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CONCENTRATION AND PRELIMINARY CHARACTERIZATION OF A CHEMICAL ATTRACTANT OF THE OYSTER DRILL, *Urosalpinx cinerea*

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Abstract—Predatory muricid gastropods, *Urosalpinx cinerea*, respond to specific chemical stimuli by creeping upcurrent. Attractant substances originate from living barnacles. Newly hatched snails have no prior predatory experience but respond strongly to attractants. We report here methods for rapidly extracting and desalting attractants from seawater. Attractants from living barnacles are relatively large, at least partially proteinaceous, heat-stable molecules (>1000 but <10000 daltons) that adsorb onto Amberlite XAD-7, a polyacrylate water purification resin, at neutral pH. Attractants remain adsorbed to the resin during a wash with deionized water and can be eluted in a small volume with 100% methanol. Attractant substances are effective in the bioassay in μg/liter concentrations (octa- to nanomolar range). Potency is destroyed by nonspecific proteases (carboxypeptidase and pronase) but not by trypsin. Attractant is not sequestered within barnacles.

Key Words—Attraction, muricid gastropods, barnacles, chemical sensing, *Semibalanus balanoides, Balanus eburneus, Urosalpinx cinerea,* oyster drill, snail, predator–prey relationships.

INTRODUCTION

The majority of studies performed on the chemical nature of attractants for aquatic organisms have been on feeding attractants. These studies have been

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of two types: (1) tests and analyses of extracts or homogenates of prey (Shelton and Mackie, 1971; Atema et al., 1980; Carr, 1967; Townsend, 1973; Castilla, 1972; Gurin and Carr, 1974; Collins, 1975); and (2) characterization of molecular constituents of washings (Shelton and Mackie, 1977; Atema et al., 1980; Carr, 1967b; Hanscomb et al., 1976) or extracts of living animals and molecular analysis of individual compounds in synthetic mixtures (for review see Bardach, 1975; Atema, 1980). Both approaches indicate the presence, and some of the types of, molecules composing prey that are attractive to predators or scavengers (mainly amino acids and proteins). However, behavioral and biochemical identification of highly specific attractants such as those indicating specific prey are obscured by either approach. Homogenization and extraction generate complex organic mixtures from which trace organic molecules are not easily retrieved. Reconstitution of active mixtures is limited to those molecules readily detected, characterized, and commercially obtainable. Neither approach answers questions on the nature of a native attractant released from intact prey.

To paraphrase William Herrnkind (personal communication), in nature predators do not usually encounter extracted or homogenized prey, nor do they locate mixtures of readily available shelf chemicals. Furthermore, a specific behavioral response to molecular stimuli suggests that specific information must have been conveyed. Specific information requires a unique molecular structure to code that information. It might be expected that specific attractants would be similar in size and complexity to other information-conveying molecules, for example, hormones. Therefore, a basic premise in the investigation reported here is that specific attractants can be purified and characterized from the seawater bathing living intact prey.

Oyster drills, *Urosalpinx cinerea* (Say), have a well-documented ability to locate intact prey from a distance by creeping upcurrent in response to chemical cues (for a thorough review of the early literature see Carriker, 1955; Blake, 1962; Wood, 1968; Pratt, 1974, 1976; Ordzie and Garafalo, 1980). Newly hatched *Urosalpinx cinerea* from the Delaware Bay region have a specific propensity for a molecule(s) from barnacles (Rittschof et al., 1983). This propensity is maintained in adults even after they have fed exclusively upon oysters for over a year (Rittschof and Gruber, unpublished data). Indeed, if barnacles were a commercially important shellfish, oyster drills would probably be commonly called barnacle drills.

As part of an ongoing project with the objective of assessing the potential of chemical biocontrol measures for oyster drills, we reported (Rittschof et al., 1983) the development of a bioassay useful in monitoring attractants from prey. The assay uses newly hatched drills and their response to attractants in seawater. The assay was developed specifically for use in the determination of the chemical nature of attractant molecules. Here we report on the concentration, purification, and initial characterization of the attractant originating

from balanoid barnacles. Identification of the active substance(s) is in progress.

The rapid, simple procedure reported here for purifying and concentrating attractants as well as preliminary information on the nature of the molecule have applications in many areas of chemosensory inquiry. Our bioassay has both a defined response and a highly concentrated and partially purified stimulus and is being put to other uses. The concentration and desalting step that uses Amberlite XAD-7 may have general applicability for extraction and desalting of other complex bioactive molecules. Several coworkers have already communicated positive results with this technique.

