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THE PLASMA MEMBRANES OF BOVINE CIRCUMVALLATE PAPILLAE ISOLATION AND PARTIAL CHARACTERIZATION

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SUMMARY

1. A procedure has been established to isolate plasma membranes and other subcellular membranes from bovine circumvallate papillae by means of discontinuous sucrose density gradient centrifugation. From bovine circumvallate, which is the papilla that contains the most taste buds, about 240 μg plasma membrane proteins per g tissue, or 3.1 μg membrane protein per mg homogenate protein, was obtained.

2. Electron microscopy and marker enzyme studies confirmed the purity of the membrane fractions. Among the cellular membranes, the plasma membranes have the highest specific activities of ouabain-sensitive (Na^+–K^+–Mg^{2+})-ATPase (EC 3.6.1.4) and 5′-nucleotidase (EC 3.1.3.5).

3. [^{14}C]Fructose was found to bind preferentially to the taste papillae plasma membranes when mixed with the particulate fraction of the whole homogenate. The [^{14}C]fructose-bound plasma membranes could be subsequently isolated by sucrose density gradient centrifugation. On the other hand, plasma membranes from mount papillae which contain no taste buds failed to show such preferential binding with the taste stimulant fructose.

4. The preferential binding of ^{14}C-labeled fructose to the plasma membranes of circumvallate papillae suggests that taste receptors of taste tissues may be located in the plasma membranes.

INTRODUCTION

The sensations of sweet, bitter, sour and salty tastes are familiar experiences to man. Electrophysiological studies have documented the neural specificities in taste responses[1,2]. But the physiological and chemical processes at the tissue level that lead to these neural responses are unknown. Peripheral receptor(s) at the site of contact with taste stimulant has long been postulated, but not yet identified[2–4]. Attempts have been made to isolate specific "taste-receptor protein" from tongue epithelium[5–9]. But the authenticity and merits of these "taste proteins" are doubtful, and their reproducibility have been questioned[10,11].

The plasma membranes are dynamic structures and perform a wide range of physiological functions, to name a few, the sites for cellular specificities such as antigenic recognition, hormonal receptors, and viral-tumor transformations (*e.g.* polyoma

virus-transformed BHK-21 cells)[12], are largely located in the plasma membranes of various cells. One is led to speculate that taste specificity may be one of the functions of the plasma membranes of taste receptor tissues, *i.e.* the taste papillae on the tongue surface. This reasoning prompted me to study the plasma membranes of taste papillae. The circumvallate papillae from bovine tongues were used in this study because they are rich in taste buds. Behavioral studies have also shown that cows can distinguish sweet sugars from non-sweet substances[13]. In order to study the interactions of taste stimulants and the plasma membranes of taste papillae, a procedure was developed to isolate plasma membranes from bovine circumvallate papillae. The isolation procedures and partial characterizations of the plasma membranes are given in this report. Evidences for a receptor role for the plasma membranes are also presented.

MATERIALS AND METHODS

Materials

Fresh bovine tongues were purchased from a local slaughterhouse and were kept on ice during transportation.

All chemicals were reagent grade. All nucleotides were purchased from P. L. Biochemicals; cytochrome *c* and bovine serum albumin, from Sigma Chemical Co.; [U-^{14}C]fructose, scintillation grade toluene and 2,4-dioxane, from New England Nuclear; PPO and dimethyl-POPOP, from Packard Instrument Company, Inc.

Methods

Preparation of plasma membranes from bovine circumvallate papillae

Various means to disrupt the tongue tissues and subsequently to isolate their plasma membranes for the special case of taste papillae were investigated in order to optimize yields and minimize destructions. The following procedure is the final adopted method for our investigations.

After arriving at the laboratory, the fresh bovine tongues were quickly cut from excess back tissues and cleaned with deionized water. The tongues were dissected in an ice-filled tray. The circumvallate papillae in two rows (approximately 20–24 pieces) at the posterior part of the tongues were quickly removed to a petri dish on ice. Equal numbers of mount papillae located at the central posterior region of the tongue were taken as control materials since they contain no taste buds. Ten tongues yielded approximately 1.7 g and 0.5 g of fresh tissues of circumvallate and mount papillae, respectively. The tissues were finely chopped while kept on ice. An aliquot of deionized water buffered with Tris at pH 8.4 was added to the tissues, which were allowed to stand at room temperature for 3 min. Then the samples were quickly brought to isotonic conditions with ice-cold sucrose in 5 mM Tris–1 mM EDTA, pH 8.4 (Medium A). All subsequent procedures were carried out at 4 °C. A 10% tissue homogenate in 0.25 M sucrose–5 mM Tris–1 mM EDTA, pH 8.4 (Medium B) was prepared with Tenbroeck all-glass hand homogenizer with 5–8 strokes. This part of the procedure involving hypotonic treatment and homogenization was completed in less than 7 min. The homogenate, filtered through three layers of cheese cloth, was ready for ultracentrifugation and membrane fractionations.

