EXCISED HONEYBEE ABDOMENS

and the biosonic analyzer system aid pharmacological and toxicological investigation

ROY J. PENCE

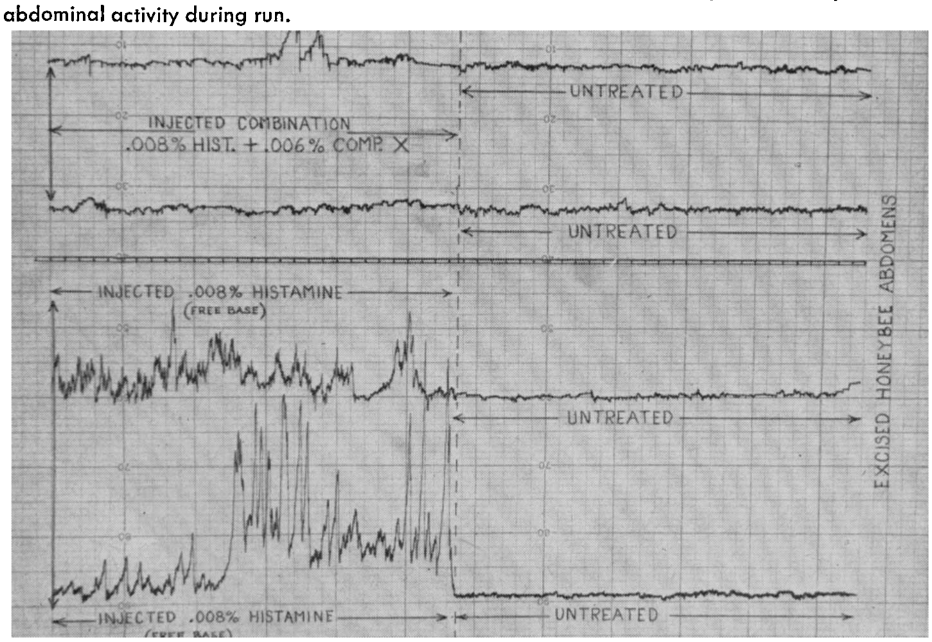
For economic reasons as well as to satisfy physiological requirements, new and innovative pharmacological test systems are under constant investigation. Researchers at the Department of Agricultural Sciences at UCLA have been investigating physiological similarities between insects and higher organisms in response to pharmacological agents. This initial report demonstrates the advantages of one entomological test system (utilizing honey bees) as analyzed with electronic instrumentation.

Because insects are available in greater numbers and have comparatively shorter life cycles than higher animals, they are ideally suited for screening neuroactive substances. Their physiological and biochemical similarities to higher animals greatly offset their morphological differences.

It has been learned that excised honeybee abdomens are excellent bio-assay systems for pharmacological studies (the science involving the action of drugs on living things)—particularly when results can be evaluated with recording instruments capable of faithful transduction and amplification. A number of drugs and pesticides have been studied biosonically with this technique and instrumentation. The most important of these studies reveal new information on histamine and on a newly classified "compound X" that significantly inactivates histamine at its site of liberation.

Honeybee abdomens used for these studies were from bees leaving the hive with empty crops. When such abdomens were carefully severed from the thorax, at the pedicel of living CO_2-anesthetized bees, they became unstressed bio-assay systems maintaining steady pulsitive activity for 24 hours or longer. Honeybee abdomens are exceptionally well suited for the pharmacological investigation of histamine and other neuro-active substances for several reasons. Each abdomen has two large tracheal sacs which expand and collapse in response to the expansion and contraction of the rigid parts of the body wall. From these air sacs the tracheae continue as smaller branching tracheoles that ramify to all parts of the internal organs, like bronchi in higher animals which also branch and become smaller to where they eventually carry out gaseous interchanges with the blood. Severe histamine poisoning in higher animals causes a broncho-constriction and swelling of lungs, leading to respiratory failure. Similar symptoms occur in the honeybee.

Biosonic pen tracings of injected honeybee abdomens. Operator visually and aurally monitors abdominal activity during run.

