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AN ENERGY-TRANSDUCTION MECHANISM IN CHEMORECEPTION BY THE BARK BEETLE, SCOLYTUS MULTISTRIATUS[1]

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SUMMARY

Findings from long-term studies of the field biology and behavior of *Scolytus multistriatus* (Marsh.) as involved in chemical-messenger aspects of host tree acceptance and non-host rejection have provided a necessary basis for studying chemoreception by this insect. Subsequent investigations of chemical attractants and feeding stimulants for this beetle have shown that many quinols (e.g., hydroquinone) serve as such positive stimuli. Similar experiments aimed at repellency and/or feeding inhibition have proven that many quinones (e.g., *p*-benzoquinone) are negative stimuli. Results presented, or summarized, in this paper allow the characterization of the energy-transduction mechanism involved in quinone inhibition of *S. multistriatus* feeding as being formation of an energy-transfer complex between the quinone and the receptor site, sulfhydryl group, in the sensory nerve membrane protein; the oxidation of the sulfhydryl; and the reduction of the quinone. Such a mechanism is known to bring about a change in the conformation of macromolecules in membranes. Changes in conformation alter inorganic ion flow through membranes and this may generate the action potential necessary to fire the neuron. This mechanism is compatible with the observed order of relative inhibitory activities of variously substituted 1,4-naphthoquinones.

INTRODUCTION

Chemoreception by insects necessitates an exchange of energy between the chemical messenger and the receptor site in the chemosensitive sensillum. Interpretation of such energy-transduction mechanisms in animals has been only theoretical (e.g., Amoore, 1), but Norris (19, 20) recently presented biologically and chemically based data to support a quinol-quinone (electron-transfer) system as such a mechanism in the chemoreception of the beetle *Scolytus multistriatus* (Marsh.).

The detailed knowledge (Fig. 1) of the biology and behavior of *S. multistriatus* as related to its acceptance of host trees and rejection of non-host trees, as summarized by Baker and Norris (2, 3), served as essential background for developing an understanding of this chemoreception mechanism. Our extensive field investigations of the interrelationships among *S. multistriatus* and *Scolytus quadrispinosus* Say, and obviously vigorous host and non-host trees indicated that these beetles are not attracted, or do not respond by chemokinesis, over significant distances (i.e., more than a few centimeters) to such host trees (14, 18). *Scolytus multistriatus* feeds in the twigs of *Ulmus*, especially *Ulmus americana* L. in the United States; and *S. quadrispinosus* feeds similarly in *Carya* spp. (hickories). In studying the flight of these two *Scolytus* spp. in the mixed hardwood forest, as many, or more, of each species were trapped in the periphery of the crown of healthy non-host trees as in the edge of the crown of vigorous host trees (14, 18). Thus, flight dispersal of the beetles was random with regard to healthy trees (host or non-host).

With the flight dispersal of these beetles into the periphery of the crown

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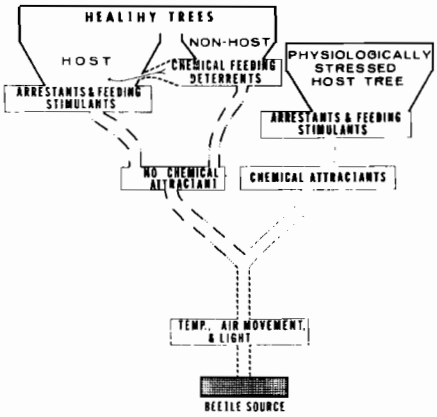

FIGURE 1. Diagrammatic summary of interacting influences of various physical and chemical stimuli in the environment on the host acceptance, and non-host rejection behavior of *Scolytus multistriatus*.

of vigorous trees being random with regard to host and non-host trees, the mechanism for preventing *Scolytus* from alighting and feeding in non-host trees was next investigated. These studies (3, 12, 13) indicated that each investigated non-host tree species contained one or more chemicals in its tissues which served to repel or inhibit feeding by the *Scolytus* on that tree. The discovery (12, 13) that juglone (5-hydroxy-1,4-naphthoquinone) in *Carya* spp. repelled *S. multistriatus* from these non-host trees has been our most thoroughly researched example of chemical repellency in non-host trees. When juglone was removed from the extract of *Carya* tissues, *S. multistriatus* fed vigorously on the remaining chemicals from hickories.