A diagram of the isolation scheme is shown in Fig. 1.

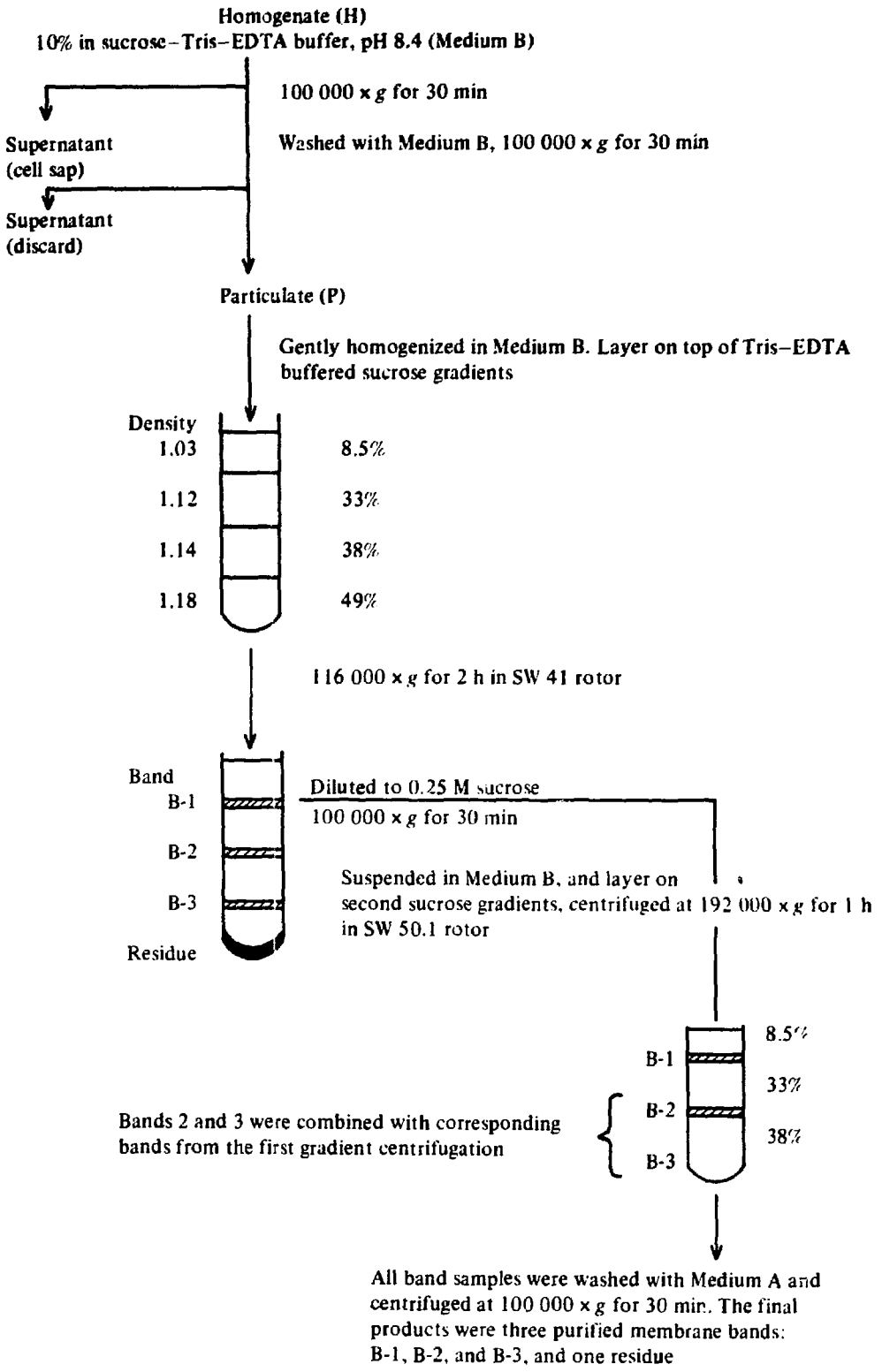

Fig. 1. Schematic diagrams for the preparation of plasma membranes from bovine circumvallate papillae. Detailed procedures are given in the next.