METHODS AND MATERIALS

Reagents. Deionized water was produced by a Millipore Corporation Milliq system. The Amberlite XAD-7, XAD-4, and XAD-2 resins used were generous gifts of the Rohm and Haas Corporation. Cellulose was Sigmacel 50 from Sigma Chemical Corporation. Methanol was HPLC grade (Bodman Chemicals, Media, Pennsylvania). All other reagents were reagent grade. Enzymes were purchased from Sigma immediately prior to use. Trypsin was from bovine pancreas (Sigma T-2884), carboxypeptidase was from bovine pancreas (Sigma C-0386), and pronase E (Sigma P-5147) was from *Streptomyces griseus.*

Preparation of XAD Resins and Cellulose. XAD resins were washed thoroughly with water and then with methanol. Methanol removed a flocculent white material (antistatic agent, according to the manufacturer) and was a necessary step in their preparation. Columns were prepared by back-flushing as recommended by the manufacturer. Batch treatment of resins was by the same washing procedure. Cellulose, batch washed in a Buchner funnel, was washed extensively with water, ethanol, and either acidified or basified ethanol. After washing and sequentially rewashing the material with methanol and water, it was dried under vacuum.

Column Procedures. XAD columns were run at rates from 1 ml/min to >1 liter/min. Slow flow rates were used in analytical procedures. High flow rates were used in preparative procedures.

Pressure Dialysis. Pressure dialysis was performed with an Amicon Corporation 180-ml ultrafiltration cell with or without a 2-liter reservoir. Membranes were prepared and handled according to the instructions of the manufacturer. Filtrations requiring longer than 30 min were conducted at 4°C. Short-term dialysis was performed at ambient temperatures (20–23°C).

Test Snails. Newly hatched oyster drills, *Urosalpinx cinerea* (Say), were harvested from our laboratory hatchery as previously described (Rittschof et al., 1983). They were stored at 8–13°C without food. Snails used one day for

one experiment were sometimes reused another day in a separate experiment as described in Wood et al. (1983). Williams et al. (1983) showed that *U. cinerea* exposed to attractants for several hours may have a slightly reduced response to that attractant on a subsequent day. Snails were not exposed to attractant for more than 15 min on any particular day. Snails were stored in aged seawater when not being tested.

Barnacles. Semibalanus balanoides and *Balanus eburneus* were sources of attractant. *B. eberneus* were obtained locally and stored in a 16-liter polycarbonate container with aerated seawater. Bricks and rocks on which *S. balanoides* had set were collected from the inner breakwater of Delaware Bay and Indian River Inlet. In all, several hundred living intact *B. eburneus* averaging over 1 g total weight each (including shell) and many thousands intact living *S. balanoides* weighing about 0.1 g each were used. Rocks encrusted with *S. balanoides* were maintained in a 40-liter glass aquarium with aeration and fed several times a week with algae from the University of Delaware's Mariculture Facility. Attractant was in seawater in which barnacles were living independent of whether or not they were fed.

Preparation of Attractant Water. Attractant-free seawater was 1-μm-filtered seawater that had aged by standing for several days. The response to this water in the bioassay is less than 15%.

Attractant was prepared in two ways: (1) intact living barnacles were bathed in aged, 0.4-μm polycarbonate-filtered seawater (30–32 ppt.) for a minimum of 3 hr; (2) less-crude stimulus was produced by first 0.2-μm-polycarbonate filtering and then purifying the aged seawater by passing it through XAD-7 (Rohm and Haas resin). Barnacles were rinsed in aged filtered seawater, then in three changes of XAD-7 "scrubbed" water, and finally bathed in fresh polycarbonate-filtered and XAD-7-scrubbed seawater. After a 3-hr incubation, the water surrounding the barnacles was filtered through glass fiber filters (1 μm nominal pore size) and 0.4 μm polycarbonate filters. This was the starting material. Preparations were bioassayed at several log dilutions to determine potency.

Bioassay. Bioassay procedures used were those described by Rittschof et al. (1983) and Wood et al. (1983) with the exception of a microbioassay. The latter assay was used when only small volumes of attractant were available. The microassay apparatus was designed for use with microliter amounts of stimulus and milliliter volumes of seawater. It consisted of a Desage syringe pump modified to hold six disposable 10-ml syringes. Each syringe was fitted with a 0- to 200-μl disposable automatic pipetter tip. Borosilicate glass 1-ml pipets were cut in half and bent as previously described (Rittschof et al., 1983). The response criteria were the same as previously described for the larger assay apparatus, but the total volume of seawater necessary to run a 10-min assay was 1.7 ml.

