Disc Electrophoresis of Extracts from the Taste Buds Located in Circumvallate Papillae of Rat Tongues

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ABSTRACT   The epithelium of the circumvallate papillae of rat tongues was stripped off by treatment with 0.005% elastase in a state when many taste buds were present. The taste buds were isolated from the stripped epithelium by further treatment with 0.005% elastase and 0.08% trypsin. A protein which was thought to be characteristic of taste buds was found from semi-micro disc polyacrylamide gel electrophoretic studies of the stripped epithelia with and without taste buds. This result was supported by micro disc polyacrylamide gel electrophoretic studies of isolated taste buds.

INTRODUCTION

Many investigators of taste receptors have postulated, mainly from electro-physiological and behavioral studies, that the taste substances would interact with a protein, denoted receptor protein, in the taste receptors. Dastoli and Price (1) extracted from bovine tongues a protein which combined with the substances which taste sweet to man and which they denoted "sweet-sensitive protein." Since then, several biochemical studies on taste receptors have been reported (2–9). These reports, however, are not conclusive and contradict each other. In general, a quantity of taste receptors is small and it is hard to separate them without contaminating other tissues. This may be the cause of these contradictions. If we can obtain a preparation containing a large proportion of taste receptors, biochemical studies may result in a reliable analysis.

In the present paper, I will describe a method for obtaining a preparation rich in taste receptors from the circumvallate papillae of rat tongues, and will show disc electrophoretic characteristics of extracts from the preparation.

MATERIAL AND METHODS

Epithelia Containing Taste Buds

Female Wistar king rats, 6- to 7-wk old, were anesthetized with an intraperitoneal injection of sodium barbital. The tongues were removed from the bodies after blood-

letting by cutting the hearts. Four types of papillae and region A without any taste buds are shown in Fig. 1. A circumvallate papilla contains hundreds of taste buds in a limited region and so is an appropriate papilla for the preparation rich in taste buds. The dorsal dermis with the circumvallate papilla was removed from the tongue with a razor on a layer of beeswax in a Petri dish. The papilla was cut off as cleanly as possible (2 × 2 mm² in area, 1–1.5 mm in thickness) from the isolated dorsal
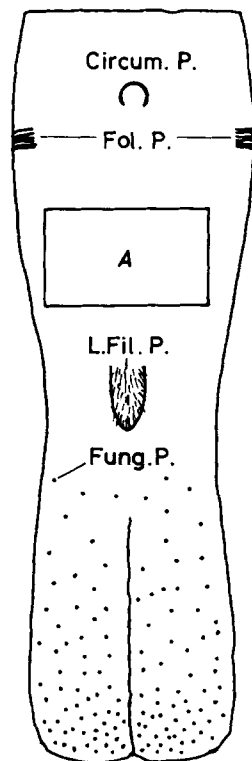

Figure 1.   Schematic representation of a rat tongue. Circum. P., circumvallate papilla; Fol. P., foliate papilla; L Fil. P., largest filiform papilla; Fung. P., fungiform papilla. Taste buds are contained in Fol. P. and Fung. P. and most plentifully in Circum. P. Region A is the dorsal dermis without any taste bud.

dermis under a binocular stereomicroscope. Fig. 2 shows the cross section of the papilla and it is observed that taste buds exist in the epithelial layer of the trench. 10–20 papillae were prepared together, washed with Hanks's balanced salt solution (pH 7.0, referred to as H(+) sol), and then treated with 0.005 % elastase (Grade 1, Batch 23, Miles-Servac [Pty] Ltd., Maidenhead, Berks, England) in 5 ml H(+) sol at 4 °C for 6 h at 120 vibrations per min with the aid of a shaker (Fig. 3, middle). Thereafter, a split was made between the epithelium and the underlying dermis from the edge up to a region close to the trench with forceps. These papillae were treated once more with 5 ml of the new enzymic solution at 60 vibrations per min for 6 h. Then, the epithelia were completely stripped off. Stripped epithelia could be obtained in this
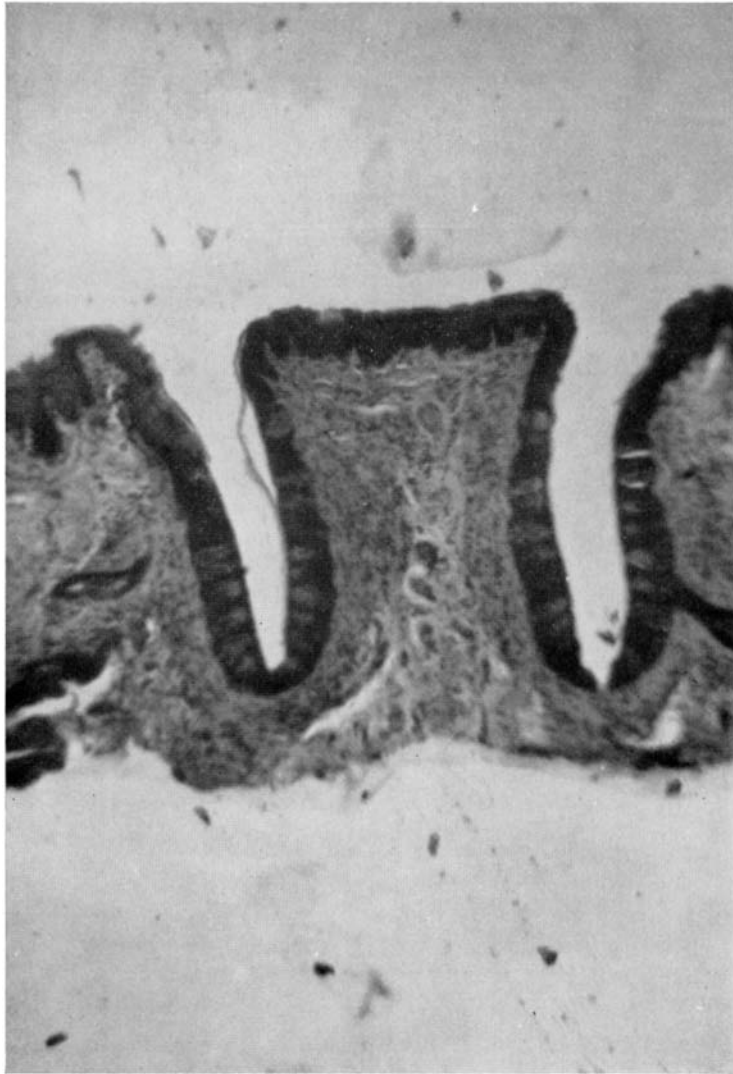
FIGURE 2.   Cross section of a circumvallate papilla. Taste buds exist in the epithelial layer of the trench. Fixed with glutaraldehyde, and stained with hematoxyline and eosin.