The filtered whole homogenate was centrifuged at 100000 × *g* for 30 min in Spinco 65 rotor in a Beckman Model 65L ultracentrifuge. The resultant supernatant is the cell sap, while the pellet contains all the particulate cellular components plus

cell debis. The pellet was washed once with Medium B, and recentrifuged as before. The washed pellet was suspended in a suitable volume of Medium B (density = 1.03) by gentle hand homogenization. The suspension was layered on top of discontinuous sucrose gradients composed of 3 ml each of 49% sucrose (density = 1.18), 38% sucrose (density = 1.14), and 33% sucrose (density = 1.12). These three sucrose solutions were prepared in Medium A. The gradient samples were then centrifuged at 116000 × *g* for 2 h in Spinco SW 41 buckets. Three membrane bands were obtained at the interphases of *d* = 1.03/1.12 (B-1), *d* = 1.12/1.14 (B-2), *d* = 1.14/1.18 (B-3), respectively, and a residue at the bottom of the tube. The membrane bands were carefully aspirated into separate tubes. The residue was washed once with saline and stored as saline suspension. B-1 was diluted to 0.25 M sucrose with Medium A and sedimented at 100000 × *g* for 30 min in a Spinco 65 rotor, and was further purified by a second sucrose density gradient centrifugation. The second sucrose gradients consisted of 2.5 ml each of 33% and 38% sucrose in Medium A. The sample in Medium B was layered at the top and was centrifuged at 192000 × *g* for 1 h in Spinco SW 50.1 buckets. The first membrane band at *d* = 1.03/1.12 was the plasma membrane Fraction B-1. The second faint membrane band at *d* = 1.12/1.14 was combined with B-2 of the first gradient. The pellet in trace amount was combined with B-3. All three membrane fractions were diluted and sedimented as before, followed by washing once with 0.15 M NaCl in Medium A. The pellets were immediately processed according to the needs of subsequent experiments.

Electron microscopy

The sedimented membrane samples were immediately pre-fixed for 1 h in cold freshly prepared half-strength Karnovsky fixative containing 2.5% glutaraldehyde and 2% paraformaldehyde in 50 mM cacodylate buffer (pH 7.2), followed by washing twice with cold 8% sucrose–140 mM veronal acetate buffer (pH 7.4). Then the samples were post-fixed for 1 h in 1% OsO_4 in sucrose–veronal acetate buffer, and treated with 0.5% uranyl acetate in veronal acetate buffer at pH 5.2 for 2 h. The specimens were dehydrated through graded ethanol solutions and embedded in Epon according to Luft[14]. Ultrathin sections from Epon-embedded samples were mounted on carbon-covered grids and stained with 1% uranyl acetate in 50% ethanol and lead citrate[15]. Electron micrographs were taken with a Philips EM 300 electron microscope.

Enzyme assays

The activities of marker enzymes for each membrane fraction were studied to assess the purity of the plasma membranes. Membrane pellet samples were washed and suspended in a small volume of isotonic saline solution and divided into several portions. Each portion was diluted to a convenient volume with appropriate buffer for each individual enzyme assay system. Aliquots were also taken for protein determinations. The ouabain-sensitive (Na^+–K^+–Mg^{2+})-ATPase (EC 3.6.1.4) activities were assayed according to Wallach and Ullray[16] with modifications. The reaction mixture consisted of 50 mM Tris buffer (pH 7.6), 0.5 mM EDTA, 150 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, 2 mM Tris–ATP (pH 7.6), 0.1 mM ouabain, and about 20 μg membrane proteins. 5′-Nucleotidase (EC 3.1.3.5) activities were assayed according to Michell and Hawthorne[17]. The reaction mixture consisted of 50 mM Tris buffer (pH 7.4), 100 mM KCl, 10 mM $MgCl_2$, 10 mM sodium–potassium tartrate, 5 mM AMP, and about 20 μg membrane protein. Reaction mixtures for both enzymes were incubated at 37 °C for 15 min and were terminated by boiling in

a water bath for 2 min. P_i released from the enzyme reactions was determined by the method of Chen *et al.*[18]. The activities of microsomal NADH:cytochrome *c* reductase (EC 1.6.99.3) were determined according to Mackler[19] in a mixture containing 40 mM potassium phosphate buffer (pH 7.5), 10 mM NaN_3, 0.01% NADH, 0.1% cytochrome *c*, and membrane protein in 1% sodium cholate. The mitochondrial succinate:cytochrome *c* reductase (EC 1.3.99.1) activities were determined according to Tisdale[20] in a mixture of 10 mM potassium phosphate buffer (pH 7.4), 1 mM NaN_3, 2 mM EDTA, 0.005% bovine serum albumin, 1 mM potassium succinate, 0.1% cytochrome *c*, and membrane proteins. Both the microsomal and mitochondrial enzyme reactions were followed at 15-s intervals for 3 min at 37 °C.

Chemical analyses

Protein was determined either by the method of Lowry *et al.*[21] or by the microbiuret method of Itzhaki and Gill[22], depending on the amount of protein to be assayed. Protein samples were first precipitated with cold 10% trichloroacetic acid, washed twice with the same reagent, followed by extraction twice with ether–ethanol (3:1, v/v) and twice with ether–ethanol (3:2, v/v). The protein residues were finally dissolved in 1.0 M NaOH. Aliquots in duplicates were used for assay.

