their subjective judgments of taste by having them draw lines to reflect the strength and the pleasantness of a taste (*8*). We tested ten individuals twice, once after a 14-hour fast (just before breakfast) and next, just after lunch (*9*). The taste stimuli were six concentrations each of glucose, NaCl, citric acid, and quinine sulfate (*10*). Three confirmatory replicate judgments were obtained for each quality (sweet, salty, sour, bitter), each concentration, each attribute (intensity, pleasantness), and each condition (fasting, after eating) (*9*). Small paper cups holding about 10 ml of solution were presented in a random order of concentration at room temperature. The subject was required to rinse his mouth with water between samples.

Subjects rated the more concentrated solutions as tasting stronger (Fig. 1). Since many of the functions appear to have long linear segments in log-log coordinates, the middle range of concentrations seems to produce power functions relating subjective taste intensity to physical molarity (*11*). In contrast, the ratings of preference are more curvilinear (*12*). The preferences for sour and bitter are anomalous. Whereas Western populations find the taste of citric acid and quinine to be unpleasant, the Karnataka laborers reported that citric acid became increasingly pleasant as the concentration was increased, and that quinine sulfate was exceptionally pleasant at low concentrations and became less pleasant only with large increases of concentration. We found no significant differences between ratings of pleasantness made under the two hunger conditions, in contrast to the hypothesis of alliesthesia (*13*), which predicts that satiety reduces the pleasantness of the sweet taste (*13, 14*).

Our findings bear upon two major issues in responses to simple sensory stimuli: (i) the influence of dietary (or genetic) factors and (ii) the influence of body state (hunger or satiety). The anomalously high pleasantness ratings for citric acid and quinine sulfate differ from the ratings for similar compounds made by Western subjects (*1*), and of Indian medical students tested in the same locale by similar procedures (*15*). The Karnataka group scored intensity and pleasantness of glucose and NaCl in a manner similar to that of Western populations, so that the anomaly is not due to the method of measurement. Dietary history recommends itself as a partial explanation. The tamarind fruit chewed by these laborers is slightly sweet but exceptionally sour. It seems reasonable to assume that these subjects were able to distinguish between the samples (aqueous solutions of test substances) and real foods, so that the high preference ratings given to citric acid probably reflect an actual preference for sourness. Another possibility is that genetic inbreeding of these laborers may yield a population which finds sourness acceptable; that hypothesis requires extensive further investigation.

HOWARD W. MOSKOWITZ*
Food Science Laboratory,
U.S. Army Natick Laboratories,
Natick, Massachusetts 01760
V. KUMARAIAH
K. N. SHARMA
Department of Physiology,
St. John's Medical College,
Bangalore, India
H. L. JACOBS
Food Science Laboratory,
U.S. Army Natick Laboratories
S. D. SHARMA
Department of Physiology,
St. John's Medical College