Temperature Treatments. In a first series of temperature experiments,

stimulus water to be tested for temperature effects on activity was glass-fiber-filtered and 0.2-μm polycarbonate-filtered. Untreated attractant was placed immediately in an ice bath. When incubation temperature was 70°C or less, undiluted attractant was incubated in an Erlenmeyer flask in a water bath set at the test temperature. A flask of equal volume containing a thermometer was incubated simultaneously. When the solution in the parallel container reached the test temperature, timing was started. Solutions treated at 30, 50, and 70°C were incubated for 30 min and then cooled to ambient temperatures with ice. Attractant to be boiled was placed in an Erlenmeyer flask with a loose glass lid to retard evaporation. The flask was placed in a boiling water bath and timing started when the solution began to visibly boil. After 5 min of boiling, the solution was cooled on ice. Aged filtered seawater was treated similarly and tested with and without addition of unheated attractant to determine the effects of boiled seawater on the snail responses. In a second series of experiments, concentrated and size-fractionated attractant was boiled in both open and sealed ampules from 0 to 90 min. Attractant was diluted in aged seawater and potency compared to that of unheated attractant.

Reagent Experiments. Reagents other than aged seawater used in experiments were high-performance liquid chromatography (HPLC)-grade methanol, deionized water, and boiled seawater. Effects of each reagent in the bioassay were tested by adding a constant small volume of cencentrated attractant and performing dilution series experiments on the reagent over the concentration range to be added to the assay. Control experiments included aged seawater alone and reagent without added attractant.

Matrix-Binding Experiments. A series of matrix-binding experiments was conducted to determine if attractant associated with barnacles could be removed from seawater. Cellulose and commercially available water treatment resins were tested.

Recovery Experiments. Experiments were conducted to determine if the biological activity removed from seawater in matrix-binding experiments could be recovered. Solvents tested for effective removal of stimulus from the resin were deionized water and HPLC-grade methanol.

Homogenization Experiments. Experiments were conducted to determine if homogenization of barnacles would improve the yield of chemoattractant. In these experiments: (1) barnacle stimulus was first prepared by our normal procedures, that is, by letting living barnacles stand in seawater and extracting the attractant from seawater with XAD-7 resin; and (2) the same barnacles that produced the attractant in step 1 were scraped from the rocks and weighed. The 125 g of barnacles was ground fine for 5 min in a mortar and pestle in 100 ml of deionized water. This homogenate and three additional 100-ml washes of the residue with distilled water were centrifuged at 3000 *g* for 5 min in a Sorval ss-34 rotor. The supernatant was filtered through a glass fiber filter and 0.4-μm polycarbonate filter. The filtrate was diluted to 3 liters

with XAD-7-treated 0.4-μm polycarbonate-filtered seawater and subjected to the same stimulus extraction, washing, and elution procedures as the stimulus prepared in step 1. All subsequent bioassays and biochemical operations were performed simultaneously on both preparations.

Enzyme Experiments. Enzyme experiments tested the ability of trypsin, carboxypeptidase, and pronase to destroy biological activity. Trypsin was tested at 200 and 2000 BAEE units/ml concentrated attractant. Carboxypeptidase was tested at 10 and 1 units/ml concentrated attractant (1 unit hydrolyzed 1.0 μmol hippuryl-L-phenylalanine/min at 25°C and pH 7.5). Pronase was tested at 1 and 0.1 units/ml concentrated attractant (1 unit hydrolyzed casein to produce color equivalent to 1.0 μmol of tyrosine/min at 37°C and pH 7.5). Separate aliquots were prepared for each incubation at 21°C (0, 0.5, 2, 4, 6, 8 hr). At each time interval an aliquot was diluted 1:250 and assayed. Controls were: seawater with no additions; seawater with the addition of each enzyme incubated for each time interval; seawater and attractant; and seawater, attractant, and each enzyme assayed immediately upon mixing.