Binding of [U-^{14}C]fructose to membrane fractions

The 100000 × *g* pellet from the whole homogenates of both circumvallate and control mount papillae were suspended by homogenization in 0.25 M fructose in Medium A containing 10 μCi [U-^{14}C]fructose (spec. act. 121 Ci/mole) per 0.5 g tissue. The mixtures were incubated at room temperature for 10 min with constant stirring. At the end of the incubation period, the fructose concentrations of the samples were adjusted to 1.1 M (38%). The samples were layered at the center of the discontinuous sucrose density gradients, centrifuged for 2 h at 116000 × *g* in a SW 41 rotor. It is important to layer the samples at the center gradient to allow the plasma membranes to spin upward in the gradients, and also to allow particles to move upward or downward in approximately equal distances. Membranes with densities of 1.14 and 1.18 will travel the shortest distances. This procedure was designed to ensure that any preferential bindings of fructose to the plasma membranes were not due to artifacts from centrifugation through density barriers. Three sucrose and one fructose density cushions, three membrane bands, and one residue were obtained. They were collected into separate tubes and were designated as S-1 (8.5% sucrose), S-2 (33% sucrose), S-4 (49% sucrose), S-3 (38% fructose), B-1 (d=1.03/1.12), B-2 (d=1.12/1.14), B-3 (d=1.14/1.18), and residue, respectively. The membrane bands were diluted and well-mixed with Medium A to fill the 13-ml tubes and sedimentated at 100000 × *g* for 30 min in a Spinco 65 rotor. The supernatants were saved as the washings from the membranes. The membrane sediments were suspended in a small volume of deionized water. The S samples of dense sucrose and fructose solutions were diluted 5-fold with deionized water. Aliquots from the membrane suspensions, together with aliquots from the diluted S samples, and the membrane washing supernatants were taken for radioactivity determinations in Bray's[23] solution at 70% efficiency in a Model 3375 Packard liquid scintillation counter. Results from the latter two solutions were combined as the final S sample values shown in Table III. It is necessary to dilute the concentrated sucrose gradient solutions before counting to avoid quenching and precipitation in the counting fluor. The remainder of the membrane suspensions were taken for protein determinations.

RESULTS

Plasma membrane isolation

A procedure has been developed for the isolation of plasma membranes from bovine taste papillae. Fig. 1 summarizes the fractionation scheme. The generally established procedures for the isolation of plasma membranes from liver, brain,