References and Notes

1. R. Engel, *Arch. Gesamte Psychol.* **64**, 1 (1928).
2. G. Ekman and C. A. Akesson, *Report of the Psychological Laboratory*, University of Stockholm, Stockholm, Sweden, No. 177 (1964); R. M. Pangborn, *Psychonomic Sci. Sect. Hum. Exp. Psychol.* **21**, 125 (1970).
3. H. R. Moskowitz, *Am. J. Psychol.* **84**, 387 (1971).
4. The method of magnitude estimation provides ratios of pleasantness, and category scaling provides intervals of pleasantness.
5. G. Chappell, *J. Sci. Food Agric.* **4**, 346 (1953).
6. H. R. Moskowitz, V. Kumaraiah, K. N. Sharma, H. L. Jacobs, S. D. Sharma, *Physiol. Behav.*, in press.
7. H. R. Moskowitz, R. A. Kluter, J. Westerling, H. L. Jacobs, *Science* **184**, 583 (1974).
8. This type of scoring or scaling, known as cross-modality matching, is a standard procedure in psychophysical measurement; the subject adjusts stimuli from one continuum (for example, numbers, the intensity of a tone, or the length of a line) until its perceived intensity matches that produced by a criterion stimulus (for example, perceived taste intensity). Ratios between line lengths are assumed to reflect ratios of perceived taste intensity or taste pleasantness. Line matching has been used by Jones, Moskowitz, Butters, and Glosser (*Neuropsychologia*, in press) to assess the perceived odor intensity of *n*-butanol by Korsakoff syndrome patients. The concept of "magnitude" is brought out clearly with the procedure, even for subjects with no concept of number or of the rules of arithmetic.
9. Lunch included rotti (bread), chappaties (whole wheat breads), and dahl (sour lentil soup). Most subjects were not vegetarians.
10. The initial concentrations were glucose, 2.0M; NaCl, 1.0M; citric acid, 0.06M; and quinine sulfate, 0.00075M. These concentrations were successively diluted (with an equal volume) five times to yield a 32 : 1 range of molarities for each compound.
11. These power functions are expressed by the equation $S = kC^n$, where S is sensory intensity and C is molarity. The lines in Fig. 1 are drawn without least squares procedures and are meant simply to show the trend of the data. Least squares estimates of the exponents, n, fitted to all of the data suggest that the rank order of power function exponents replicate the usual rank order but are lower in absolute magnitude [H. L. Meiselman, *Crit. Rev. Food Technol.* **3**, 89 (1972)].
12. Linear and quadratic trends of all the pleasantness ratings (regressed against concentration—cross-sectional data) and for the geometric mean rating of each concentration (across observers and replicates) were evaluated for significance. All trends showed significant ($P < .05$) linear components, with the exception of NaCl fasting (cross-sectional and mean data) and quinine sulfate nonfasting conditions (cross-sectional and mean data). The following conditions showed significant ($P < .05$) quadratic trends: NaCl fasting (cross-sectional and mean data), NaCl nonfasting (cross-sectional and mean data), citric acid nonfasting (mean data), quinine sulfate fasting (cross-sectional and mean data), and nonfasting (mean data).
13. M. Cabanac, *Science* **173**, 1103 (1971). Alliesthesia refers to a change in the perceived liking of a stimulus (for example, a taste, odor, or temperature change) resulting from changes in the internal body state.
14. ______ and R. Duclaux, *Nature (London)* **227**, 966 (1970).
15. H. R. Moskowitz *et al.*, *Physiol. Behav.*, in press. Four groups of Indian medical students at St. John's Medical College, Bangalore, India, were tested after fasting, after breakfast, after lunch, and after ingesting a 20 percent (weight to volume) glucose solution (1.5 g per kilogram of body weight). Concentrations of the four taste substances were similar to those evaluated here.
16. We thank Dr. B. Halpern for helpful discussions on the manuscript and on the interpretations of preference measures.

* Present address: MPI Sensory Testing, Inc., 770 Lexington Avenue, New York 10021.

1 April 1975; revised 9 July 1975

Cochlear Tuning Properties: Concurrent Basilar Membrane and Single Nerve Fiber Measurements

Abstract. *Removal of perilymph from the cochlea has been reported to destroy the sharp tuning of cochlear neurons. That these changes are mechanical in origin is refuted by the concurrent recording of sharp neural tuning with broad basilar membrane responses from the same region of the partially drained cat cochlea. A second cochlear filter is therefore necessary.*