Subsequent bioassay studies (19, 20) with a group of related 1,4-naphthoquinones indicated that all tested quinones significantly inhibited *S. multistriatus* feeding when added in a range of concentrations to otherwise stimulatory extracts of elm (host) tissues or to known chemical stimulants (Fig. 2). The extents to which given concentrations of the various 1,4-naphthoquinones reduced the amount of *S. multistriatus* feeding when added to a standard stimulant solution (19) depended on (a) the presence or absence of substituent groups on the 1,4-naphthoquinone; (b) the type(s) of substituent group(s); and (c) the position(s) of the substitution(s). The effects of substituents upon the properties of molecules are not readily generalized (17), but in these specific studies (19, 20) the substitution of OH in the 5 or 5, 8 positions (Fig. 3) increased feeding inhibition, and in the 2 position it decreased inhibition. Molecules with substitutions in the 2 or 2,3 positions were less inhibitory than the unsubstituted 1,4-naphthoquinone. At the 2 position, $OH > CH_3 > Cl$ in inhibitory effect. According to Morton (17), substitution in the 2 position of 1,4-naphthoquinones reduces the redox potential much more than substitution at other positions. However, the redox potential is actually increased by substitution of Cl at the 2,3 positions (25). Thus, if redox potential were solely responsible for the relative inhibition of variously substituted 1,4-naphthoquinones to *S. multistriatus* feeding, then the order of inhibition among those tested compounds with substitutions in the 2 position would have been 2,3-dichloro-1,4-naphthoquinone > 2-methyl-1,4-naphthoquinone > 2-hydroxy-1,4-naphthoquinone. As indicated above, extensive bioassays (19, 20) have yielded data on inhibition by these chemicals which reverse this order.

Therefore, though the 1,4-naphtho-

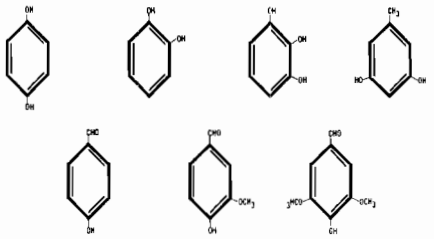

FIGURE 2. Some feeding stimulants and short-range attractants (or arrestants) for *Scolytus multistriatus*. Upper row (left to right): hydroquinone, catechol, pyrogallol, and orcinol; lower row (left to right): *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde.

TABLE 1.—Summarized methods for fixation, dehydration, and embedding of tissues of *Scolytus multistriatus* for electron microscopy

Process	Time, hr.	Temperature, ° C.
Fixation		
Paraformaldehyde-glutaraldehyde in phosphate buffer, pH 7.4	3	4
Rinse in buffer	1	4
2% Osmium tetroxide in phosphate buffer, pH 7.4	3	4
Dehydration		
30% Acetone	1	22
50% Acetone	1	22
70% Acetone	1	22
90% Acetone	1	22
100% Acetone	1	22
100% Acetone	1	22
Embedding		
3:1 Acetone:plastic*	24	22
1:1 Acetone:plastic	24	22
1:3 Acetone:plastic	24	22
0:1 Acetone:plastic	24	22
Polymerization		
In vacuum oven	24	37
In vacuum oven	48	60

* Plastic mixture: Araldite 6005 (20 ml.), Epon 812 (25 ml.), and DDSA (60 ml.). Add 3 drops of DMP-30 per ml. of plastic mixture.

were dehydrated with a graded series of acetone, and were stained with saturated uranyl acetate in 70 per cent acetone at that step in the dehydration. Beetles next were embedded in a mixture of Araldite 6005, DDSA, and DMP-30. After polymerization at 60° C. for 48 hr., thin sections were cut with a diamond knife on an ultramicrotome. Ilford's L4 emulsion was prepared and applied to sections on carbon-collodion coated nickel grids using the methods of Caro *et al.* (7). Sections were developed, after a 23-day exposure, in D-19 developer; and were double stained with lead citrate. Examination was in an A.E.I. EM 6B electron microscope at 50 and/or 60 kv.

Preparation and fractionation of antennal homogenate. Antennae from at least 5,000 male and female *S. multistriatus* or 100 to 300 male and female *P. americana* were excised at the base and frozen in 0.25 *M* sucrose until homogenized. They were homogenized in 5 ml. of ice cold 0.25 *M* sucrose in a motor-driven Teflon-glass homogenizer. The homogenation and fractionation methods were patterned after De Robertis *et al.* (9). The homogenate was filtered through glass wool into a 1×3 in. centrifuge tube, and centrifuged at 20,000 ×*g* for 45 min. After decanting, the pellet was twice resuspended, washed, and centrifuged with 5 ml. of 0.25 *M* sucrose. The final pellet was taken up in 6 ml. of 0.8 *M* sucrose and layered onto a discontinuous sucrose density-gradient composed of 6 ml. each of 1.0, 1.2, 1.5, and 1.8 *M* sucrose. The gradient tubes were placed in a SW 25.1 rotor and centrifuged at 90,000×*g* (max.) for 120 min. The particulate band located at each density interface was removed with a 5 ml. syringe, diluted to approximately 0.25 *M* with H_2O, and repelleted at 20,000×*g* for 45 min. Beginning with the band at the 0.8–1.0 *M* interface and ending with the band at the 1.5–1.8 *M* interface, the bands were designated F_1 through F_4. The protein content of an aliquot of each band was determined by the method of Lowry *et al.* (16). Another aliquot of each band was fixed in glutaraldehyde, post-fixed in osmium, dehydrated in acetone, embedded in Araldite, stained with uranyl acetate and lead citrate, and examined by electron microscopy.