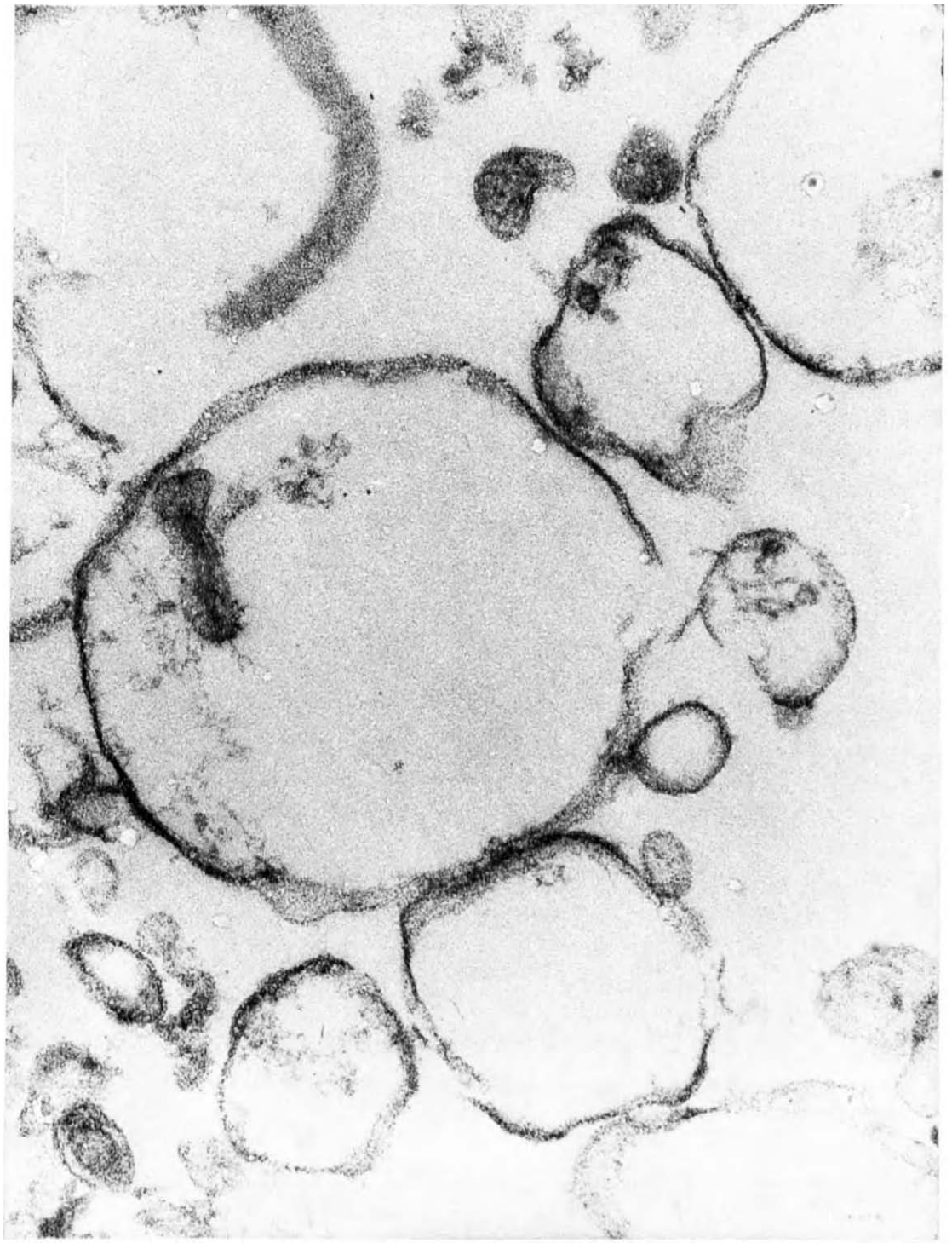

Fig. 2. Electron micrograph of thin section of bovine circumvallate membrane band B-1 (d= 1.03/1.12) from sucrose density gradient centrifugation. Stained with uranyl acetate and lead citrate. × 108800.

erythrocytes and a number of other tissues[24,26] were unsuitable for taste papillae from the bovine tongue. One difficulty was that the epithelial papillae tissue was too tough to be broken apart by simple hand homogenization in isotonic sucrose or NaCl solution. Even with the help of ultrasonication, it was still tedious and time consuming. This problem was solved by a brief hypotonic exposure of the tissues followed by hand homogenization in an all-glass apparatus. The brief hypotonic treatment rendered the papillae susceptible to homogenization without damaging the integrity of the plasma membranes as shown by their intact morphology and enzyme activities.

Another difficulty was that the papillae tissues absorb water and become swollen in the homogenization medium. In the initial low speed centrifugation steps required by the conventional plasma membrane isolation procedures[24,25], separation was impossible. In the present procedure, the initial centrifugation was performed at high speed to precipitate all the homogenate particulate fractions, from which plasma membranes were to be isolated. When subjected to discontinuous sucrose gradient centrifugation, the homogenate particulate yielded three membraneous fractions and a residue. A second sucrose gradient centrifugation was employed to purify the plasma membrane fraction and to minimize the contamination by the endoplasmic reticulum and mitochondria. The complete fractionation procedure produced five fractions, the cell sap, three membrane bands (B-1, B-2, and B-3), and one residue.