There is still controversy concerning the origin of the sharply tuned characteristics exhibited by the single cochlear nerve fibers of all mammalian species so far investigated. Comparisons of the filtering characteristics observed in measurements of the amplitude of basilar membrane vibration with those of frequency threshold curves (FTC's or tuning curves) of single cochlear fibers in the same species show evidence of a substantial discrepancy [guinea pig (*1*–*4*); squirrel monkey (*5*)]. On this, and on a number of other grounds, it has been suggested that cochlear filtering is a two-stage process: the first filter (that of the basilar membrane) being followed by a physiologically vulnerable second filter (*1*–*3*; *6*). The validity of these comparisons between basilar membrane and cochlear nerve measurements, however, has recently been questioned by Robertson (*7*), who demonstrated that drainage of the scala tympani, as is required for some of the mechanical measurements in the guinea pig, can eliminate the normal sharp tuning of cochlear neurons. He suggested, as one possible interpretation, that the basilar membrane may be sharply tuned only under those conditions which give rise to sharp neural responses; that is, when the scala tympani is sufficiently filled with perilymph.

We consider this suggestion unlikely on the grounds that Rhode (*8*) obtained his basilar membrane data from the squirrel monkey under conditions in which the scala tympani was full of perilymph, and Johnstone, Taylor and Boyle (*9*) obtained similar data under conditions in which the scala tympani was not drained, or drained only to an extent where normal tuning properties of single cochlear fibers in the guinea pig were found (*10*). In contrast, in these basilar membrane studies (*8, 9*), the functional condition of the cochlea was not monitored (for example, by threshold measurements of the gross cochlear action potential). Mechanical interference with the cochlea or long-term drainage of perilymph from the scala tympani can cause deterioration in the tuning properties of cochlear fibers (*1, 7*). In fact, it may even be possible to obtain deterioration in the tuning of single cochlear fibers which would not be reflected in the threshold of the gross cochlear action potential, provided that a small part of the cochlea is damaged.

A purely mechanical explanation has a number of other difficulties (*4*), such as the observation of an amplitude of vibration independent of fluid accumulation, the close correspondence between differential cochlear microphonic responses and the mechanical responses of the drained basilar membrane (*3*), and the similar phase characteristics of the mechanical and neural responses.

The crucial experiment, however, must be to control the basilar membrane measurements by concurrent recording of the FTC's of single cochlear fibers emanating from the same region of the cochlea. We have done this in our experiments with the cat, where conditions of drainage, the physiological condition of the animal, and cochlear nerve recording can be optimized more easily than in the guinea pig.

Four cats were used in our experiments, although completely satisfactory concurrent recordings were obtained in only two of the animals, those in which stable conditions of cochlear drainage could be maintained. Full details of the preparation, sound system, and single cochlear fiber recordings have been described (*1*). In brief, the cats were anesthetized with pentobarbitone sodium. Blood pressure, temperature, and end-tidal CO_2 were kept within normal limits. Fibers were identified, and their thresholds as a function of frequency were determined with an automatic, computer-controlled paradigm [as in (*11*)]. Tone bursts of 40-msec duration, with 5-msec rise and fall times, were presented every 130 msec (Fig. 1A). Their frequencies and intensities were adjusted by the computer to scan the frequency threshold curve first downward and then upward in frequency and in 1-db sound pressure level (SPL) steps. The threshold criterion was two extra spikes during the tone stimulus compared with an identical period immediately following the tone burst. Under this paradigm it took about 30 seconds to sweep across a frequency range of three octaves; the sweep in the reverse direction was included in order to avoid bias due to any hysteresis introduced by this rapid sampling technique.

For the basilar membrane measurements, a capacitive probe (*12*) was modified so that the most basal 9 mm of the basilar membrane could be measured through the round window without remov-