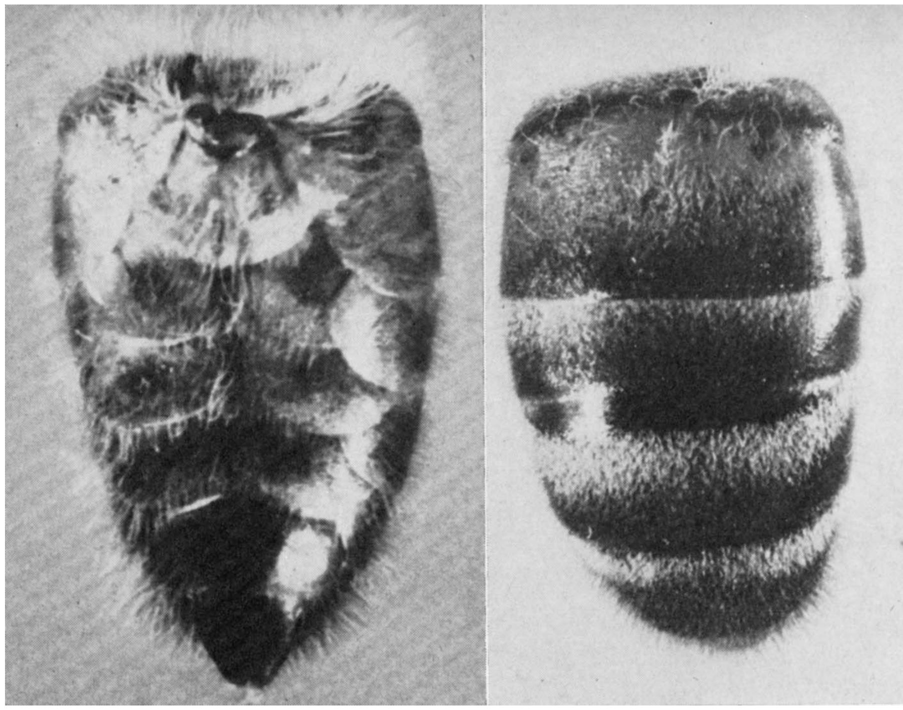


Histamine production

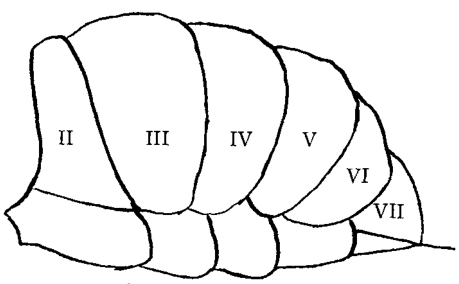
Another important advantage in using the abdomens is that honeybees, like higher animals, also manufacture and utilize histamine for their own body needs. Histamine is the main constituent in honeybee venom. Again like higher animals, honeybees have little tolerance for histamine in excess of their body needs, and they can also be poisoned by the same substance they manufacture.

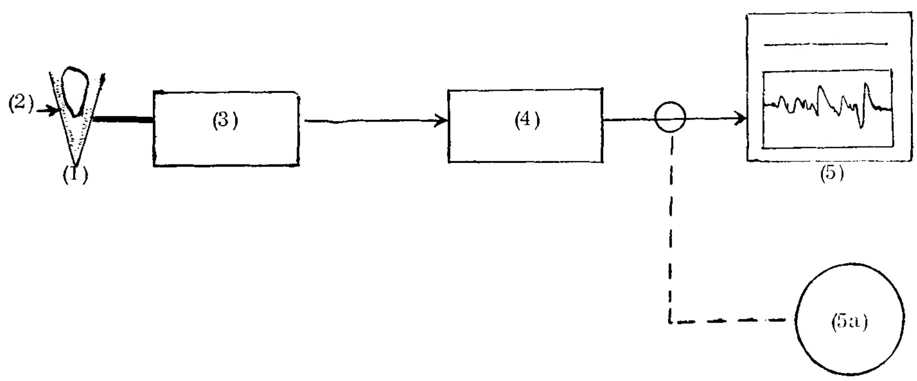
A quality B grade, free-base histamine was prepared in an aqueous concentration of 0.008 per cent, which is sublethal but irritating to the internal organs of the honeybee abdomen. When 0.2 lambda of the 0.008 per cent solution is administered into the esophageal tube leading to the crop, the entire abdomen reacts with rapid oscillation and strong intermittent muscular thrusts. Presumably this reflex action is caused by hyperactive automatism of the stomodeal valve as it attempts to reject the administration. Inevitably some passes through the valve, enters the ventriculus, and affects the entire nervous system, because the motor mechanism becomes greatly accelerated. The crop, which normally writhes and pulsates to stir the pollen-nectar bolus, also becomes hyperactive, adding to overall oscillation.

Hyperactivity of a treated honeybee abdomen may be visually observed but



Dorsal and ventral views (right photos) of excised honeybee abdomens showing setae-covered plates which move over sandpaper surface of bioanalyzer receptacle. Sketch below shows segments of abdomen and diagram to right shows operation of the biosonic analyzer: (1) receptacle cone of fine grade sandpaper with (2) abdomen in place, sending vibrations to (3) piezo-electric element, producing electrical signal to (4) amplifier from which it is transcribed by (5) servo-chart recorder—or can be heard in (5a) earphones. Lower photo shows author checking the recorder chart of the biosonic analyzer while also listening for changes in frequency of vibration through earphones.





not transcribed unless it is converted into measurable energy. For this purpose a high-gain, low-noise-level amplifier was designed with an ultrasensitive transducer to pick up minute motions of a treated and untreated abdomen over a fine sandpaper surface. The amplified signal is relayed to a servo-pen chart recorder which records all motion, and change in motion—much like an electrocardiogram.