Definition of Activity Unit. Units are defined arbitrarily based upon the known response of test snails (Rittschof et al., 1983; Williams et al., 1983; Wood et al., 1983). One unit is the attractant required per milliliter to evoke a 37% response in the assay. Because of the nonlinear nature of the cumulative percent response assay (Williams et al., 1983), 0.1 units evoke a 13% response. Dilution series bioassays were performed on preparations throughout purification procedures in order to determine location, amount, concentration, percentage recovery, and units per milligram dry weight of stimulus. When assaying preparation of unknown potency, we made and tested dilutions until responses between 13 and 38% were obtained. Units were estimated from plots of percentage response against the log of the dilution.

RESULTS

Manipulations. Biological activity was always observed in water bathing either living *Semibalanus balanoides* or *Balanus eburneus*. Relatively potent preparations (1–5 units) were used to examine the effects of manipulations on stimulus activity. Stimulus could be centrifuged at 10,000 *g* for 20 min at 4°C with no detectable loss of activity. Vacuum aspirator filtration through 1-μm glass-fiber filters also had no appreciable effect on potency. However, filtration through polycarbonate filters (0.2 μm) with vacuum filtration sporadically produced marked increases in potency (often more than doubling in apparent number of activity units). The same result could be obtained by a period of vacuum treatment without filtration. This phenomenon was observed only with crude attractant preparations that could be diluted and still retain activity but that did not evoke high levels of response at any

dilution. The normal log relationship between stimulus concentration and snail activity (Rittschof et al., 1983) was not observed. That is, dilution of the stimulus resulted in much higher estimates of the number of units in a preparation than did assay of the concentrated material. Assay of the material after vacuum treatment resulted in the normally observed relationship.

Water that had not been polycarbonate-filtered to remove bacteria rapidly lost potency upon standing at room temperature. One-micron-filtered attractant lost 50% of its potency in 6 hr at room temperature. The 0.2-μm polycarbonate-filtered attractant retained potency for at least 6 hr at room temperature.

Molecular Size Determination. Two types of dialysis experiments were employed to estimate the size of molecule of the barnacle stimulus. First, 100 ml of approximately 5 activity units of stimulus were placed in a dialysis bag (20 Å pore size, approximately 10,000-dalton exclusion limit) and dialyzed against 300 ml of 0.2-μm polycarbonate-filtered seawater for 4 hr. Bioassays of the dialysate showed stimulus activity ($P < 0.005$). Second, a series of experiments employing Amicon pressure dialysis membranes was conducted. Bioassayable activity passed through 10,000- and 5000-dalton membranes and was retained by the 1000-dalton membrane and by the 500-dalton membrane (Figure 1).

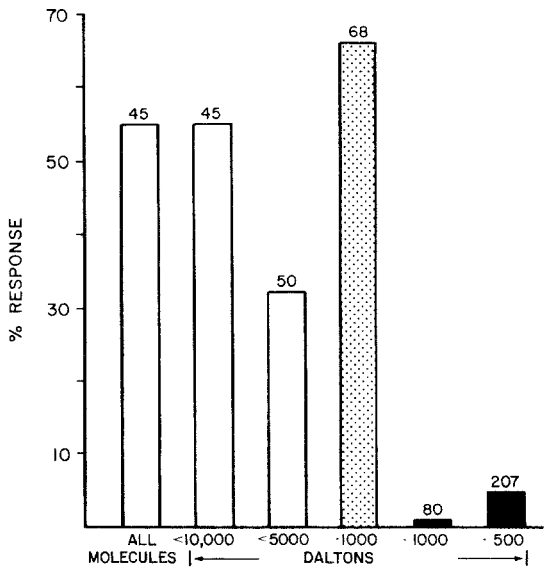

FIG. 1. Molecular size of barnacle attractant. Pressure dialysis with Amicon membranes followed by bioassay indicates that attractant is approximately 5000 daltons. Numbers above bars are numbers of snails tested.

Matrix-Binding Experiments. Experiments were conducted to determine a nonionic matrix that binds stimulus activity. Following Blake's lead (Blake, 1962, observed stimulus was bound to cellulose-containing products), we tried cellulose matrices. Additionally, we tested two water-purification resins, Amberlite XAD-2 that adsorbs nonpolar substances and Amberlite XAD-7 that adsorbs both polar and nonpolar substances. Significant ($P < 0.005$) amounts of activity were removed by untreated Sigmacel cellulose and base-washed cellulose, but not by acid-washed cellulose. Although there was significant retention of the attractant, cellulose matrices had low capacity. Of the Amberlite resins, XAD-2 had low apparent capacity, while XAD-7 was very effective (>95% retention) (Figure 2). Subsequent tests showed XAD-7 resins had a high capacity for attractant while XAD-2 and a second XAD-4 had limited capacity. We passed 1800 units of activity through a column containing 0.3 g of XAD-7 resin with less than a 5% leak rate at flow rates of 4 ml/min. XAD-7 resin was used in subsequent testing.