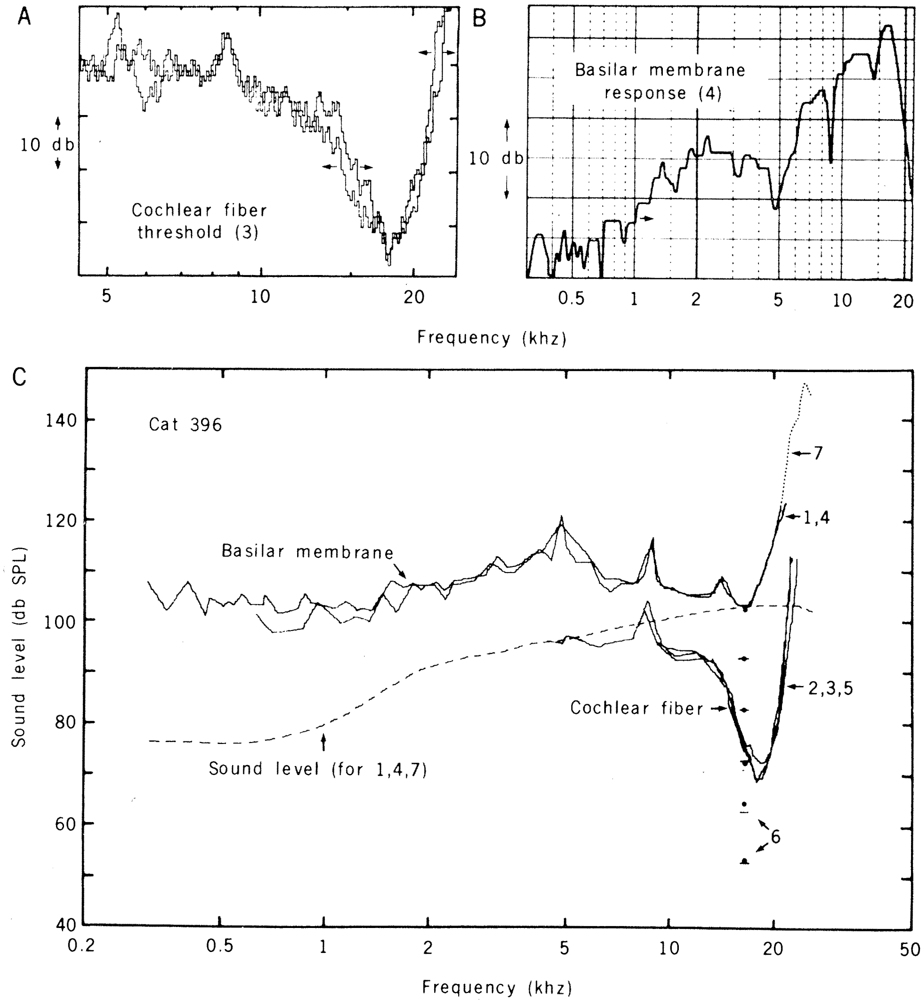

Fig. 1. Interposed sequence of measurements from the basilar membrane and from a single nerve fiber from the same region of a partially drained cochlea of cat 396. (A) Computer-controlled plot of signal voltage level required to reach neural threshold criterion as frequency was stepped first downward and then upward (arrows). A smooth curve through this plot corrected to sound level at the tympanic membrane (with open bulla and septum) is plotted with repeat determinations as curves 2, 3, and 5 in C. (B) Automatic plot of basilar membrane response at constant signal voltage level. This curve is also corrected for the sound system, inverted (assuming linearity) to represent an isoamplitude curve (330 Å root mean square) for comparison with the neural data, and plotted with repeat determination as curves 1 and 4 in C. (C) Comparison of neural and mechanical tuning properties. The sequence of measurements is indicated by the numbering of the curves made during a single stable drainage. The basilar membrane measurements 1, 4, and 7 were made at the sound levels indicated by the dashed curve and arbitrarily positioned to coincide with it at the most sensitive frequency. Curves 1 through 5 were all made within 14 minutes; then, 6, basilar membrane amplitudes made at the peak response frequency of 16 khz (dots) were measured at various SPL's (short bars) indicating almost complete linearity to well below neural threshold; finally, 7, the basilar membrane curves were extended by lock-in measurements. The basilar membrane measurements were all made at 5.6 mm from the basal extremity of the membrane. The slightly higher frequency (but much sharper) tuning of the cochlear fiber suggests that it arises from a slightly more basal and presumably more drained position.

ing any bone from the cochlea. A reduced tip diameter (50 μm) and working distance ($\sim$ 5 μm) improved spatial resolution and eliminated the correction factor required with larger probe tip sizes. Responses were recorded automatically (Bruel & Kjaer 2305 level recorder through a B & K 2020 slave filter, set at 3.16-hertz bandwidth) with frequency swept continuously from 20 hertz to 20 khz (Fig. 1B). This range was extended with a lock-in amplifier (Brookdeal 401).