way, and the epithelial layer of the trench did not seem to be damaged. Fig. 4 is a photograph of the ventral view of a whole stripped epithelium. The two dark semicircles are the epithelial layers on both sides of the trench. Fig. 5 *a* and *b* shows the cross sections of the stripped epithelium and of the rest of the papilla, respectively. As observed in this figure, taste buds were contained completely in the stripped epithelium and none were left in the rest of the papilla. This stripped epithelium, therefore, is one of the preparations rich in taste buds as stated in the Introduction. On the
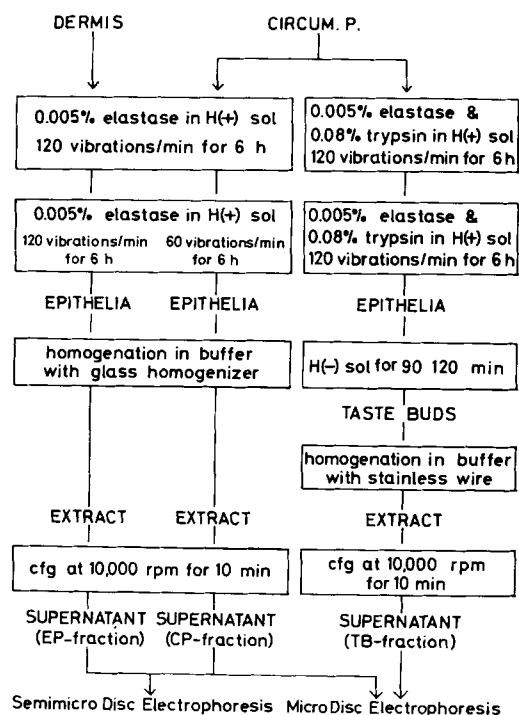
FIGURE 3. Procedures used to obtain extracts for disc electrophoresis. See text for preparations of the circumvallate papillae and of the dermis (dorsal dermis of region A in Fig. 1). Usually 10–20 circumvallate papillae and 5–10 sections of dorsal dermis were used. H(+) sol, Hanks's balanced salt solution (pH 7.0); H(−) sol, Hanks's balanced salt solution free of Ca^{++} and Mg^{++} (pH 7.0); buffer, 0.1 M phosphate buffer solution (pH 7.0). See text for EP-, CP-, and TB-fraction. All procedures were done at around 4°C.

other hand, 5 to 10 sections of dorsal dermis of region A in Fig. 1 were cut into pieces and the epithelia were stripped off just after treatment with 0.005 % elastase at 120 vibrations per min for 12 h (Fig. 3, left).