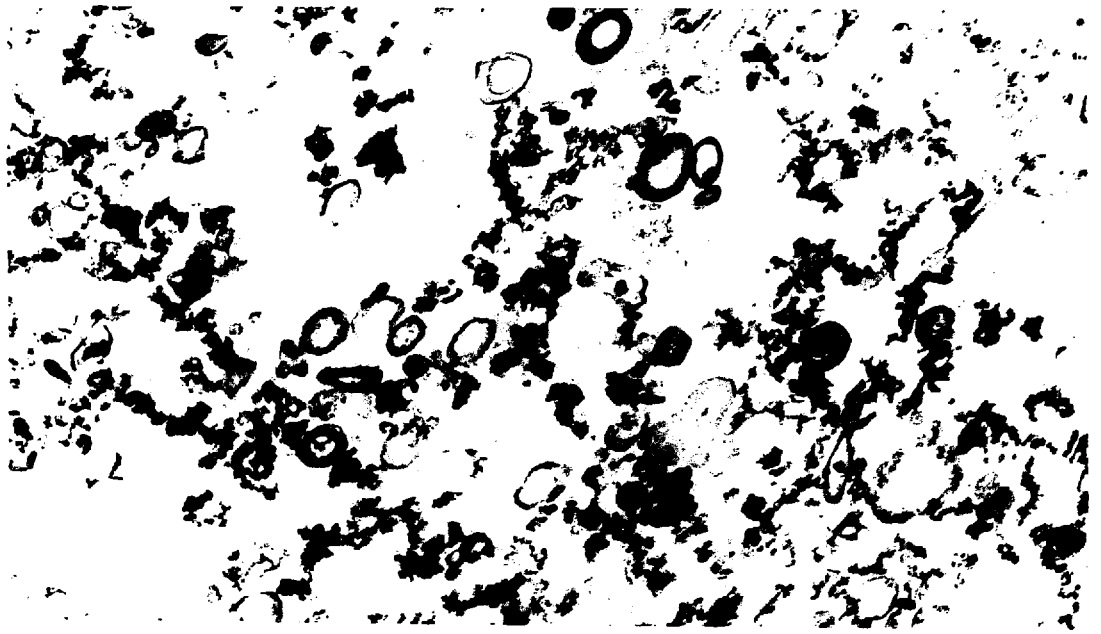

Fig. 3. Electron micrograph of bovine circumvallate membrane band B-2 (d= 1.12/1.14) from sucrose density gradient centrifugation. Thin section. ×24000.

The morphology of the three membrane bands, as seen from the electron micrographs in Figs 2–4, indicates that membrane B-1 (d=1.03/1.12) of the bovine circumvallate papillae consists largely of plasma membranes, membrane B-2 (d= 1.12/1.14) contains the microsomal fraction of the cell, and membrane B-3 (d= 1.14/1.18) shows clearly the structures of mitochondria.

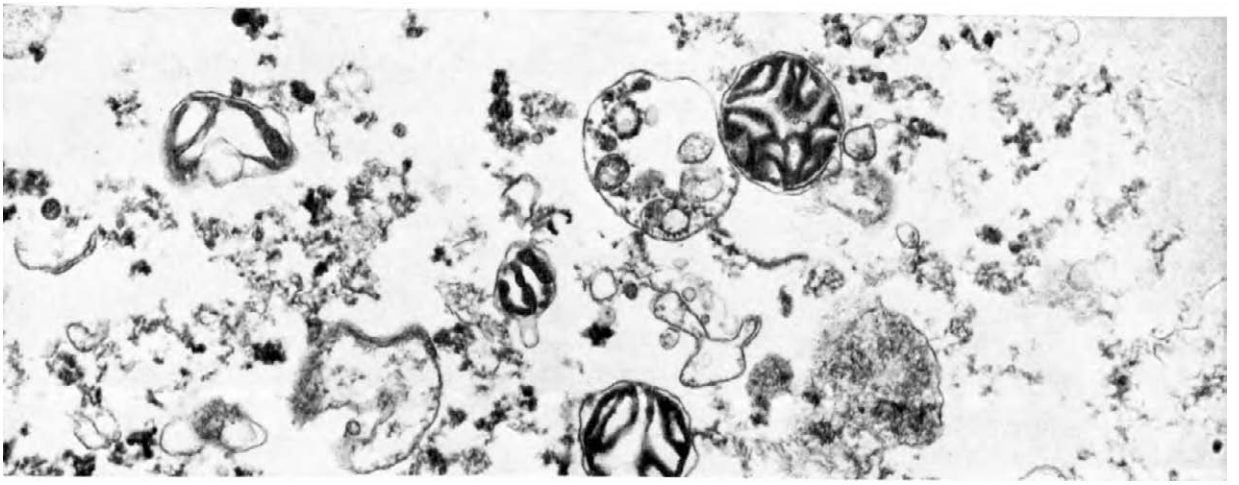

Fig. 4. Electron micrograph of bovine circumvallate membrane band B-3 (d= 1.14/1.18) from sucrose density gradient centrifugation. Thin section. × 24000.

Protein distributions

The protein distributions of the circumvallate and mount papillae are shown in Table I. An average of 78 mg protein per g tissue was obtained from both the circumvallate and the mount papilla tissues. The average plasma membrane yield was 243 μg membrane protein per g circumvallate tissue (3.1 μg membrane protein per mg homogenate protein), and 147 μg membrane protein per g mount papillae (1.9 μg membrane protein per mg homogenate protein). The overall protein distribution patterns were similar in both tissues.

TABLE I

PROTEIN DISTRIBUTIONS OF BOVINE TONGUE PAPILLAE

Protein contents of subcellular fractions from bovine circumvallate and mount papillae are presented in mg protein per g fresh tissue ± S.E., except that the values for membrane proteins are given in μg per g tissue ± S.E. Results (in parentheses) are also expressed as % of total homogenate protein. The results are from the mean values from 8–10 experiments. 8–10 fresh tongues were used for each experiment. Detailed fractionation procedures were given in the text and in Fig. 1.