Throughout the experiments, the gross physiological condition of the cochlea was monitored periodically by determining the threshold, to 10-μsec clicks, of the gross cochlear action potential recorded from a wire placed just below the round window.

A micropipette of 20 to 40 megohms impedance was inserted into the posterior third of the cochlear nerve in order to sample fibers with characteristic frequencies most suitable for the comparison study (10 to 20 khz). The round window membrane was removed, and a silicone rubber tube (0.35 mm outside diameter) was inserted through the round window opening, along the wall of the scala tympani (well away from the basilar membrane) until the tip lay about 9 to 10 mm from the basal end of the basilar membrane. This tube was connected to a motor-driven syringe, which allowed manual adjustment or constant suction to drain and refill the scala tympani. Cat cerebrospinal fluid warmed to 37°C served as artificial perilymph.

Preliminary measurements established that draining the perilymph thoroughly so that no meniscus was visible produced in most units the effects noted by Robertson (7), that is, loss of the low threshold, sharply tuned segment of a cochlear fiber's FTC. In a few of the units, however, the loss was only partial. With the drainage tube inserted slightly less far, many more units fell into the latter category. Under these conditions, in which the meniscus was visible at about 7.5 to 9 mm, curious changes of shape in the FTC's could be obtained, which, in some cases, resulted in sharper than normal tips. It would seem that a length of the basilar membrane was covered by a thin layer of fluid, which, although difficult to observe, was presumably deeper toward the drainage tube tip than toward the hook end of the basilar membrane.

