quantitative und qualitative Geschmacksvergleiche zwischen frisch zubereiteten und ins Gleichgewicht gebrachten Lösungen von Fruchtzucker, Mannose, Malzzucker, Xylose und Rhamnose angestellt. Erfahrene Versuchspersonen stellten in bestimmten Zeitintervallen die Geschmackswirkung der Lösung fest, die mit polarimetrischen Zeitmessungen verglichen wurden.

R. M. PANGBORN and R. B. CHRISP[21]

College of Agriculture, University of California, Davis (California, USA), March 14, 1966.



Intensity of sweetness and bitterness of 4 concentrations of freshly prepared and equilibrium solutions of α-D-mannose. (0 = none; 8 = extreme.) Each point represents 48 individual judgments.

[20] M. R. KARE and W. MEDWAY, Poult. Sci. *38*, 119 (1959).

[21] We gratefully acknowledge the participation of the subjects and the technical assistance of Mrs. IDA M. TRABUE.

About a Possible Participation of Nucleic Acids in Synaptic Transmission

Some time ago, while studying the parasympathetic regulation of cardiac rhythm in *Rana temporaria*[1,2], some evidence was obtained which might imply an involvement of RNA in synaptic function. This finding is now reported in detail. The choice of neuromyocardial synapses for experimentation was suggested by several data found in the literature. An effect of nucleic acids and nucleotides on the conduction system and on the refractory period of the heart was observed by DRURY and SZENT-GYORGYI[3] and by DRURY[4]. In 1956 ROBB[5] reported that, after stimulation of the vagus nerve, nucleic acid derivatives in the dog heart differed from those present when the sympathetic nerve was stimulated. The neuromyocardial system had also the advantage of being well known and readily accessible to quantitative experimentation.

The experiments were performed on *R. temporaria* hearts perfused with Ringer's solution. The cardiac rhythm was blocked by stimulating the nuclei of the vagus nerve or the sensory area of the splanchnic by means of electrodes[2]. Blockage was also obtained by acetylcholine ($1.0 \cdot 10^{-6} M$). Neutral trypaflavine[6] ($1.0 \times 10^{-4} M$) was dissolved in Ringer's solution.

Figure 1 shows that trypaflavine $1.0 \cdot 10^{-4} M$ is capable of inhibiting the blocking action on the heart caused by electric stimulation of the nuclei of the vagus or of the receptive field of the splanchnic; it can also inhibit the blocking action of acetylcholine. The duration of the trypaflavine effect is longer in the case of electrical stimulation of the splanchnic area than in the case of the

[1] P. VOLPE, Boll. Soc. ital. Biol. sper. *7*, 4 (1962a).
[2] P. VOLPE, Boll. Soc. ital. Biol. sper. *7*, 5 (1962b).
[3] A. N. DRURY and A. SZENT-GYORGYI, J. Physiol. *68*, 213 (1929).
[4] A. N. DRURY, Physiol. Rev. *16*, 292 (1936).
[5] J. S. ROBB, Am. J. Physiol. *187*, 626 (1956).
[6] A mixture of 2,8-diamino-10-methylacridinium chloride and of 2,8-diaminoacridine containing, when dried at 105 °C for 2 h, not less than 13.3% and not more than 15.8% of Cl. The nitrogen is about 16.2%.