Epithelia stripped from 10–20 circumvallate papillae were washed with 0.1 M phosphate buffer solution (pH 7.0) and homogenized in 0.1 ml buffer solution with a glass homogenizer of about 0.12 ml volume, which was made from a 0.25 ml injection syringe. The crude extract was centrifuged at 10,000 rpm for 10 min, and the supernatant was denoted CP-fraction. The final volume of CP-fraction was about 0.08 ml. Similarly, supernatant was prepared from the epithelia stripped from 5 to 10 sections of dorsal dermis of region A and was denoted EP-fraction. Its volume was about 0.3 ml. 25 to 100 μl and 0.5 to 1.5 μl of these supernatants were submitted to semimicro and micro disc electrophoresis, respectively. Protein concentration of the supernatants was determined after Lowry et al. (10) and was adjusted to 1 and 2 mg/ml for CP- and EP-fraction, respectively. All procedures were done at around 4°C.
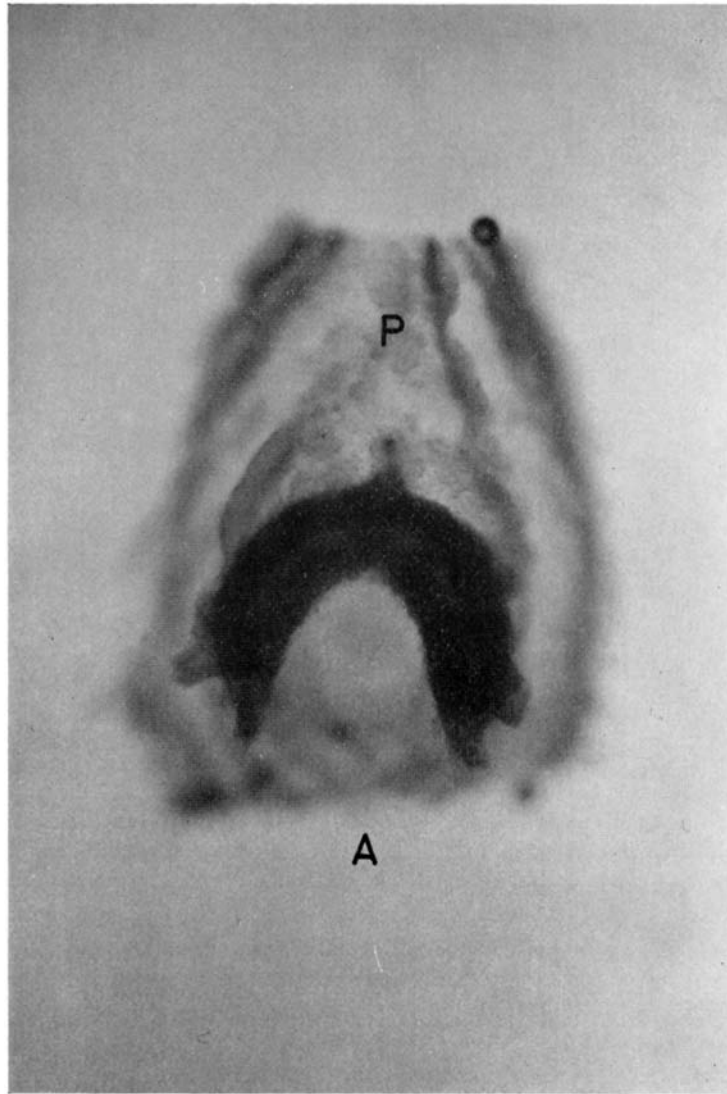
FIGURE 4. A ventral view of a whole epithelium stripped from the circumvallate papilla. The two dark semicircles are the epithelial layers on both sides of the trench, the bottom of which looks a little bright between the dark semicircles. A, anterior end of the papilla; P, posterior end.

Isolation of Taste Buds

5–10 circumvallate papillae were treated twice with 0.005% elastase and 0.08% trypsin in 5 ml H(+) sol as described in the previous section (Fig. 3, right). The stripped epithelia, containing many taste buds, were washed several times with Hanks's balanced salt solution free from Mg^{++} and Ca^{++} (pH 7.0, referred to as H(−) sol) and soaked in 20 ml of solution for 90–120 min. Then, the stripped epi-
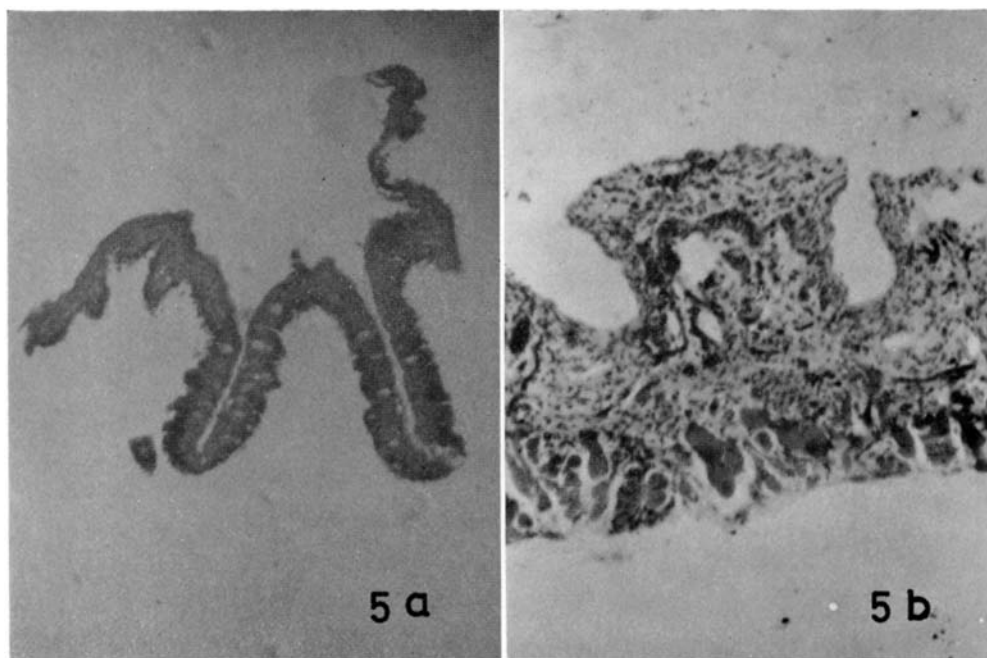
FIGURE 5.   (a), Cross section of an epithelium stripped from the circumvallate papilla. The taste buds are seen in the epithelial layer of the trench. (b), Cross section of the dermis of the circumvallate papilla after stripping the epithelium. No taste bud is found on the surface of the dermis.