Investigation of complexing between

2-(methyl-^{14}C)-1,4-naphthoquinone and particulate bands from antennal homogenate. To determine the binding affinity of the feeding inhibitor, 2-(methyl-^{14}C)-1,4-naphthoquinone, for particulate material in the density-gradient bands F_{1-4} from the antennal homogenate, the following procedure was used. The antennal homogenate was prepared as previously described, but before the centrifugation procedure, it was incubated for 15 min. at room temperature with 0.1 μc. of 2-(methyl-^{14}C)-1,4-naphthoquinone (final concentration was $2\times10^{-6}M$). The incubate was centrifuged at 20,000$\times g$ and the pellet obtained was resuspended in 0.25 M sucrose and recentrifuged three times to remove radioactivity not bound to particulate matter. Aliquots of each wash were analyzed for radioactivity. The final pellet obtained was fractionated on the density gradient as described previously. Samples for counting were placed in 10 ml. of methyl cellusolve-based counting solution (toluene, 500 ml.; methyl cellusolve, 500 ml.; PPO, 5.5 g.; and POPOP, 300 mg.) and counted in a Packard 3380 liquid-scintillation counter. Each sample vial was counted twice for 10 min. and the count was averaged. Quenching was monitored with an external standard.

Ultraviolet spectroscopy of binding of naphthoquinones to components of the supernatant and particulate fractions of the antennal homogenate. Antennae were homogenized in a Potter-Elvehjem homogenizer containing 23 ml. of ice cold 0.05 M Tris buffer, pH 7.0. The homogenate was filtered through glass wool to remove cuticular debris and rinsed with 15 ml. of Tris. The homogenate then was subjected to the same centrifugation procedure as described for the radiolabelled naphthoquinone-binding study. The initial decantate (supernatant) from the centrifugation procedure was saved for study of the complexing (binding) of its soluble and particulate components, not sedimented at 20,000$\times g$, with inhibitory naphthoquinones. Bands removed from the sucrose gradient were diluted with Tris to 50 ml., and then centrifuged at 32,800 $\times g$ for 2 hr. The resultant pellets were each resuspended in 4 ml. of Tris.

Ultraviolet spectroscopy methods patterned after Dastoli and Price (8) and Donovan (10) were used to determine the relative binding affinity of the particulate fractions and decantate for the feeding inhibitors, 2-methyl-1,4-naphthoquinone, 1,4-naphthoquinone, or 5-hydroxy-1,4-naphthoquinone. Absorption measurements were made with a Cary Model 15 spectrophotometer using a 0.0 to 0.1 scale expansion. The dynode setting was 2, and the amplifier sensitivity was 2. Absorption cells with a path length of 1 cm. were used. Baselines (zero absorbance as a function of wavelength) were determined with an aliquot of a fraction or the decantate in both the sample and reference cells. The four particulate fractions (bands F_{1-4}) and the decantate were tested at concentrations producing an optical density of 0.2 at 280 nm. These concentrations were obtained by diluting each aliquot with Tris. Enough naphthoquinone (0.13 mM solution in deionized water) was added to the solution of the aliquot in the sample cell to yield a final concentration of $1.7\times10^{-7}M$ naphthoquinone. The decrease in optical density induced at 280 nm. was then recorded immediately. Corrections for absorption of the naphthoquinone at 280 nm. were made by subtracting the absorbance of each chemical alone in Tris from the absorption value obtained with the naphthoquinone in combination with the antennal fraction. All naphthoquinones used were recrystallized from redistilled benzene.

As stated by Szent-Györgyi (24), in energy transfer two events are to be considered: (a) the extent of coming together (complexing) of involved molecules, and (b) the actual transfer of energy. To further investigate the extent of complexing of inhibitory quinone with receptor sites (i.e., chemicals) in aliquots from the antennal preparation, or with model receptor chemical (i.e.,

quinones are functioning as oxidation-reduction systems in their inhibition of *S. multistriatus* feeding, other properties and reaction capabilities of each specific quinone are significantly involved. The fact that OH substitution on 1,4-naphthoquinone, whether at the 5; 5,8; or 2 positions, unfailingly yielded a degree of inhibition greater than that indicated by the relative redox potential of the given chemical led Norris (19) to suggest that hydrogen-bonding capabilities, brought to the naphthoquinone by OH substitution, could account for the greater feeding inhibition of these chemicals than their redox potentials would indicate. Prior to the more recent studies (11), the relatively poor inhibition by 2,3-dichloro-1,4-naphthoquinone remained unexplained; however, we now can present physical-chemical explanations for the feeding inhibitory action of 1,4-naphthoquinone to *S. multistriatus;* and for the relative inhibitions of the variously substituted 1,4-naphthoquinones.