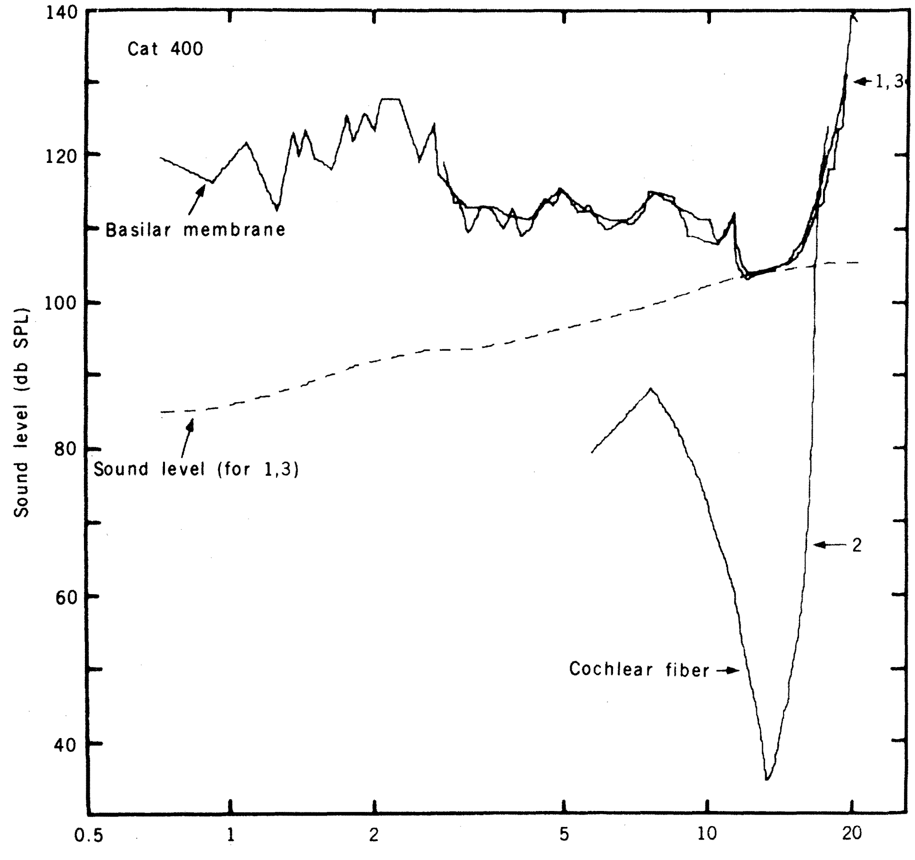

Fig. 2. Comparison of mechanical and neural tuning properties for cat 400, the former measured at a more apical (6.5 mm) position on the basilar membrane (isoamplitude, 400 Å root mean square). Measurements were made during stable drainage over a period of 10 minutes, in the sequence given. In this case, the neural threshold is as low as that normally encountered with an intact cochlea; the frequencies of tuning in the selected neural and mechanical data correspond closely.

In two cats it was possible to obtain sufficiently stable partial drainage that the capacitive probe could be placed in position, and the basilar membrane response measured concurrently with repeated determinations of the FTC's of cochlear fibers of characteristic frequencies (CF) close to or equal to the peak response frequencies of the basilar membrane. While we determined the cochlear fiber FTC's, the capacitive probe was left in position and operative. Under these conditions many sharply tuned fiber response measurements were obtained interposed between measurements of broadly tuned basilar membrane responses (Figs. 1 and 2).

Examples of the automatically plotted FTC's of cochlear fibers (Fig. 1A) and of the basilar membrane response (Fig. 1B) are shown. They and other repeat determinations of both cochlear nerve and basilar membrane responses have been corrected to SPL at the tympanic membrane (with open bulla and septum) and reduced to the same scale for comparison (Fig. 1C). Figure 2 shows a similar comparison from a more apical position of measurement in a different animal. Here, the optimal frequencies of neural and mechanical tuning correspond closely, and the neural threshold is low. The findings demonstrate that the low-pass basilar membrane characteristic obtained with the capacitive probe technique can be obtained concurrently with sharply tuned cochlear fibers under stable conditions of partial drainage of the scala tympani. Similar sharp responses were obtained during concurrent measurements in 22 other fibers with CF's distributed over the range 13 to 30 khz.

Not all fibers obtained under these conditions, however, were sharply tuned. It is therefore of importance that concurrent measurements of broadly tuned basilar membrane and sharply tuned cochlear fibers were made on fibers with CF's above (Fig. 1) and below as well as at the frequency (Fig. 2) of optimal basilar membrane response. This eliminates the possibility that measurements were not made from corresponding regions if, for example, optimal neural tuning is at some distance from the maximal basilar membrane response.

Mechanical measurements were also made with different degrees of drainage exceeding the range over which the neural responses changed dramatically. Under no condition was there any tendency for the basilar membrane responses to change in sharpness of tuning.

The influence of draining the perilymph from the first half turn of the scala tympani on the gross cochlear action potential threshold supports the more detailed picture provided by the single fiber recordings. The minimum drainage necessary to obtain mechanical measurements, which left most of the corresponding fiber thresholds intact, had little influence on the gross cochlear action potential threshold. Draining the perilymph column to about 10 mm from the basal end of the basilar membrane, however, which dramatically affected fiber characteristics in the 10- to 20-khz region, led to a gross action potential threshold about 20 db higher than normal. This then supports the contention of Wilson and Johnstone (*4*) that in the conditions under which they obtained capacitive probe measurements in the guinea pig, the cochleas were rarely sufficiently drained to be subject to the effect which Robertson (*7*) reported. As a similar condition of drainage was also used by Evans (*10*), his null finding with cochlear nerve fibers is consistent with these and Robertson's data.

