Isolation of the Sex Attractant of the American Cockroach

Abstract. The sex attractant of the female American cockroach, *Periplaneta americana* (L.), has been collected and isolated by a combination of extraction, distillation, and chromatographic procedures. Progress through these procedures has been followed by bioassay and by ultraviolet spectrophotometry. The attractant has been isolated by gas chromatography, and characterization by mass spectrometry is proceeding.

Specific sex attractants are produced by numerous animal species. Their identification has been attempted for many years (*1*), and two which are highly potent have been identified recently (*2*). The attractants of the gypsy moth and silkworm moth are active in quantities of 10^{-13} to 10^{-15} g; and we find that the attractant of the American cockroach, *Periplaneta americana* (L.), elicits a response from the male at levels of 10^{-17} g or less. By contrast, the queen substance of the honey bee (*3*) is attractive (*4*) to the drone at relatively high dose levels (10^{-4} g).

The virgin female American cockroach emits its specific sex attractant in minute quantities (*5*); this attractant has been the object of study in these laboratories for some years. Previous reports (*6–8*) have dealt with the behavioral response of the male to extracts of active material, with conditions affecting its production, and with the development of a bioassay. The present report concerns the chromatographic isolation of the attractant.

The initial steps in the isolation procedure take advantage of the fact that the attractant is distillable with water. Aqueous extracts of paper (Whatman No. 1) on which virgin females had been kept were made 0.1*N* in sodium hydroxide. After 15 minutes the extract was acidified to a *p*H of 5.0 to 5.5 with sulfuric acid and then heated until 60 percent had been distilled with complete recovery of the attractant. The distillate was treated with dilute lead nitrate to remove fatty acids. This treatment also prevented the *p*H of subsequent distillates from increasing. The distillate was redistilled and the process repeated until the volume of the final distillate was reduced to a convenient amount for extracting. The active material in the distillate was extracted completely with isopentane as shown by bioassay (*7*).

Purification and final isolation of the attractant compound was accomplished by two different chromatographic techniques. In the first, five liquid-adsorption columns and one gas-liquid partition column were used. In the second, two liquid-adsorption columns were followed by two gas-liquid partition columns. The efficacy of the procedures was evaluated through the course of the separations by bioassay and ultraviolet analysis.

In procedure No. 1 the concentrated attractant solution was applied first to an acid-washed alumina column. After elution with water, the attractant was retained on the column and the column was extruded, added to water, and the mixture distilled. The distillate containing the attractant was extracted with isopentane for purification through the following sequence of columns; florisil, silicic acid–celite, florisil, alumina, all of which were developed and eluted with graded concentrations of ether in isopentane ranging from 0 to 10 percent. The active eluates from each column were evaporated to dryness and taken up in isopentane, then applied to the next column.

Throughout the separations performed on the liquid-adsorption columns, over 90 percent of the attractant activity was recoverable from each column as determined by bioassay. In this sequence of columns, ultraviolet-absorbing contaminants present in initial active fractions were shown to be separated from the activity, and the final eluate was without significant ultraviolet absorption.

Gas chromatography was accomplished with a conventional packed column apparatus employing a radium-argon ionization detector. Eluates from the column were collected in specially designed coiled traps (*9*) in a Dry Ice-alcohol bath at a temperature of −80°C. Contents of the traps were recovered by washing with isopentane. The active material was shown by bioassay to be

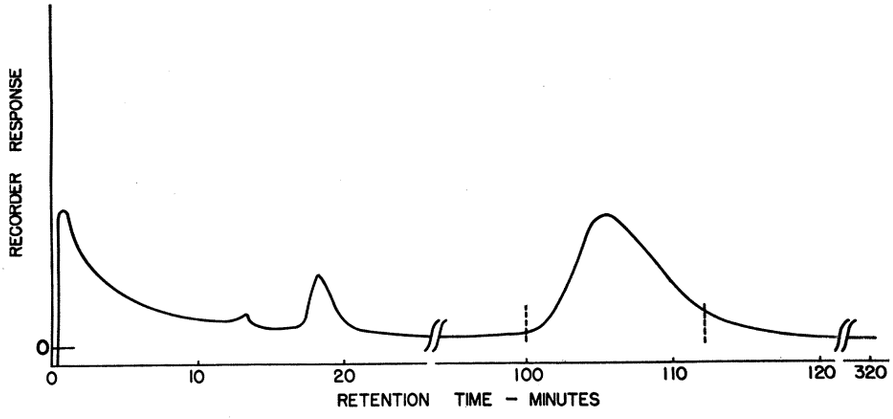

Fig. 1. Gas chromatogram of the isopentane solution of the attractant material after purification by five liquid adsorption columns.

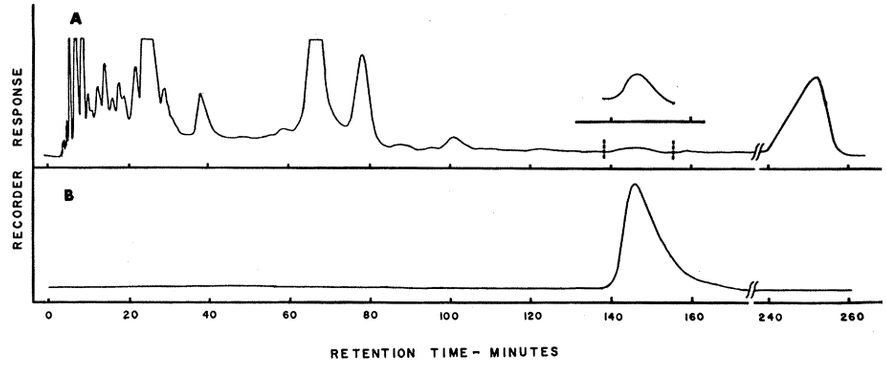

Fig. 2. (*A*) Gas chromatogram of attractant material (one-half total) after two liquid adsorption columns. Adjusted to sensitivity 1×. In superposition, graph of active peak is shown at sensitivity 10×, as actually observed. (*B*) Rerun of total trapped active material. Sensitivity 30×. Four-foot Chromosorb W column (80 to 100 mesh) coated with 5 percent by weight Apiezon M. Column temperature, 130°C; detector temperature, 150°C; inlet temperature, 150°C; argon flow rate, 110 ml/min.