thelia were washed several times with 0.1 M phosphate buffer solution and put in the small glass slide chamber filled with buffer solution. The layer containing taste buds was carefully and gently scratched under a binocular stereomicroscope with a stainless wire needle of 200 μm diameter, the tip of which was tapered with nitric acid and coated with 5% silicone varnish in toluene. The taste buds were isolated by this treatment. Many of the isolated taste buds were of onion-like shape as *in situ*, both axes of which were 40–70 μm (Fig. 6 *a*). Some of them were damaged during isolation of the taste buds and changed in shape (indicated by an arrow in Fig. 6 *a*). A single taste bud is shown under a higher power in Fig. 6 *b*. The tip of the taste bud is indicated by an arrow. As many as 100 taste buds could be isolated from one stripped epithelium without heavy deformation.

The taste buds were isolated from two stripped epithelia at a time. The isolated taste buds of intact shape were selected and collected in a region in the chamber packed as closely as possible. A suspension of taste buds was sucked in a micropipette (150 μm in diameter at the tip) and transferred into a capillary tube (about 10 μl; 80 mm in length, 400 μm in diameter), which was filled with the suspension three-quarters full. This tube contained less than 100 taste buds, and was then centrifuged at 800 rpm for 10 min. The supernatant without taste buds was discarded. A suspension of taste buds in the stock was added to the remainder in this tube, centrifuged, and the supernatant discarded. After this procedure was repeated 5–10 times, the tube
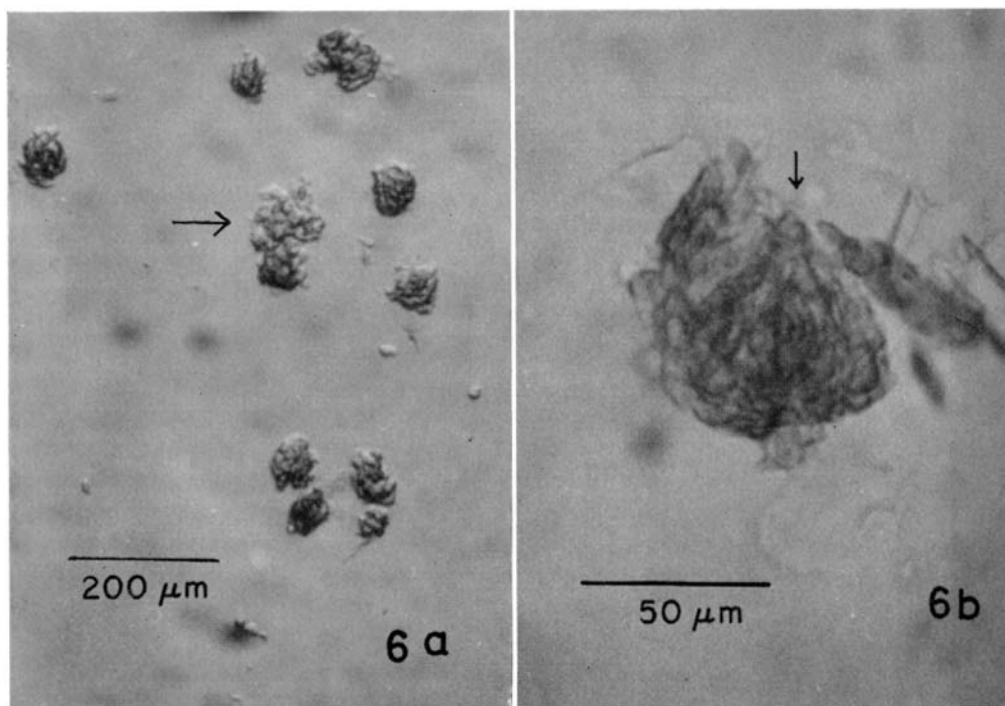
FIGURE 6. (a), Several isolated taste buds. The arrow indicates one or two deformed taste buds. (b), Single isolated taste bud under a higher power. The arrow indicates the tip of the taste bud.

contained hundreds of taste buds. The collected taste buds were washed twice with buffer solution, and the tube was half filled with buffer solution. The upper part of the tube was broken off. A loop of stainless wire, 5 cm in length, 30 μm diameter, attached to a motor-driven blender, was introduced into the tube and the taste buds were homogenized at 12,000 rpm for 3 min. The homogenate was then centrifuged in this tube at 10,000 rpm for 10 min. Thus, 5 μl of the supernatant was obtained. This supernatant was denoted TB-fraction and submitted to micro disc electrophoresis. Protein concentration of the supernatant was determined after Lowry and was 0.1–0.5 μg/μl. All procedures were done at around 4°C.