Our experiments, therefore, indicate that the normally sharply tuned characteristics of single cochlear fibers can be obtained under those conditions in which broadly tuned basilar membrane responses are also measured. Thus some second filter mechanism is required to account for the sharp neural tuning. Whether this mechanism, which is affected so rapidly by complete drainage of perilymph from the appropriate segment of the cochlea, depends upon electrical (*4, 7, 13*) or other influences remains to be determined.

E. F. EVANS
J. P. WILSON

*Department of Communication,
University of Keele,
Keele, Staffordshire ST5 5BG, England*

References and Notes

1. E. F. Evans, *J. Physiol. (Lond.)* **206**, 14P (1970); *ibid.* **226**, 263 (1972).
2. ______ and J. P. Wilson, in *Basic Mechanisms in Hearing*, A. R. Moller and P. Boston, Eds. (Academic Press, New York, 1973), pp. 519–551.
3. J. P. Wilson, in *Facts and Models in Hearing*, E. Zwicker and E. Terhardt, Eds. (Springer-Verlag, Berlin, 1974), pp. 56–63.
4. ______ and J. R. Johnstone, in *Hearing Theory 1972* (Instituut voor Perceptie Onderzoek, Eindhoven, Netherlands, 1972), pp. 172–181; *J. Acoust. Soc. Am.* **57**, 705 (1975).
5. C. D. Geisler, W. S. Rhode, D. T. Kennedy, *J. Neurophysiol.* **37**, 1156 (1974).
6. J. P. Wilson and J. R. Johnstone, *Nature (Lond.)* **241**, 206 (1973).
7. D. Robertson, *Science* **186**, 153 (1974).
8. W. S. Rhode, *J. Acoust. Soc. Am.* **49**, 1218 (1971).
9. B. M. Johnstone, K. J. Taylor, A. J. Boyle, *ibid.* **47**, 504 (1970).
10. E. F. Evans, *J. Physiol. (Lond.)* **208**, 75P (1970).
11. N. Y.-S. Kiang, E. C. Moxon, R. A. Levine, in *Sensorineural Hearing Loss*, G. E. W. Wolstenholme and J. Knight, Eds. (Churchill, London, 1970), pp. 241–268.
12. J. P. Wilson, *J. Sound Vib.* **30**, 483 (1973).
13. J. R. Johnstone, B. M. Johnstone, G. K. Yates, *J. Acoust. Soc. Am.* **57**, 1210 (1975).
14. Supported in part by the Medical and Science Research Councils. We thank J. S. Corbett and J. B. Ruscoe for technical assistance.

4 August 1975

Flavonoid Localization in Epidermal Papillae of Flower Petals: A Specialized Adaptation for Ultraviolet Absorption

Abstract. *In petals of various species of angiosperms that differ in ultraviolet absorption and reflectance, flavonoids were concentrated at surfaces that absorb ultraviolet light. Microscopic examination of intact, dried petals treated with sodium methoxide revealed dense concentrations of flavonoid material in the tips of specialized epidermal papillae. Scanning electron micrographs confirmed this cellular localization and disclosed structural features of the cuticle which are associated with ultraviolet absorption. These papillate epidermal cells have an adaptive complex of chemical and structural features which function in ultraviolet absorbance patterning on the surfaces of flower petals and assist in visual discrimination by insects.*

Ultraviolet (UV) patterning is present on the petals and other floral parts in a substantial number of flowering plants (*1, 2*). These patterns serve as nectar guides to many UV-sensitive insect pollinators (*3*) and as functional cues regulating the behavioral orientation of flower-foraging bees (*4*). Relatively little attention has been devoted to why some plant structures absorb UV while others, with the same visual appearance to humans, reflect it (*2, 5*).