MATERIALS AND METHODS

In the investigations of the energy-transduction mechanism operative in inhibition of insect feeding by quinones, parallel experiments were frequently conducted on *S. multistriatus* and *Periplaneta americana* (L.). The American cockroach was involved because of its similar responses to 1,4-naphthoquinones (21) and its large antennae which make studies of receptor chemicals in chemosensitive sensilla less laborious than with the relatively small elm bark beetle.

Ultrastructure of chemoreceptor sensilla on antennae. Antennae of *S. multistriatus* were either dissected and immediately placed in fixative (Table 1), or the head of the insect was amputated with a razor blade while held in a solution of the fixative. These methods yielded comparable degrees of fixation. The entire process employed in fixing, dehydrating, and embedding tissues of *S. multistriatus* for electron microscopy is summarized in Table 1. Further details are given by Borg and Norris (4, 5).

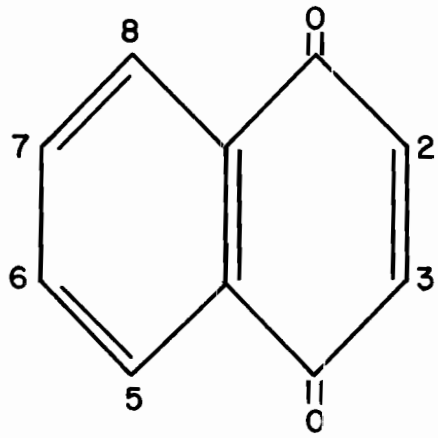

FIGURE 3. 1,4-Naphthoquinone and derivatives investigated as feeding inhibitors of *Scolytus multistriatus*: 2,3-dichloro; 2-methyl; 2-hydroxy; unsubstituted 1,4; 5,8-dihydroxy; and 5-hydroxy.

Specimen tissue was sectioned on a Sorvall MT-2 ultramicrotome with a Dupont diamond knife. All sectioned tissue was stained with a saturated, aqueous solution of uranyl acetate. Sections next were washed gently with distilled water, and then were blotted with filter paper. Tissue then was stained with cold lead citrate for 5 to 10 min. using the technique of Reynolds (23). After staining in lead citrate, grids again were gently washed with distilled water and examined with a JEM-7 electron microscope at 80 kv.

Penetration of chemical messenger into the sensory sensillum. Catechol (1,2-dihydroxybenzene) (Fig. 2) is a feeding stimulant for *S. multistriatus* (6). To determine whether such a chemical messenger penetrates into chemosensitive sensilla of the insect, 10 live adult beetles were bathed in tritiated (500 mc./m*M*) catechol ($4.5 \times 10^{-3} M$) in 80 per cent ethanol for time periods of 5, 10, or 60 min., and then were rinsed in Millonig's phosphate buffer. Beetles next were fixed for 3 hr. in Karnovsky's fixative (15) in Millonig's phosphate buffer, rinsed in buffer, and post-fixed in 2 per cent OsO_4 in Millonig's buffer. The specimens then

reduced glutathione), an inhibitor to insect gustation, *p*-benzoquinone or 1,4-naphthoquinone, was added to aliquots of F_2 or decantate, or to reduced glutathione, in buffer solution, and each mixture was frozen in a covered glass container. This is one method of increasing the degree of complexing between the candidate molecules. When frozen, a preparation was removed and observed microscopically for a change of color from those of the frozen control solutions of the individual test components. In some cases, after melting, the preparation was analyzed in the spectrophotometer for changes in absorbance. This analysis was aimed at determining whether an energy-transfer reaction had occurred between complexing components.

Dropping-mercury-electrode polarography of energy-transfer complexing in a model system. In the absence of sufficiently purified quantities of receptor chemical from antennae for polarographic study of energy-transfer complexing with quinones, the interaction between a model receptor chemical (i.e., reduced glutathione); and 1,4-naphthoquinone with or without a sulfhydryl group-inhibitor chemical (*N*-ethyl maleimide) was investigated. Reduced glutathione was chosen as a model receptor-site chemical because the complexing and/or oxidizing reactions of quinones with sulfhydryl groups of proteins are documented (25), and the sulfhydryl-containing reduced glutathione is an important constituent of neural tissues (22).