confined to a well-separated peak with a reproducible retention time, illustrated within dashed vertical lines in Fig. 1. Fifteen to thirty percent of the active material was recoverable with this trapping procedure.

In procedure No. 2 the first two liquid adsorption columns were used as previously, and the concentrated active eluates from the second column applied in two aliquots to a freshly packed gas chromatographic column. Trapping of the effluent stream was now carried out at −160°C in a bath of isopentane and liquid nitrogen. The two aliquots of attractant solution gave reproducible chromatograms of the form shown in Fig 2*A*, the attractant peak being denoted by the dashed vertical lines. The longer retention time in Figs. 2*A* and 2*B*, as compared to Fig. 1, is attributable to the fact that a separately prepared batch of column packing was used.

The graph of Fig. 2*A*, adjusted to an amplifier gain of 1, shows that the amount of active substance was minute compared with the contaminants. The trapped active material from the two aliquots was combined and rerun in the same column at a gain of 30, as shown in Fig. 2*B*. This graph shows that the attractant was effectively separated in the first gas chromatogram and is now free from evident contamination. Seventy-five percent of the total peak area attributable to the attractant on the chromatograms of the two aliquots (see Fig. 2*A*) was observed on the chromatogram obtained from the combined eluates (see Fig. 2*B*), and identically 75 percent of the activity was trapped as measured by bioassay. The remaining 25 percent of the activity was accounted for as a residual on the implements used for transferring the material. This complete accountability of activity indicates, firstly, that a direct relation exists between the peak area and the attractant, and, secondly, that the attractant passes through the column unaltered. Based on the response of the detector and the recovery, the amount of attractant isolated was calculated to be approximately 28 μg. The fact that the chromatogram of the isolated attractant compound shows no peak before or after the attractant peak indicates that the compound has been obtained in a highly purified state. For the detector response used, the sensitivity was of the order of 0.02 μg.

Infrared spectra obtained by means of microspectrophotometric techniques (*10*) indicate that the compound is aliphatic in nature. The presence of an ester carbonyl is also indicated. Characterization of the attractant is proceeding by means of mass spectrometric analysis of the isolated compound.

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Generation of Electric Potentials by Bone in Response to Mechanical Stress

Abstract. The amplitude of electrical potentials generated in stressed bone is dependent upon the rate and magnitude of bony deformation, while polarity is determined by the direction of bending. Areas under compression develop negative potentials with respect to other areas. Similar results were obtained both in living and dead bone. Removal of the inorganic fraction from bone abolishes its ability to generate stress potentials. It is probable that these potentials influence the activity of osseous cells directly. Furthermore, it is conceivable that they may direct, in some manner, the aggregation pattern of the macromolecules of the extracellular matrix.

Malaligned fractures in children usually straighten in time as new bone is deposited on the concave side of the deformity and old bone is removed from the convex side (*1*). Similar behavior has been described during healing of fractures in vitro (*2*). These and other examples indicate that mechanical factors may direct the pattern in which bone is deposited and removed. While the relationship between stress and bone architecture has long been recognized (*3*), the mechanisms by which force may influence cellular activity in bone are not completely known. Despite the fact that mechanical factors have been demonstrated to have a direct action on osteogenic cells in vitro (*4*), other regulatory processes may be in operation. Since bioelectric or direct-current fields have been linked with cellular migration (*5*), tumor formation (*6*), morphogenesis (*7*), and regeneration of amphibian limbs (*8*), it seemed desirable to investigate whether mechanical deformation could produce measurable d-c potentials in bone. Both mammalian and amphibian bone were employed in our study. Potentials were measured with matched silver–silver chloride electrodes and direct-coupled amplifiers having input impedances ranging from 10^6 to 10^{14} ohms. There was minimal electrode polarization during the procedure.

Initial observations were made on fresh preparations of feline fibulae from which the soft tissues surrounding the central portion of the shafts had been removed by subperiosteal dissection. Care was exercised to prevent drying and to limit artifacts produced by electrode movement during deformation of the bone. With one electrode placed on the posterior aspect and another one opposite it on the anterior aspect at midshaft, stress was applied so that the thin fibula bowed—concave posteriorly. The posterior electrode instantly became negative with respect to the anterior one and remained so, though with a slowly decreasing difference, until the deforming force was removed. The bone immediately returned to its normal shape but the anterior electrode became briefly negative with respect to the posterior, and then returned to a state of isopolarity. An equal deformation of the bone in the opposite direction produced a reversed polarity of equal magnitude. The amplitude of the potential was dependent upon the rate and magnitude of the bony deformation, while its polarity was determined by the direction of bending. Subsequently, freshly removed specimens of cat fibula, rat femur, and bullfrog tibiofibula (Fig. 1)—fixed in insulated clamps—demonstrated the same phenomena. The potentials generated in these stressed bones apparently were not dependent upon cell viability, since frozen and thawed or air-dried specimens behaved like the fresh prepara-