Once attractant was adsorbed to the XAD-7 resin, washing to remove salt and recovery of the activity was attempted. A larger column that had a theoretical capacity of over 230,000 units was constructed of approximately 40 g of XAD-7 resin, and run at flow rates of 300 ml/min. We passed 18,000 units through this column and assayed the flow in fractions and found no detectable activity. Gradual decrease in salinity and distilled water washing produced no detectable release of activity. However, 100% methanol applied

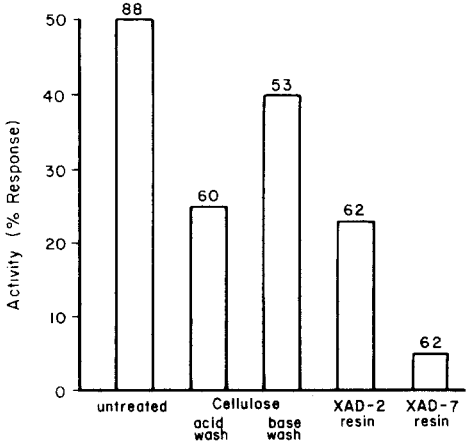

FIG. 2. Matrix binding of attractant. A known amount of activity was passed through different matrices and assayed for activity. Acid-washed cellulose and XAD-2 and XAD-7 resins removed at least some activity (compare to untreated control). Amberlite XAD-7 reduced assay responses to levels observed in attractant-free seawater. Numbers above bars are numbers of snails tested.

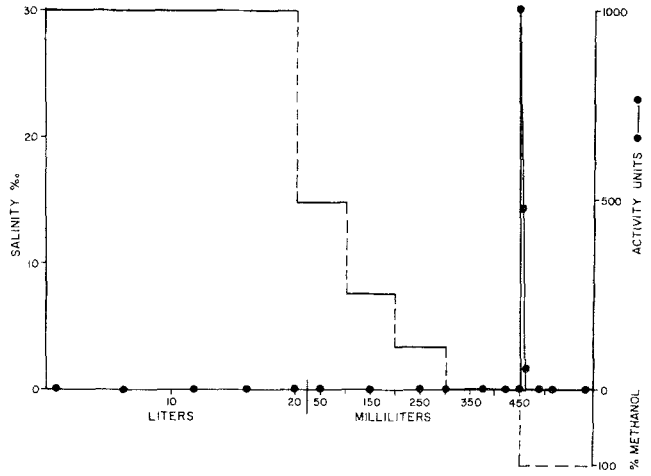

FIG. 3. Concentration and desalting of specific attractant with Amberlite XAD-7 resin. Attractant is adsorbed onto XAD-7 resin (100 ml/min onto a 2-cm ID × 25-cm bed), salinity is reduced to 0, and attractant is stepped off column at interface between deionized water and 100% methanol.

to the column released at least 65% of the units loaded on the column attractant eluted at the interface between the water and methanol (Figure 3, Table 1). About 30 min was required to pass 20 liters of seawater through the column, 10 min to wash away salts and nonadsorbed material, and 5 min to collect concentrated attractant.

Temperature Experiments. Temperature stability of the attractant was tested prior to further purification and characterization. First experiments were conducted with crude preparations that had been 0.2-μm polycarbonate-filtered. These experiments demonstrated that biological activity was relatively heat stable. There was no detectable change with 30 min of heating at 30, 50, or 70°C. Boiling crude attractant for 5 min reduced assay potency at high concentrations but had no effect at low concentrations. Boiling seawater alone inhibited assay responses.

Next a series of experiments was conducted on concentrated attractant. Attractant was heated at 100°C in sealed and open vials for 30, 60, and 90 min. Biological assays compared the activity of the different boiled samples to that of unboiled stimulus and to aged seawater controls. Whereas there was significance reduction of biological activity in samples boiled open to the air (G statistic $P < 0.005$), samples heated in sealed ampules showed full biological activity.