Preparation of Gels and Electrophoresis

Commercial monomers of acrylamide and bisacrylamide were recrystallized after Loening (11). Electrophoresis was carried out at around 4°C.

(A) SEMIMICRO DISC ELECTROPHORESIS   The inner walls of the glass tubes (2 mm ID, 7 cm in length) were coated with Driwell (photo wetting agent, Fuji Photo Film Co., Ltd., Tokyo, Japan). 7.5 % gels at pH 8.9 were prepared in the glass tubes after Ornstein (12) and Davis (13). 25–50 μl of CP- or EP-fraction was put on the spacer gel. Electrophoresis was carried out at 0.5 mA per tube for 80–100 min. The gels were stained with 0.5 % amido black in 7.5 % acetic acid solution and destained

with 7.5% acetic acid. The destained gels were photographed, while the optical densities of the stained protein discs in the gels were recorded.

(b) MICRO DISC ELECTROPHORESIS The electrophoretic technique used was a micro scale modification (14, 15) of the original disc electrophoretic method of Ornstein and Davis.

The inner wall of 5-μl glass capillaries (440 μm in diameter, 33 mm in length. Drummond Scientific Co., Broomall, Pa.) were coated twice with 0.05% methyl cellulose (4,000 cps, Katayama Kagaku Kogyo Co., Ltd., Osaka, Japan) in 30% formic acid-formaldehyde solution (1:5) (16).

Stock solutions

Stock A: pH 8.9; 4.3 g Tris, 0.315 ml N,N,N',N'-tetramethylethylenediamine, 3.6 N H_2SO_4 to pH 8.9, and water to 50 ml. Stock B: pH 6.7; 2.85 g Tris, 1 M H_3PO_4 to pH 6.7, and water to 50 ml. Stock C: 6.67 g acrylamide, 0.067 g N,N'-methylene-bisacrylamide, 1.25 mg $K_3Fe(CN)_6$, and water to 12.5 ml. Stock D: 70 mg $(NH_4)_2$ S_2O_8, 25 ml 2% Triton X-100, and water to 50 ml. Stock E: pH 6.7; 2.99 g Tris, 0.23 ml N,N,N',N'-tetramethylenethylenediamine, 1 M H_3PO_4 to pH 6.7, and water to 50 ml. Stock F: 400 mg $(NH_4)_2S_2O_8$, 10 ml 2% Triton X-100, and water to 20 ml. Electrode buffer: pH 8.5; 3.0 g Tris, 14.4 g glycine, and water to 500 ml.

Preparation of Gels

80 mg acrylamide and 4 mg hydantoin were added to the mixture of 0.1 ml stock A, 0.3 ml stock C, and 0.4 ml stock D. This solution gave a 30% polyacrylamide gel for the lower gel. The mixture solution of 0.15 ml stock E, 0.05 ml stock C, 0.05 ml stock F, and 0.28 ml water gave a 5% polyacrylamide gel for the upper gel.

Each of the capillaries was filled with 30% gel solution to half its volume with the aid of capillary action. The polymerization proceeded overnight at around 4°C. Before each run, the 5% gel solution was carefully added to the 30% gel solution, to measure 2 mm, with a micropipette. This gel was polymerized for 10–20 min. Superfluous water on the top of the gel was sucked off, and stock B solution diluted to 1:8 with water was added to the top of the capillaries and removed after a few minutes. Then, 0.5–1.5 μl of the supernatant was applied with a micropipette. The electrophoresis was carried out at a constant current of 30 μA per capillary, and terminated after about 30 min when the bromophenol blue band had migrated 10 mm into the lower gel. Then, the gels were immediately pushed out of the capillaries into 7.5% acetic acid. After staining for 5 min, the gels were destained in 7.5% acetic acid. The gels were photographed while the optical density of the stained protein discs was recorded with Joyce-Loebl-Double-Beam Micro-densitometer (Joyce, Loebl & Co., Inc., Burlington, Mass.). The ratio between the height of the gel in the capillary and the length of the recording paper was 20.

RESULTS

Semimicro Disc Electrophoresis

More than 20 bands could be detected in the gel separation patterns resulting from electrophoresis (pH 8.9) of each fraction. Figs. 7 A, B, and C represent
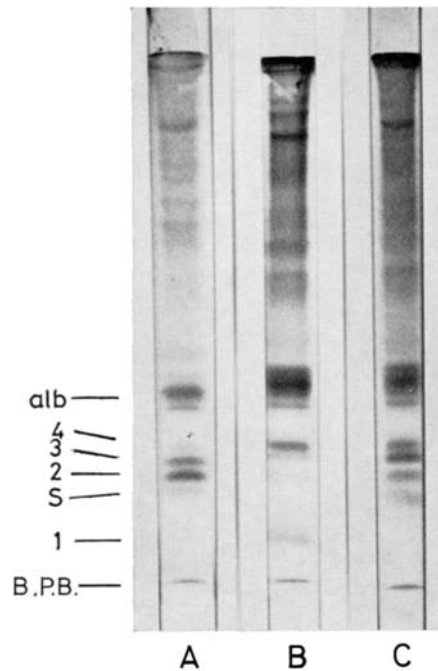
FIGURE 7. Gel separation patterns resulting from semimicro disc electrophoresis at pH 8.9. (A), CP-fraction. (B), EP-fraction. (C), mixture of CP- and EP-fraction. alb, albumin band. B.P.B., bromophenol blue band. Numbers are attached to the bands of fast moving proteins in the order of their speed. The amount of the protein applied to the gel was 35 μg in (A), 60 μg in (B), and 35 μg of CP-fraction and 25μg EP-fracin (C). Band S is missing in (B). Band 1 cannot be seen in (A) (this band could be found by increasing the protein of CP-fraction applied to the gel to 100 μg). The pattern in (C) shows the overlapped pattern of both of CP- and EP-fraction, although band 1 can scarcely be seen.