The solutions to be polarographed were prepared as follows: (a) 100 ml. of 0.1 *M* phosphate buffer, pH 7, were deoxygenated in a covered glass system for 25 min. with prepurified nitrogen; (b) then the weighed amount of test chemical(s) was added to the buffer solution; (c) nitrogen was bubbled through the preparation for 15 min.; (d) avoiding unnecessary contact with air, 20 ml. of the preparation was transferred to a proper glass vessel for polarography; (e) nitrogen was passed through the solution in the vessel for 15 min.; and (f) with the nitrogen stream removed from the test solution, but with a thin stream of nitrogen running across the surface of the liquid, the polarographic analyses were run. All studies were conducted using a Type PO4 Radiometer Polariter with the dropping-mercury electrode.

RESULTS

Ultrastructure of chemoreceptor sensilla on antennae. Three types of chemosensitive sensilla, sensilla basiconica (type A), sensilla basiconica (type B), and sensilla trichodea, were found on the antennae of both males and females of *S. multistriatus*. Detailed descriptions of the ultrastructure of these sensilla are given by Borg and Norris (5). Both types of sensilla basiconica had many more-or-less flask-shaped pores in the cuticular wall. These appear rather similar to pores reported in previously described sensilla basiconica. The bottom of each pore was lined with a plasma membrane which was multiple invaginated in the form of finger-like tubules. Overstaining with uranyl acetate showed that the membrane was continuous around the inner boundary of the cuticular wall of each sensillum (Fig. 4). Based on our total microscopic investigations of the sensilla basiconica on *S. multistriatus* antennae, we concluded that the dendritic branches of sensory neurons in these sensilla are directly exposed, via the finger-like tubules and the flask-shaped pores in the cuticle, to a certain chemical environment which may surround the insect.

Penetration of chemical messenger into the chemosensory sensillum. The tritiated feeding stimulant, catechol (1,2-dihydroxybenzene) (Fig. 2), penetrated into the lumen of sensilla basiconica of live *S. multistriatus* and bound especially with nerve membranes (Fig. 5). Each time exposure (i.e., 5, 10, or 60 min.) of the beetles to the tritiated catechol resulted in significant label entering these sensilla. Numerous autoradiographs showed clearly that the feeding stimulant entered these sensilla via the combined