Homogenization Experiments. Attractant extracted from seawater was compared to stimulus prepared by homogenizing and extracting tissues of the

TABLE 1. PURIFICATION OF BARNACLE STIMULUS

Fraction	ml	U/ml	mg/ml	U/mg	Total units	Recovery (%)	Purification	13% A[a] (M)	37% A (M)
Starting	4000	3.0	34.800	0.086	12,000	100	1	10^{-4}	10^{-3}
XAD-7	47	150.0	0.560	267.140	6,964	58	3,106	10^{-7}	10^{-6}
YM5 FT	43	100.0	0.083	1204.820	4,300	36	14,009	10^{-9}	10^{-8}
UM2 Ret[b]				1266.666	2,406		14,728	10^{-9}	10^{-8}

[a] Molar concentrations were estimated assuming a minimum molecular weight of 1000 and using the dry weight of each fraction.

[b] Material tested was from a separate purification and was passed through a YM5 membrane prior to concentration on the UM2 membrane. This material was assayed and then dried and weighed as were all the other preparations.

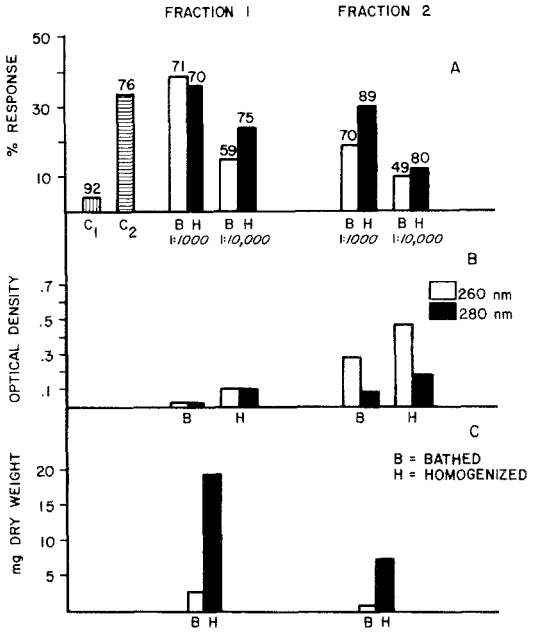

FIG. 4. Comparison of homogenization and seawater extraction of attractant. (A) Bioassay of active material from each preparation. C1 is attractant-free seawater control, C2 is response to a standard preparation. Fractions 1 and 2 account for 80–95% of the recoverable activity in the preparations. Numbers above bars indicate number of snails tested. (B) Relative absorbance at 260 and 280 nm of each of the active fractions for each preparation. (C) Total dry weights for each of the active fractions.

same barnacles. Both preparations contained activity. Relative potencies were similar in the two preparations (Figure 4). Both preparations had similar OD 260/280 (Figure 4B) ratios and showed consistent differences between fractions 1 and 2. Absorbances for the homogenized preparation were markedly higher in each fraction.

Homogenization resulted in approximately a 10-fold decrease in the specific activity of the preparation as a result of the increase in inactive material from soft tissues of the barnacle copurifying with the stimulus (Fig. 4C).

Reagents and Bioassay. Discovery that attractant could be bound to XAD-7 resin, desalted, and eluted in concentrated form suggested that the bioactive compounds were stable after treatment with methanol and to low ionic strength solutions. However, since reagents can alter snail bioassay behavior, experiments were designed to determine their effects on the assay. Potency was examined in both a dilution series of the reagent alone and with a constant addition of barnacle stimulus and a dilution series of the reagent to

be tested. The three additions to the bioassay during test procedures were boiled water, deionized water and methanol. Reagents were tested separately at dilutions likely to be encountered during subsequent steps in the purification. As the stimulus was concentrated, maximum concentrations of additives in the bioassay were likely to range downward from a 1:100 ($\simeq$0.3 M) dilution, in all probability to a range less than a 1:1000 ($<<$ 0.03 M) dilution. Of the reagents tested, none stimulated drills to creep. Methanol, however, had a negative effect on the assay at higher concentrations (G statistic $P << 0.005$). There was an inverse relationship between the amount of methanol and the response to attractant. Dilutions of methanol greater than 1:500 (0.06 M) had insignificant effects on responses (Figure 5).

Response to Partially Purified Concentrated Attractant. Partially purified and concentrated attractant was tested for biological activity over a range of dilutions (example, Figure 6). Response of snails was optimal at dilutions of attractant that corresponded to approximately 10^{-8} m to 10^{-9} m concentrations of attractant by weight. Concentrations above the optimum evoked lower percentage responses as did concentrations that were less than the optimum. Responses to optimal concentrations approached 100% when assay conditions (temperature, care of snails) were attended to carefully.