the photographs of the gel separation patterns of CP-, EP-fraction, and mixture of CP- and EP-fraction, respectively, where numbers are attached to the bands of the fast moving proteins in the order of their speed. The patterns of CP- and EP-fraction are schematically shown in Figs. 8 A and B, respectively. The pattern of CP-fraction remarkably resembled that of EP-fraction as a whole. Any distinct differences could not be found in the cathodic portion between them, but some differences were found in bands 2, 3, and 4. Differences were also found in the bands near the albumin one. These differences, however, were slight and would not be able to characterize CP- or EP-fraction. Band 1 in EP-fraction was far denser than that in CP-fraction; this is not seen in Fig. 2 A. This band may be characteristic of EP-fraction. Moreover, a special band was always found between bands 1 and 2 of CP-fraction, but could not be found in the pattern of EP-fraction. This band was denoted band S. Even when the protein of EP-fraction applied to the gel was
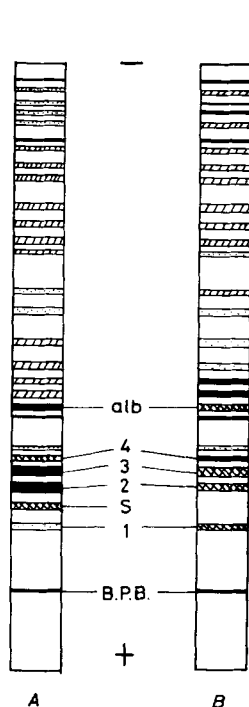
FIGURE 8   FIGURE 9

FIGURE 8. Schematic representation of Fig. 7. (A), CP-fraction. (B), EP-fraction. Symbols attached to each band are the same as in Fig. 7.

FIGURE 9. Densitometric recordings of gel separation patterns resulting from semi-micro disc electrophoresis at pH 8.9 (not the same preparations which were used in Fig. 7). Ordinate, distance from the origin of the separation gel. (A), EP-fraction. (B), CP-fraction. Symbols attached to each peak are the same as in Fig. 7. Note that the peak of band S is missing in (A). The peak of band 1 is faintly seen in (B).
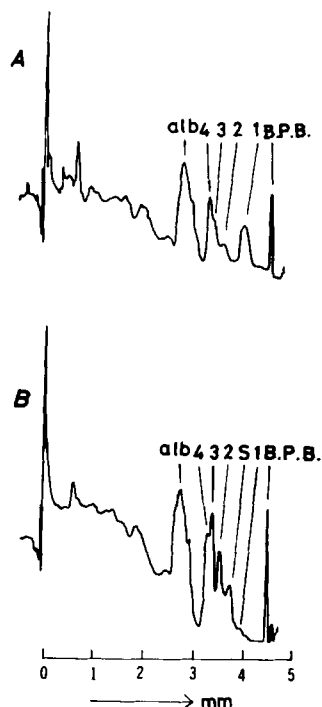
increased to as much as 100 μg and even when the gel was stained with Coomassie brilliant blue R-250, band S could not be found in the pattern of EP-fraction. Since band S was located near band 2, a mixture of CP- and EP-fraction was submitted to electrophoresis to inspect the possibility that band S in CP-fraction could be identical to band 2 in EP-fraction. The resulting pattern (Fig. 7 C) showed the completely overlapped pattern of both CP- and EP-fraction. This result indicates that band S was not band 2 in EP-fraction. Thus, band S was concluded to be characteristic of CP-fraction.

Fig. 9 A and B shows the densitometric recordings of the electrophoretic patterns of EP- and CP-fraction, respectively. The symbol attached to each peak corresponds to that in Fig. 7. It is obvious that the peak of band S was missing between the peaks of bands 1 and 2 in EP-fraction (Fig. 9 A). The

peak of band 1 in CP-fraction (Fig. 9 B) was almost missing, but was clearly found by increasing the amount of CP-fraction applied to the gel. Since CP-fraction contained a large proportion of the proteins originating from the taste buds, it was suggested that the protein of band S originated from the taste buds. This suggestion was confirmed by micro disc electrophoresis of isolated taste buds, as shown below.

No differences could be found between CP- and EP-fraction in the semi-micro disc electrophoresis at pH 4.5.