Fraction	*mg protein/g tissue ± S.E.*	
	Circumvallate papillae	*Mount papillae*
Homogenate	77.5 ± 7.2 (100)	78.1 ± 2.2 (100)
Cell sap	22.5 ± 1.7 (29.0 ± 2.3)	16.4 ± 1.4 (21.1 ± 1.3)
Particulate	41.2 ± 2.0 (56.1 ± 2.5)	43.9 ± 4.0 (56.4 ± 5.5)
Membranes		
B-1	243.4 ± 28.8 (0.32 ± 0.06)	146.6 ± 12.1 (0.19 ± 0.05)
B-2	180.0 ± 24.6 (0.23 ± 0.06)	176.2 ± 13.9 (0.23 ± 0.04)
B-3	206.5 ± 38.3 (0.27 ± 0.08)	169.1 ± 33.1 (0.22 ± 0.05)
Residue	37.2 ± 1.9 (48.4 ± 3.6)	39.5 ± 3.7 (51.2 ± 5.7)

Enzyme activities

The morphological findings from the electron microscopy were corroborated by the distributions of activities of marker enzymes associated with various membrane fractions. As shown in Table II, membrane B-1 exhibited the highest specific activities of both 5'-nucleotidase and ouabain sensitive (Na^+–K^+–Mg^{2+})-ATPase. The 5'-nucleotidase activities are comparable to those found in plasma membranes from rat liver reported by Emmelot *et al.*[26] (32.2 μmoles P_i/mg protein per h), but the circumvallate ATPase activities are higher than those reported by these authors (they reported 11.6 μmoles P_i/mg protein per h)[26]. Membrane Fraction B-2 exhibited the highest activity of the microsomal enzyme NADH:ctytochrome *c* reductase, whereas Fraction B-3 was the only fraction that contained appreciable activity for in the mitochondrial enzyme succinate dehydrogenase.

TABLE II

SPECIFIC ACTIVITIES OF CELLULAR MEMBRANE-ASSOCIATED ENZYMES FROM BOVINE TONGUE PAPILLAE

Membrane samples from sucrose density gradient centrifugations were washed with 0.15 M NaCl and suspended in appropriate buffer solutions. Enzyme activities (mean values from 5 duplicate determinations) were determined immediately at 37 °C.

Tissue	*Fraction*	*μmoles P_i/mg per 15 min ± S.E.**		*μmoles cytochrome c reduced/mg per min ± S.E.*	
		*Ouabain-sensitive (Na^+–K^+–Mg^{2+})-ATPase**	*5'-Nucleotidase*	*Microsomal NADH: cytochrome c reductase*	*Succinate: cytochrome c reductase*
Circumvallate papilla	Homogenate	2.6 ± 1.2 (1.77)	0.5 ± 0.1	0.06 ± 0.02	0.02 ± 0.01
	Membrane				
	B-1	13.5 ± 6.0 (1.41)	5.9 ± 3.0	0.21 ± 0.05	negligible
	B-2	7.5 ± 3.0 (1.21)	1.1 ± 0.3	0.44 ± 0.08	0.06 ± 0.01
	B-3	2.3 ± 0.5 (1.21)	0.8 ± 0.4	0.28 ± 0.06	0.14 ± 0.01
Mount papilla	Homogenate	0.8 ± 0.37 (1.47)	0.7 ± 0.2	0.07 ± 0.04	—
	Membrane				
	B-1	2.5 ± 1.0 (1.50)	3.1 ± 1.1	0.12 ± 0.02	—
	B-2	0.7 ± 0.03 (1.05)	1.2 ± 0.5	0.25 ± 0.06	—
	B-3	1.5 ± 0.5 (1.12)	1.3 ± 0.5	0.18 ± 0.04	—

* Values in the parentheses are ratios of (Na^+–K^+–Mg^{2+})-ATPase to Mg^{2+}-ATPase activities.

Binding of [^{14}C]fructose to cellular membranes

In order to demonstrate the role, if any, of the plasma membrane of taste papillae in taste perception, the homogenate particulate fraction was incubated with ^{14}C-labeled fructose, then subjected to density gradient centrifugation. The binding profile of the radioactive taste stimulant to the three cellular membrane fractions were shown in Table III. As expected, the S samples contained over 90% of the total radioactivity. The significant finding is that the plasma membranes (B-1)

TABLE III

BINDING OF [^{14}C]FRUCTOSE TO CELLULAR MEMBRANES OF BOVINE TONGUE PAPILLAE

[^{14}C]Fructose (spec. act. 121 Ci/mole) was incubated for 10 min at room temperature with the particulate fraction in Tris–EDTA buffer (pH 8.4) containing 38% fructose. Samples were layered at the center of centrifuge tubes containing sucrose density gradients, and centrifuged for 2 h at 116000 × *g* in SW 41 rotor. In addition to the three membrane bands (B-1, B-2, B-3) and a residue, the sucrose gradient cushions were also collected and were combined with the washings from the membrane bands, designated as S-1, S-2, S-3, and S-4, respectively. Aliquots of the S-samples and membrane suspensions were counted in Bray's solution. Radioactivities are presented as total cpm × 10^{-3}. Specific activities are given as cpm × 10^{-3} per mg protein.