Flavonols were reported as the pigments responsible for the UV absorption in the basal part of the petal (ligule) of *Rudbeckia hirta* (*6*) and an absence of flavonoids was associated with the UV reflectance in the distal part. A similar distribution pattern of another class of flavonoids, chalcones, coincides with the UV absorbing pattern in the nectar guide of *Oenothera hookeri* (*7*). We observed that both the upper and lower surfaces of the flavonoid-containing petals of *Impatiens balsamina* reflect UV, while in the flavonoid-containing petals of *Primula sinensis* both surfaces absorb UV. Moreover, ligules of

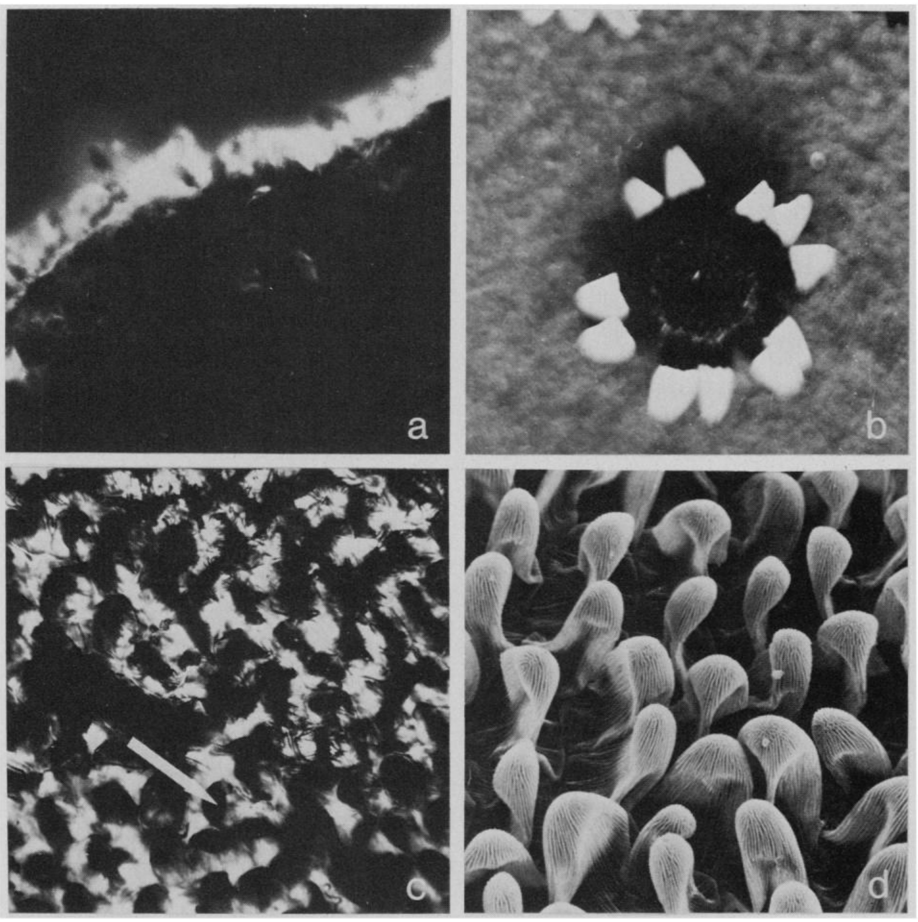

Fig. 1. (a) Aluminum chloride fluorescence reaction of the papillate epidermal cell layer on the UV-absorbing upper surface of *Rudbeckia hirta* ligule (cross section, × 400). (b) Ultraviolet absorbance pattern of *Lasthenia chrysostoma* ligules (× 5). (c) Surface view of papillate cells of UV-absorbing epidermis of *L. chrysostoma* (× 600). Sodium methoxide-stained flavonoids (arrow) are localized in the tips of the papillae. (d) Scanning electron micrograph of papillate epidermal cells of dried ligules of *L. chrysostoma* (× 700).