Micro Disc Electrophoresis

The concentration of 30% of the separation gel was found to be the optimum to demonstrate the bands characteristic of CP-fraction in the micro disc electrophoresis, and the following micro disc electrophoresis was carried out at this concentration. Fig. 10 B, C, and D shows the photographs of the gel separation patterns resulting from micro disc electrophoresis (pH 8.9) of CP-, EP-fraction, and a mixture of CP- and EP-fraction, respectively. The patterns of CP- and EP-fraction are schematically shown in Fig. 11 A and B, respectively. Numbers are attached to the fast moving proteins. Major bands were separated over a range from the origin to 4 or 5 mm in the separation gel, where about 20 bands were counted. Several dispersed bands were found at the more anodic portion, which were omitted in Figs. 10 and 11. The pattern of CP-fraction remarkably resembled that of EP-fraction as a whole, as expected from the results of semimicro disc electrophoresis. But a band characteristic of CP-fraction could also be found at an anodic portion
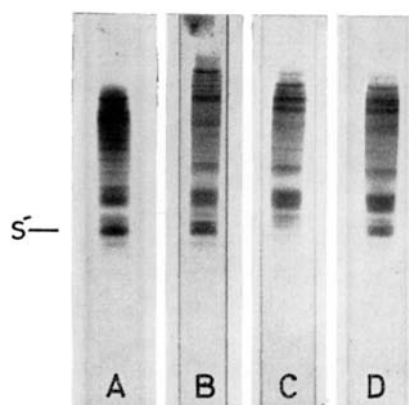

FIGURE 10. Gel separation patterns resulting from micro disc electrophoresis at pH 8.9. (A), TB-fraction; (B), CP-fraction; (C), EP-fraction; (D), mixture of CP- and EP-fraction. The amount of protein applied to the gel was 0.2–0.4 μg in (A), (B), and (C), and 0.15 μg of each CP- and EP-fraction in (C). Band S', indicated, is seen in (A), (B), and (D), but not in (C). The pattern in (D) shows the overlapped pattern of both CP- and EP-fraction. Some bands of fast-moving proteins are omitted.
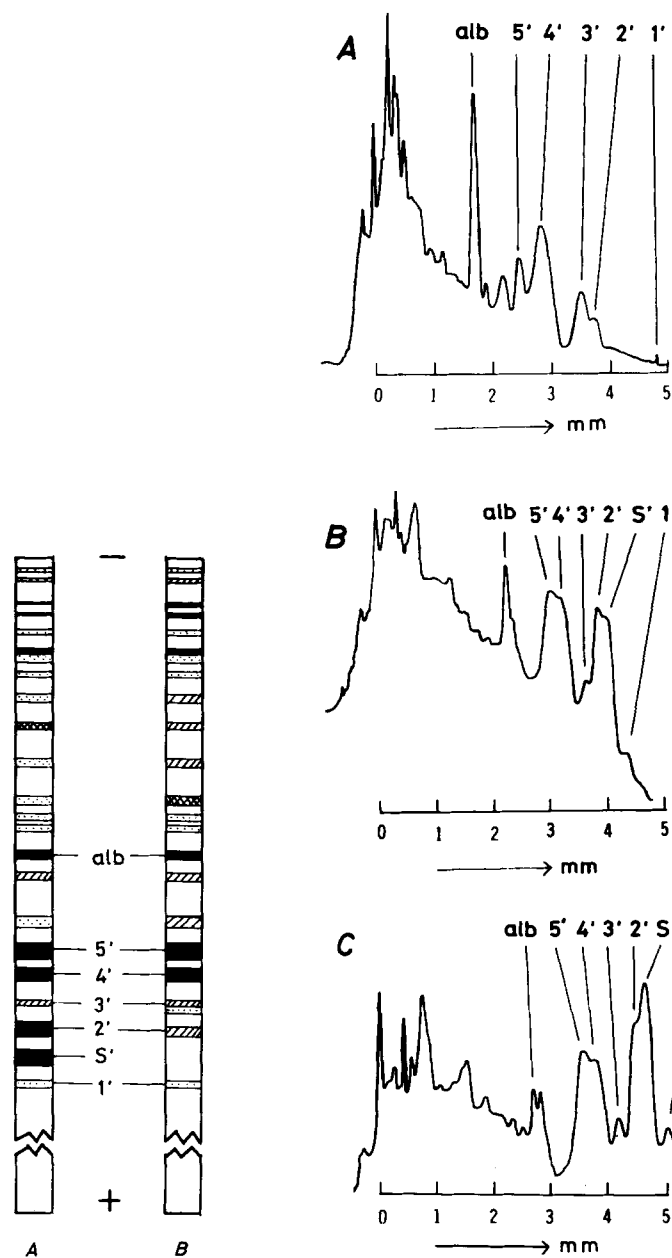
FIGURE 11

FIGURE 12

FIGURE 11. Schematic representation of gel separation patterns in micro scale. (A), CP-fraction; (B), EP-fraction. Symbols attached to each band are the same as in Fig. 7.