Fraction	*Circumvallate papilla*		*Mount papilla*	
	cpm × 10^{-3}	*Specific activity*	*cpm* × 10^{-3}	*Specific activity*
Particulate	19 700.0	856.0	17 600.0	835.0
S-1	2 591.0		2 588.0	
B-1	150.0	217.0	7.3	120.0
S-2	6 039.0		8 130.0	
B-2	140.0	140.0	19.9	218.0
S-3	4 547.0		4 865.0	
B-3	13.6	25.3	6.4	71.6
S-4	810.0		224.0	
Residue	5.9	0.46	45.0	4.78
Recovery (%)	72.5		90.0	

of taste papillae, circumvallate, contained the highest specific radioactivity among the three cellular membrane fractions, despite the fact that B-1 had to travel the farthest to reach equilibrium with its buoyant density, because the samples were layered with the 38% gradient at the center of the gradient centrifuge tube. The ^{14}C-labeled sugar showed a definitive preference to be associated with the plasma membranes of a taste receptor tissue, the circumvallate. In contrast, fructose binding to the membranes from the mount papillae devoid of taste buds was of a non-specific nature; not only that the B-1 plasma membrane fraction had less counts than B-2, the radioactivity distribution pattern correlated inversely with the relative distance from where the sample was layered. The preferential binding can also be demonstrated by other means, such as by gel filtration technique on Sephadex column. Isotope binding activities of isolated membranes are being studied.

DISCUSSION

This report describes, for the first time, a procedure for the isolation of plasma membranes from taste receptor tissue, the circumvallate papillae from bovine tongues. This report also provides, for the first time, experimental evidence suggesting that the plasma membranes of a bovine taste papilla contain receptor sites for fructose.

By precipitating all the particulates from crude tissue homogenate, we elimin-

ated the stepwise fractionations required by most plasma membrane isolation procedures, thus saving operation time. The sucrose density gradient centrifugation steps can be achieved either by spinning samples downward or upward, except in the isotope binding experiments, the samples were layered at the center of the tube. The second gradient centrifugation is needed to further purify the plasma membrane fraction.

The presence of taste receptors in the taste cell membranes has been postulated by earlier investigators[3]. Our finding is the first positive demonstration of a preferential binding between a taste stimulant and plasma membranes of a taste receptor tissue. In the procedures described for the isotope binding studies, the plasma membrane fraction had to travel the longest distance to reach its buoyant density equilibrium, yet it carried with itself the highest specific radioactivity against two gradient barriers. Membranes from non-taste tissue bound only non-specifically with the same stimulant (Table III). These findings suggest that only the plasma membranes from bovine taste papillae contain fructose receptors which are absent in the other intracellular membranes of the same tissue, and are also absent in the neighboring mount papillar tissue devoid of taste buds. Recently, Cagan[27] reported that sucrose binds better with crude whole homogenates of bovine taste papillae than with non-taste tissue homogenates.

Although bovine taste buds are concentrated in the circumvallate (Davies, R. O. and Kare, M. R., unpublished), the B-1 fraction obtained from the whole circumvallate papilla undoubtedly contained plasma membranes from cells other than the taste buds. This could have been a serious criticism to this report. However, the finding that among all the intracellular particulate species, B-1 of the circumvallate has the highest specific binding activity toward a sweet substance, and the finding that B-1 from control papillae failed to exhibit a similar function leave little doubt that sweet taste receptors exist on the plasma membranes of a taste receptor tissue, namely the bovine circumvallate papilla. The question that remains to be answered is whether the sweet receptors are restricted to plasma membranes of taste buds, or the plasma membranes of all cell types within the taste papilla are able to bind with taste substances.

Other reports on the isolation of plasma membranes from chemosensory receptor tissues, to my knowledge, are by Koch[28] and Koyama *et al.*[29]. Koch[28] reported the isolation of plasma membrane (E_2) from rabbit olfactory epithelium at the interphase of 0.8 M–1.0 M sucrose (27–34%) on sucrose density gradients. The E_2 fraction contained 0.1 mg/g tissue and with an ouabain-sensitive ATPase activity of approximately 21 μmoles P_i/mg protein per 30 min. Recently, Koyama *et al.*[29] obtained a plasma membrane (B-II) fraction from bovine olfactory epithelium at the 30–33% sucrose interphase on density gradients. They gave no protein values, but reported a specific ATPase activity of 7.48 μmoles P_i/mg per h. In our studies, the specific activity of ATPase was 13.5 ± 6.0 μmoles P_i/mg protein per 15 min at 37 °C. The circumvallate membranes also had a 5′-nucleotidase activity of 5.9 μmoles P_i/mg protein per 15 min at 37 °C.

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