The anatomical configuration of the honeybee abdomen is ideal for detecting external motion activated by underlying muscles. The abdomen has six tergal and sternal plates (see diagram) connected by intersegmental membranes. During normal respiration, abdominal excursion is bidirectional. The mechanism is made up of protractor muscles which pull and push the segments apart, and lateral compressor and dilator muscles which draw and expand the tergum and sternum in a vertical direction. It is this pregenital musculature which is responsible for lateral and longitudinal movement of the setae-covered tergal and sternal plates over the granular surface of the abdominal receptacle. The abdominal receptacle is a cone of fine-grade sandpaper connected by a needle to a piezo electric element which directs its output signal to the high impedance biological signal amplifier—and is instantly transcribed by the servo-chart recorder (see diagram).

An untreated abdomen alone is capable of generating a strong signal, but injection of *any* neuro-active substance causes it to become hyperactive, resulting in specific characteristic frequency and amplitude variations from the normal rhythmic wave form.

Testing

Testing excised honeybee abdomens with the Biosonic Analyzer System made it possible to determine the degree of histamine irritation as well as the degree of *counter* irritation caused by compound X when it was combined with the same amount of histamine *causing* irritation. With this dual technique it has been learned that 0.006 per cent compound X physiochemically inactivates 0.008 per cent histamine when both are administered simultaneously into living honeybee abdomens, and that this inactivation is comparable to what occurs at the site of histamine liberation. Evidence of this is illustrated in the graph. It is also interesting to note that these data corroborate results observed in the field where histaminergic skin conditions on higher animals have been corrected by topical applications of experimental compound X.

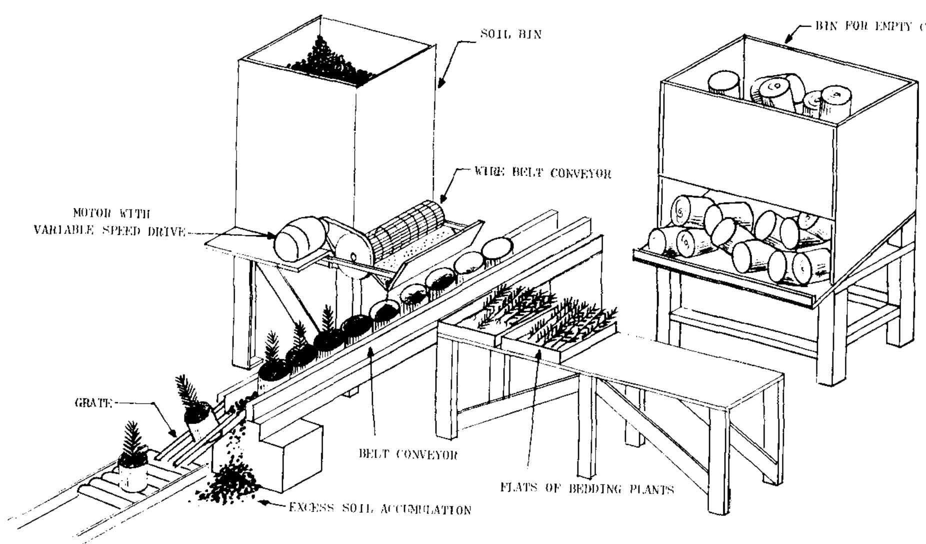
Research is still in progress and additional drugs and pesticides are currently being evaluated. Investigations are also underway to relate the physiological and pharmacological similarities in mechanisms of drug action at the mammalian level. The Biosonic Analyzer System, utilizing insect bio-assays, appears to be a potentially significant contribution to rapid screening and evaluation of pharmacological and toxicological substances of agonist, antagonist and depressant nature.

Roy J. Pence is Specialist, Department of Agricultural Sciences (Entomology), University of California, Los Angeles.

Through cooperation with Physionics Corporation, Southern Pines, North Carolina, an updated unit (photo) of greater capability than the apparatus herein described, is currently available for laboratory and teaching purposes.

MECHANIZED POT GALLON CON speeds nursery of ornamentals

F. K. ALJIBURY R. G. CURLEY W. H. HUMPHREY



CONTAINER PLANT CANNING MACHINE SKETCH

THE PRODUCTION OF ORNAMENTAL PLANTS in gallon-size containers is a major enterprise in California. An estimated 350 nurseries in the state produced over 80 million dollars worth of container grown plants in 1967 (not including cut flowers). The potting of plants in gallon containers is one of the principal operations in most nurseries and at the same time a major labor requirement. Potting in gallon cans is still largely a hand operation although in a few cases it has been partially mechanized. The project described here attempts to better mechanize this operation and to thus minimize cost and labor requirements.

To be successful, a canning machine must: (1) handle the soil mix and meter it into the cans; (2) feed the can into the machine; (3) handle the plants and plant them in the containers; and (4) remove and handle the final product.

In 1964 a simple, low-cost system was devised for metering soil mix into gallon cans. The machine was developed in cooperation with Select Nurseries at Brea, California. The schematic diagram shows the basic parts of the system: a conveyor