FIGURE 12. Densitometric recordings of gel separation patterns resulting from micro disc electrophoresis at pH 8.9 (not the same preparations which were used in Fig. 10). Ordinate, distance from the origin of the separation gel. (A), EP-fraction; (B), CP-fraction; (C), TB-fraction. Symbols attached to each peak are the same as in Fig. 11. 0.005–0.01 µg of bovine serum albumin was added to the sample applied to the gel in (A) and (B). Note that the peak of band S' is missing between the peaks of bands 1' and 2' in (A). The relative value of the optical density of band S' to the total is larger in (C) and (B) are considerably larger than in (A).

between bands 1' and 2', as indicated by band S' in Fig. 10. Even when the gel was stained with Coomassie brilliant blue, band S' could not be found in the pattern of EP-fraction. A mixture of CP- and EP-fraction was submitted to electrophoresis. The result (Fig. 10 D) showed the completely overlapped pattern of the CP- and EP-fractions. Besides band S', band 2' in CP-fraction was significantly more prominent than in EP-fraction. Band 2' may also be characteristic of CP-fraction. Although band 1, characteristic of EP-fraction, was found in semimicro scale, such a band could not be found in micro scale.

Since the bands characteristic of CP-fraction could be found in micro scale, TB-fraction was submitted to micro disc electrophoresis. Fig. 10 A shows the pattern of TB-fraction. Band S' could also be found. This band in TB-fraction was seen to be slightly denser than in CP-fraction. Band S' in TB-fraction was identical to that in CP-fraction, since electrophoresis of a mixture of TB- and CP-fraction resulted in a single band S'.

Fig. 12 A, B, and C shows the micro densitometric recordings of the electrophoretic patterns of EP-, CP-, and TB-fraction, respectively. The samples of EP- and CP-fraction applied to the gel in Fig. 12 contained 0.005–0.01 μg of bovine serum albumin as a marker protein. The peak of the albumin band was slightly changed in location with recordings, mainly because of slight differences between the states of the inner walls of the glass capillaries coated with methyl cellulose. It was clearly indicated, however, that the peak of band S' was missing between the peaks of bands 1' and 2' in EP-fraction and that the relative value of the optical density of band S' to the total was larger in TB-fraction than in CP-fraction. Besides, the relative value of the optical density of band 2' in TB- and CP-fraction was larger than in EP-fraction. The amount of protein of band S in CP- and TB-fraction was roughly estimated to be 6 and 10% of the protein entering the separation gel, respectively.

DISCUSSION

Band S' could scarcely be found or not at all if the inner wall of the glass capillary was not coated with methyl cellulose. Thus, coating of the inner wall was technically an important step. Coating twice with methyl cellulose was needed to demonstrate band S' clearly.

Bands S' and S had the following similarities: (a) both bands were characteristic of CP-fraction, (b) both bands were located at a more anodic portion compared to the albumin band. The protein of these bands, therefore, was thought to be at least partially common in both bands, although the electrophoretic conditions in semimicro and micro scale were considerably different from each other.

In preparing TB-fraction, it was impossible to collect only taste buds with-

out considerable contamination of other tissues such as the germinal layer. Nevertheless, band S', characteristic of CP-fraction, was also found in TB-fraction, where the concentration of protein of this band was significantly higher than that of CP-fraction. These results strongly support the idea that the protein of band S', a part of which would be identical to the protein of band S, originated from the taste buds. The amount of protein of bands S and S' was estimated to be 10^{-9} to 10^{-10} g per taste bud if these proteins originated only from the taste buds.

The gel separation patterns resulting from semimicro disc electrophoresis of CP- and EP-fraction remarkably resembled each other except for bands S and 1, which were characteristic of CP- and EP-fraction, respectively. When commercial monomers of acrylamide and bisacrylamide were used without recrystallizing, the anodic portion of the gel separation patterns in semimicro disc electrophoresis was considerably changed. But a band characteristic of CP-fraction could also be found in these cases.

Koyama and Kurihara (7) could not find such a band characteristic of a preparation extracted either from the circumvallate papillae or from the fungiform papillae of bovine tongues in semimicro disc electrophoresis. They determined that the minimum amount of protein detectable by their electrophoretic technique was around 10^{-7} g per gel, using serum albumin as a standard protein sample. On the other hand, the amount of protein of band S was at most 10^{-9} to 10^{-10} g per taste bud in this study. If one taste bud located in the circumvallate papilla of the bovine tongue could contain this amount of protein of band S, this protein would amount to 10^{-6} to 10^{-7} g per gel in the preparation of Koyama and Kurihara (7). Besides, considering that their preparation would have contained a large quantity of tissue other than taste buds (e.g. muscle, underlying dermis of the papillae, etc.), it can be understood that a band such as band S could not be found in their preparation.

The "sweet-sensitive protein" is basic (1, 2), but the protein of bands S and S' is acidic and so the protein of these bands could not be the "sweet-sensitive protein." At present, the relation between the proteins of these bands and the "bitter-sensitive protein" (3) is unknown.

In isolating taste buds, trypsin had to be used with elastase, but did not affect the gel separation patterns resulting from semimicro disc electrophoresis. The possibility cannot be excluded that treatment with such enzymes might induce some denaturations in the proteins contained in the taste buds.

Although it could not be concluded that the protein of band S' or band S is not contained at all in the epithelium without any taste bud, it can be said that this protein would be characteristic of taste buds. It was not clarified, however, whether or not this protein related to taste reception in the taste buds. This essential problem is left open for further studies.

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