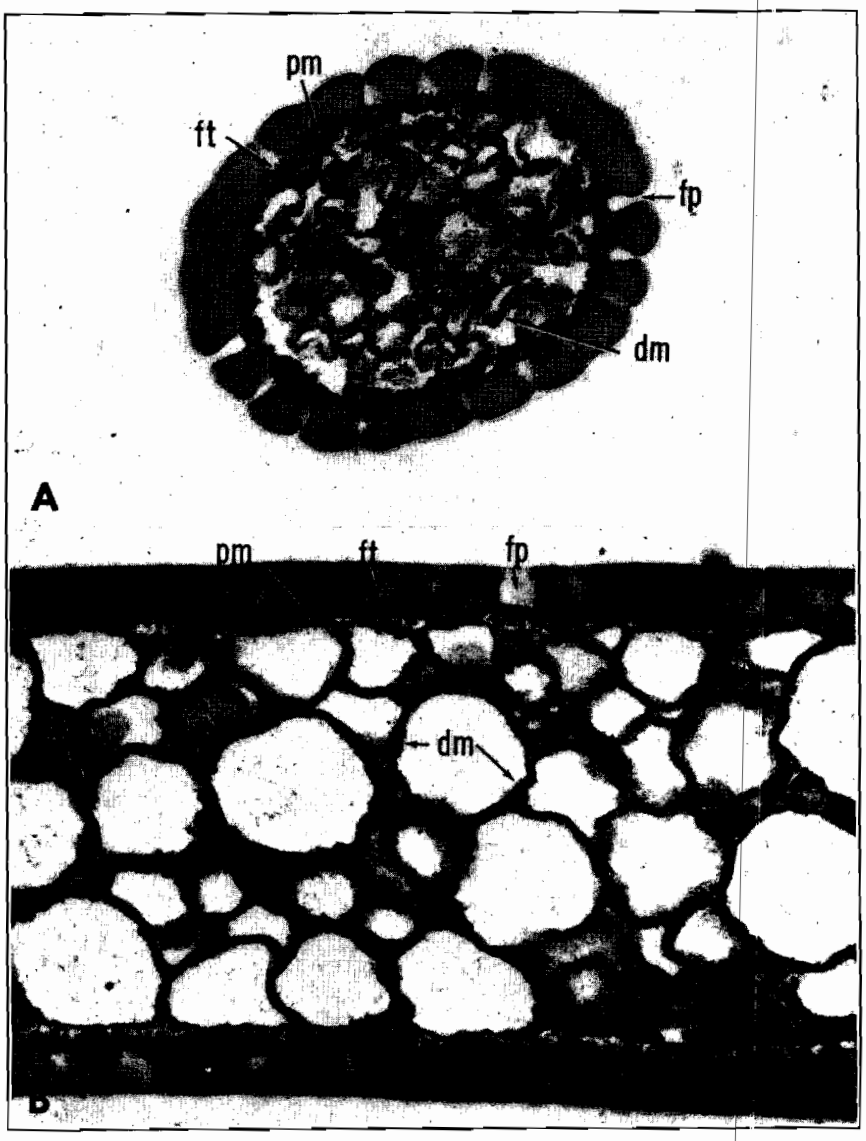

FIGURE 4. Electron micrographs of chemosensitive sensilla basiconica. (A) Cross section (60,000 ×) showing flask-shaped pores (fp) in cuticle, plasma membrane (pm) containing finger-like tubules (ft), and dendritic membranes (dm) associated with tubules and the plasma membrane. (B) Longitudinal section (45,000 ×) showing flask-shaped pores (fp) in cuticle, finger-like tubules (ft) in plasma membrane (pm), and dendritic membranes (dm) of sensory neurons in the chemosensitive sensillum.

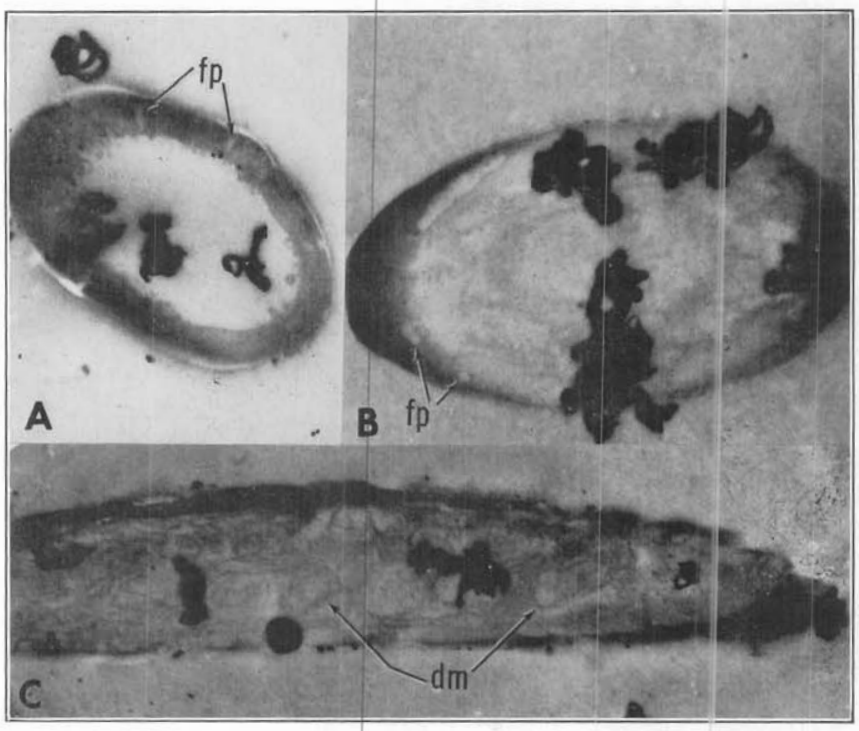

FIGURE 5. Autoradiographs showing that the radiolabel of tritiated catechol, a feeding stimulant, penetrated (A, 33,000 ×; B, 40,000 ×) through flask-shaped pores (fp) and tubules of chemosensitive sensilla basiconica on the antennae of live *S. multistriatus*; and complexed (bound) especially with dendritic membranes (dm) of sensory neurons in the lumen of the sensilla (C, 20,000 ×).

pore and tubule route through the cuticle (Fig. 5).

Complexing between 2-(methyl-^{14}C)-1,4-naphthoquinone and particulate bands from antennal homogenate. Of the recovered radioactivity, only 16 per cent was in the particulate fractions, F_{1-4}, (Table 2) from the sucrose-density gradient. Because chemoreception in gustation and olfaction must by its nature be a transient phenomenon, we expected a low level of feeding inhibitor binding to the particulate fractions. The specific activity per mg. of protein was greatest in fractions F_1 and F_2 (Table 2). Electron micrographs of aliquots of the particulate fractions (Table 2) indicated that F_1 and F_2 were rich in nerve membrane fragments (Fig. 6).

TABLE 2.—Radioactivity bound in particulate fractions isolated on a sucrose-density gradient after the antennal homogenate was incubated with 0.1 μc. of 2-(methyl-^{14}C)-1,4-naphthoquinone

Fraction	Total protein, mg.	Counts per minute per mg. protein
F_1	314	7742
F_2	157	8080
F_3	1447	5716
F_4	1078	4480
Residue	1072	5694

U.V. spectroscopy of binding of inhibitory naphthoquinones to components of antennal homogenate. The change in U.V. absorbance at 280 nm. induced by each naphthoquinone interacting with components of each particulate fraction (i.e., F_{1-4}) or the decantate from the antennal homogenate is shown in Figure 7. The greatest change in absorbance