Enzyme Experiments. Responses of snails to solutions containing enzymes were the same as those to solutions containing only seawater (0 to 4%). Responses to controls containing attractant were between 35 and 40%

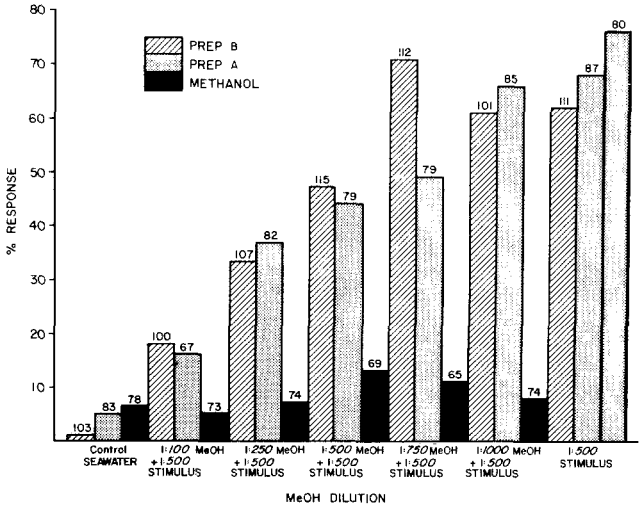

FIG. 5. Effect of methanol on activity evoked by standard attractant. Slashed bars represent one complete experiment, stippled bars represent a second replicate experiment. Solid bars are the effect of methanol in the absence of attractant on the assay. Numbers above bars indicate numbers of snails tested.

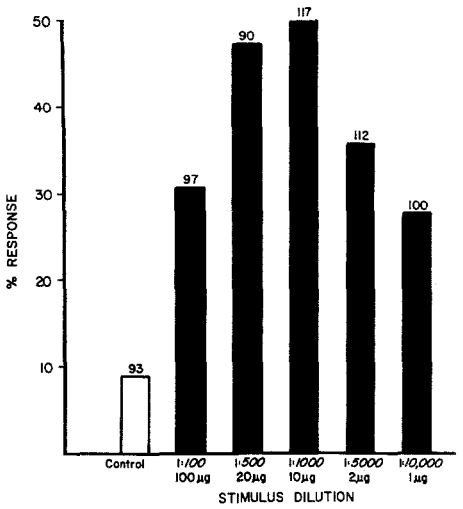

FIG. 6. Representative response to dilutions of concentrated, partially purified attractant. Attractant was concentrated and desalted with XAD-7 resin, rotary evaporated to near dryness to remove methanol, size fractionated by pressure dialysis, and tested for activity in the bioassay. Numbers above bars indicated numbers of snails tested at each dilution for that experiment. If care is taken to control temperature and absolute age treatment of snails, 90–98% response to optimal concentrations of attractant can be obtained.

(seawater plus attractant and seawater plus attractant and each enzyme assayed immediately). Trypsin had no effect on potency (experimental vs. attractant control, $G = 0.009$ NS) after 8 hr at 2000 units/ml. At all concentrations tested, carboxypeptidase and pronase E rapidly reduced potency. At 10 units carboxypeptidase significantly decreased attractant potency after 2 hr ($G = 6.88$, $P < 0.001$). Similarly, pronase E at 1.0 unit/ml significantly decreased potency after 2 hr incubation with concentrated attractant (experimental vs. attractant control, $G = 4.75$, $P < 0.05$). After 4 hr of incubation, responses to carboxypeptidase- and pronase-treated attractant were indistinguishable from responses to seawater alone.

DISCUSSION

To our knowledge, oyster drill attractant is the first marine attractant to be isolated as a by-product of living intact prey. Attractant molecules are relatively complex and temperature stable, having molecular weights between 1000 and 5000 daltons. Attractant can be destroyed rapidly by proteolytic enzymes that attack carboxy termini and or that degrade peptides to amino acids. Trypsin, a more specific serine protease that attacks only linkages

containing lysine and arginine, had no effect on attractant activity, even at enzymatic activities 200 to 2000-fold higher than those effective for the other enzymes. We conclude from these experiments that attractant is at least partially proteinaceous.