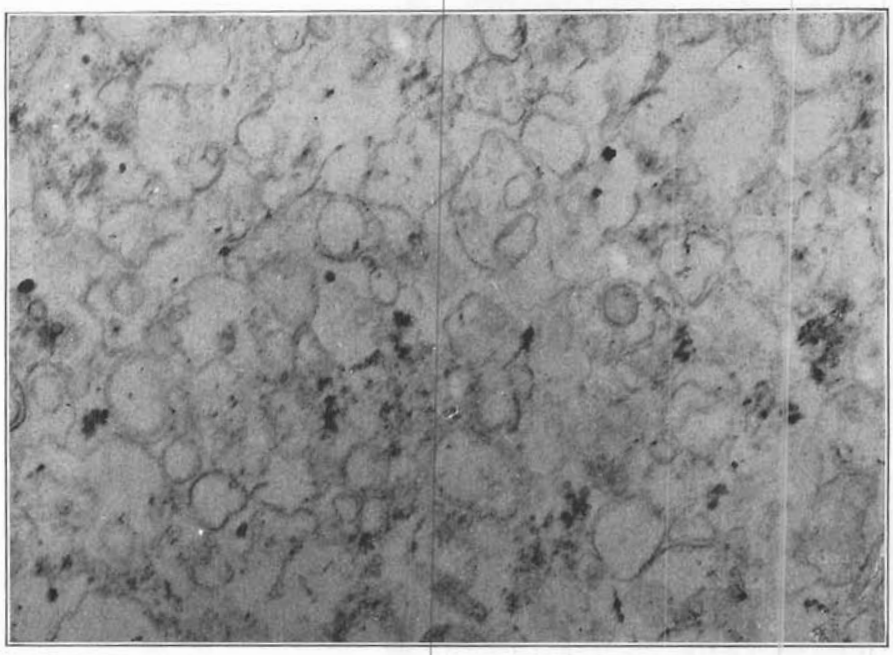

FIGURE 6. Electron micrograph of an aliquot of a particulate fraction from sucrose-density-gradient centrifugation which was rich in nerve membrane fragments.

with each naphthoquinone occurred with fraction F_2. Absorbance with F_1 was intermediate; and that with F_3, F_4, and the decantate was similar, and significantly less than with F_1. Considering the relative change in absorbance at 280 nm. associated with each naphthoquinone interacting with a given fraction or the decantate, 5-hydroxy-1,4-naphthoquinone yielded the largest absorbance change, 1,4-naphthoquinone was intermediate, and 2-methyl-1,4-naphthoquinone gave the least with fractions F_{1-2}. With fraction F_4, the pattern was reversed.

It is assumed that the magnitude of change in absorbance is directly related to the degree of binding of a naphthoquinone with components of a particulate fraction or the decantate. Because in these experiments, both the reference cell and the sample cell contained a solution of one of the particulate fractions or the decantate, any change in the conformation of components in a solution absorbing at 280 nm. after addition of the naphthoquinone to the sample cell was reflected as a change in absorbance relative to that of the solution in the reference cell.

These results established that the order of relative *in vivo* feeding inhibition by three naphthoquinones was the same as the order of their relative degrees of binding with antennal homogenate fractions F_1 and F_2 which especially contained nerve membrane fragments.

Freezing an inhibitory quinone with an aliquot of particulate fraction F_2 or with $5\times10^{-4}M$ reduced glutathione produced new spectrophotometric absorbance characteristics attributable to complexing and reduction of the feeding inhibitory quinone. When $5\times10^{-4}M$ *N*-ethyl maleimide, the specific sulfhydryl-group inhibitor, was added to the mixtures of quinone and F_2 or reduced glutathione, freezing these solutions produced color changes different from those observed in the absence of *N*-

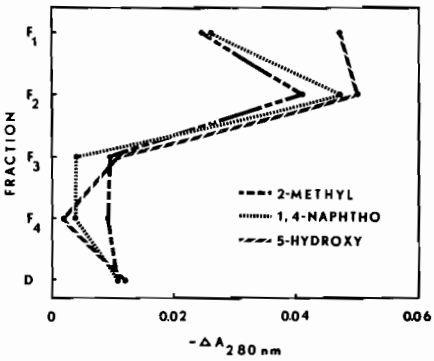

FIGURE 7. Differential absorbance at 280 nm. upon addition of the given naphthoquinone ($1.7\times10^{-7}M$) to the sample cell. The sample had been previously balanced against the reference with a concentration of a given fraction or the decantate (D) which produced an optical density of 0.2 unit.

methyl maleimide, but the color changes were still indicative of energy-transfer reactions (24). The changes in color proved that the sulfhydryl-group inhibitor, *N*-ethyl maleimide, and the feeding-inhibitory quinones were competing for the same reaction sites on F_2 and the reduced glutathione. This site would be the sulfhydryl group (i.e., -SH).