The key to isolation and concentration of attractant is its affinity for XAD-7 resin at near-neutral pH and relatively high ionic strength. This promotes virtually complete removal of stimulus even by batch procedures. Amberlite XAD-7 is a polyacrylate resin with affinity for both polar and nonpolar substances and has flow and stability characteristics that enable rapid extraction and concentration of the dilute active molecule from seawater. A second very important factor is that, once adsorbed to the resin, attractant remains bound tightly to the resin even with removal of salt by distilled water washing.

XAD resins have been used previously to extract humic substances from natural waters (Jolley, 1981). These substances compose a majority of organics in seawater and are retained poorly or not at all by XAD-7 at neutral pH. Thus extraction of seawater at neutral pH and washing of resin with deionized water selectively avoids contamination by humics. Additional reduction of contaminants is accomplished by XAD-7 extraction of seawater prior to its exposure to barnacles. This step removes inactive molecules with affinity for XAD-7 that are present in seawater.

Temperature stability and molecular size of the attractant facilitate further purification by heating under vacuum to remove methanol introduced at the resin elution step. At this step, temperature-sensitive contaminants precipitate. Molecular sizing of the temperature-treated material removes the precipitate as well as contaminants such much larger and much smaller than attractant. This partially purified material is stable and potent at a 10^{-9} M concentration by weight.

Comparison of attractant extraction by XAD-7 from seawater with attractant extraction by XAD-7 from barnacle homogenate suggests that attractant is not a major component of living barnacles and is not extensively sequestered in the organism. There are much higher levels of contamination if the starting material is homogenized soft tissues of barnacles than if it is seawater extract. This is what would be expected if levels of potentially contaminating organics are taken into account. Whereas levels of organics in seawater are on the order of milligrams per liter (Fox, 1981), organics within living organisms are many grams per liter. Thus, beginning with a homogenate can be expected, in the case of a nonsequestered or minor organic component, to elevate levels of contamination from 10^3 to 10^5 times. Further study may show that a specific tissue of barnacles is rich in attractant. Should this be the case, it could provide an acceptable alternative to seawater extraction, especially when possible copurification of degradation products by seawater extraction is taken into consideration.

With the exception of the protein detection system of *Ilyanassa obsoleta* described by Gurin and Carr (1974) and the food-finding behavior of *Aplysia californica* (Jahan-Parwar, 1975), the molar activity of even our crudely purified attractant exceeds that of published snail feeding attractants by about three orders of magnitude. *Urosalpinx cinerea* attractant appears to be similar in activity to chemoattraction systems that are mediated by peptides or proteins (Gurin and Carr, 1974; Fernandez, 1978; Schiffman and Gallin, 1979). It is apparent that *U. cinerea* attractant is not a chemical picture (Atema, 1980) because we have succeeded in discarding over 99.999% of the molecules in the preparation. This is not to say, of course, that the chemical milieu within which the molecule is solvated is not important to its activity, but rather that a relatively minor component of the chemical milieu carries specific information.

There is a good likelihood that many molecules that are detected and used in location of living, intact prey may be similar to attractant from barnacles. Straightforward adsorption techniques may therefore prove useful in concentrating and purifying other biologically active molecules. As such, adsorption techniques present an exciting perspective for the study of compounds dissolved in natural waters.

In its partially purified state, *Urosalpinx cinerea* attractant is a powerful probe. Large amounts of stable, highly concentrated attractant enable its use as a meterstick for assessment of virtually any perturbation upon snail attraction. We are using attractant and the bioassay to test and quantify additions of reagents and the effects of exposure of embryos to media from living intact prey, and to measure ambient environmental levels of attractants.

It is apparent from Rittschof et al. (1983), Wood et al. (1983), Williams et al. (1983), and this report that *U. cinerea* attractant might also be considered the molecular essence of barnacle. To withstand selection pressures resulting from millions of years of barnacle predation by oyster drills, the attractant must perform some important function for barnacles, or it is an unavoidable excretory product. We are beginning investigations on the role(s) of this molecule in the biology of barnacles. It may, for example, function in the settlement of spat (Crisp and Meadows, 1962), in the molting process of barnacles, or it may be a component of barnacle cement. We believe that discovery of the site of production of the molecule will shed light on additional functions. We agree with Pratt's (1974) conclusion that, for *Urosalpinx cinerea*, detection is the first step in a predatory act that from all indications is a sophisticated, complex, and highly redundant resource verification system.

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