Dropping-mercury-electrode polarography of energy-transfer complexing in a model system. The reaction of glutathione with 1,4-naphthoquinone resulted in the half-wave potential of the quinone wave being shifted toward the right 0.025 volt (Fig. 8). Such a small, but real, shift in potential indicated that an energy transfer and/or new compound had resulted between the chemicals in the solution. In the presence of glutathione, an anodic wave for 1,4-naphthoquinone appeared; whereas, this quinone alone yielded only a cathodic wave (Fig. 8). This indicated that the quinone had been reduced during its interaction with glutathione, and that the reduced quinol subsequently was being oxidized at the dropping-mercury electrode.

Changes in the diffusion current observed with 1,4-naphthoquinone alone versus the 1,4-naphthoquinone+glutathione indicated that complexing occurred. At a given molar concentration, the diffusion current of different chemicals should vary as the square root of their molecular weight. Glutathione+1,4-naphthoquinone gave an anodic wave current for the quinone (Fig. 8) which would indicate that the new moiety was about three times the weight of the quinone alone. The combined molecular weight of 1,4-naphthoquinone+glutathione is 465 as compared to 158 for the quinone alone. These total findings would strongly support the existence of a complex between 1,4-naphthoquinone and glutathione.

Upon addition of the specific sulfhydryl group inhibitor, *N*-ethyl maleimide, to the mixture of 1,4-naphthoquinone and glutathione, the diffusion current for the 1,4-naphthoquinone wave

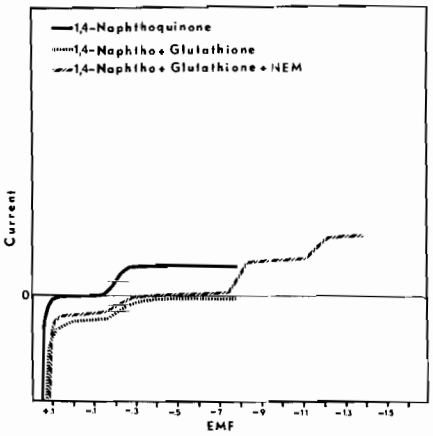

FIGURE 8. Polarograms of 1,4-naphthoquinone; 1,4-naphthoquinone+reduced glutathione; and 1,4-naphthoquinone+reduced glutathione +*N*-ethyl maleimide (NEM), at 20 μA, full-scale sensitivity, and a damping setting of 4. Concentrations of all tested chemicals were $5\times10^{-4}M$, and the buffer used was 75 ml. of phosphate buffer (pH 7)+25 ml. of 95 per cent ethanol. (Note the small shift in half-wave potential of the wave for the 1,4-naphthoquinone when mixed with glutathione or glutathione +NEM. This shift is indicative of an energy-transfer complex. Changes in diffusion current of the 1,4-naphthoquinone wave attributable to treatment also are evident.)

fell between that obtained when the quinone was alone, and when it was combined with just glutathione (Fig. 8). These results would indicate that the addition of *N*-ethyl maleimide reduced the amount of 1,4-naphthoquinone which was complexed with glutathione. These data thus prove competition between the feeding-inhibitory quinone and *N*-ethyl maleimide for glutathione, and would implicate the sulfhydryl as the reaction site on glutathione.

DISCUSSION

Data presented, or summarized, in this paper from our long-term studies of chemoreception by *S. multistriatus*, and other selected species, allow the first characterization of a naturally-operative energy-transduction mechanism involved in insect gustation and/or olfaction. This characterization also is the first for any animal.

The experimental findings indicate that the chemical messenger quinol-quinones penetrate through pores and tubules in the cuticle of chemosensitive sensilla on the antennae of *S. multistriatus*.

In the lumen of the sensillum, these regulatory ligands (i.e., quinones) complex with protein especially at the membranes of the dendrites of sensory neurons. The specific site of reaction in the receptor protein is the sulfhydryl group. In addition to the formation of the complex, the sulfhydryl group is oxidized, and the quinone is reduced. With the very recent findings of Ferkovich and Norris (11), it may now be confidently concluded that the energy-transfer complex involved in quinone inhibition of *S. multistriatus* and *P. americana* feeding entails the formation of mono- or di-adducts. The associated oxidation of the sulfhydryl and reduction of the quinone apparently are accommodated by the complex formation. This interpretation of the involved energy-transduction mechanism is compatible with the observed order of relative *in vivo* inhibition of feeding and *in vitro* binding with receptor protein by the variously substituted 1,4-naphthoquinones. An interpretation involving covalent bonding is not compatible with the observed relative *in vivo* and *in vitro* activities of these investigated inhibitory naphthoquinones. This new understanding of how the initial energy transfer essential to the generation of an action potential in the sensory neuron occurs should prove useful in many aspects of neurobiology and neurochemistry.

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