tissues may verify the correlation between anatomy and mode of transmission, and allow either electrical or chemical transmission to be inferred from the morphology of the junction. This type of inference would be of particular importance for such structures as apical dendrites where direct electrophysiological evidence is difficult to obtain (*8*).

M. V. L. BENNETT*, E. ALJURE†
Y. NAKAJIMA, G. D. PAPPAS
Departments of Neurology and *Anatomy, College of Physicians and Surgeons, Columbia University, New York*

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† Rockefeller Foundation fellow.

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Tsetse Fly Puparia: A New Collecting Technique

A glossinologist often needs to collect tsetse fly puparia for experimental use. The conventional method is to scratch tsetse breeding soil and collect the puparia brought to view. This method is time-consuming, uneconomical, and not very productive, particularly in an area with a low incidence of the fly. The searchers are unable to detect all puparia present in the soil (*1*). In a second method for separating tsetse puparia from the soil, sieves are used. This method becomes inefficient because of lumping of the soil, which blocks the sieves if the soil is slightly moist.

We tried these techniques when seeking puparia of *Glossina palpalis* (R-D) on the river Lofa, Western Province, Liberia, reported to have a low fly population (*2*), but failed to collect enough puparia to colonize this species. We describe, in this report, a new technique of tsetse puparia collection which enabled us to obtain over 5000 puparia from the same locality.

A 4-gal water barrel, a few sheets of filter paper, and a 2-inch glass vial are supplied as puparia-colecting kits to two collectors who work together. The barrel is filled with river water and carried to the possible breeding sites. Depending on the depth of breeding soil, about 0.5 to 1.5 inches of topsoil are gathered from such sites and examined for puparia by pouring the soil into water. The movement of soil and puparia in reverse directions in the water allows the puparia contained to be separated out on the surface of the water. If the soil is poured slowly almost all the puparia are set free to float, but if the process is carried out rapidly it becomes necessary to disturb the soil gently by hand in order to release the puparia trapped by the mud. The floating puparia are fished out, dried on filter paper, and stored in the glass vial containing a little sand.

The short exposure of puparia to water during collection has no bad effect on the viability of puparia, for 88.5 percent of puparia collected by this method produced live flies, a 4-percent higher emergence than that observed in the case of puparia obtained by hand-picking in the same area.

This method of puparia collection detects all the puparia in the soil examined and can be used as a method of control against this insect.

Z. H. ABEDI
MAX J. MILLER
Liberian Institute of the American Foundation for Tropical Medicine, Harbel, Liberia

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4 June 1963

Transport of Neurohormones from the Corpora Cardiaca in Insects

Abstract. *Evidence from electron microscope studies of aphid and cockroach nerves, and from the bioassay of extracts of the aortal nerves of cockroaches indicates that some neurohormones are distributed from the corpora cardiaca along nerve axons to their target organs.*

The corpora cardiaca of insects are small ganglia which are situated immediately behind the brain and connected to it by nerves. From the corpora cardiaca, nerves have been traced in various insects to the alimentary canal, salivary glands, aorta, prothoracic gland, and various muscles (*1*, *2*).

Substances produced by neurosecretory cells in the brain have been shown to pass along nerve axons to the corpora cardiaca from which they are thought to be released into the blood (*3*). In this respect the protocerebrum-corpus cardiacum system of insects is considered to be analogous to the hypothalamus-neurohypophysis system of vertebrates and the X organ-sinus gland system of crustacea.

Recent work has suggested that at least some materials may be distributed from the corpora cardiaca along nerve axons. In the fly, secretory material was observed in a nerve passing from the corpora cardiaca to the esophagus (*4*); in the cockroach it has been shown by ligaturing experiments that material passes along nerves from the corpora cardiaca to the subesophageal ganglion (*5*); and, in aphids, material staining with paraldehyde fuchsin and believed to be secretory material was traced from the corpora cardiaca along the aortal nerves and along nerves passing to muscles (*2*).

We have therefore studied the nerves leaving the corpora cardiaca in several groups of insects using paraldehyde fuchsin stain for neurosecretory material. Under the compound microscope no material was found in any of these nerves, although large amounts were sometimes present in the brain and corpora cardiaca.

Extracts of the corpora cardiaca of the cockroach can cause increased rate of heartbeat. One active factor is a peptide which is thought to stimulate the pericardial cells to release an indolalkylamine which, in turn, acts on the

heart (*6*). In the cockroach, paired aortal nerves arise from the corpora cardiaca, and pass along the sides of the heart for the whole length of the insect. They have many branches, and we have found that the pericardial cells are closely attached to them.

The aortal nerves were dissected from the abdomens of five adult *Periplaneta americana*. Care was taken to detach all pericardial cells and other tissues. The nerves of each insect were then homogenized in 0.1 ml of Ringer solution, and the extracts were assayed on standard cockroach heart preparations maintained in 5 ml of Ringer solution in an aerated tube. Four out of five of the extracts caused an increase in the rate of heartbeat. The amount of increase varied between extracts from 10 percent to 33 percent (average 27.8 percent). Boiling the extracts for 5 minutes did not impair their activity. It can be concluded that the aortal nerves contain a heat-stable factor comparable in its effect and potency to extracts of the corpora cardiaca (*6*).

In a brief account of an electron microscope study of the dorsal vessel of a caterpillar and a cockroach, Challice and Edwards (*7*) reported neurosecretory granules in axons on and near the heart. We have examined the aortal nerves of *P. americana* and the nerves passing from the corpora cardiaca in the aphid, *Myzus persicae*, with the electron microscope. In both insects many axons were packed with electron-dense granules (Figs. 1 and 2). There is little doubt that these granules represent neurosecretory material.

In cockroaches, as in aphids, there is other indication that material passes from the corpora cardiaca into several nerves. The characteristic bluish appearance of freshly dissected corpora cardiaca is probably due to neurosecretory granules (*8*), and this bluish color can be seen to extend into the aortal nerve and other nerves.

Materials present in the axons of the aortal nerves may come from either or both of two sources. They may come from brain neurosecretory cells along axons which pass through or around the corpora cardiaca, or they may come from cells within the corpora cardiaca.

Large amounts of stainable material which are found in the corpora cardiaca of cockroaches are thought to comprise material from the brain neurosecretory cells (*3*). The rapid disappearance of this material after electrical stimulation and enforced hyperactivity has previously been interpreted as indicating release into the blood (*9*). We suggest as an alternative explanation that the brain material may act locally in the corpora cardiaca to stimulate the intrinsic cells of that organ; perhaps it becomes a precursor of their secretory product.

Materials elaborated by these cells may then pass from the corpora

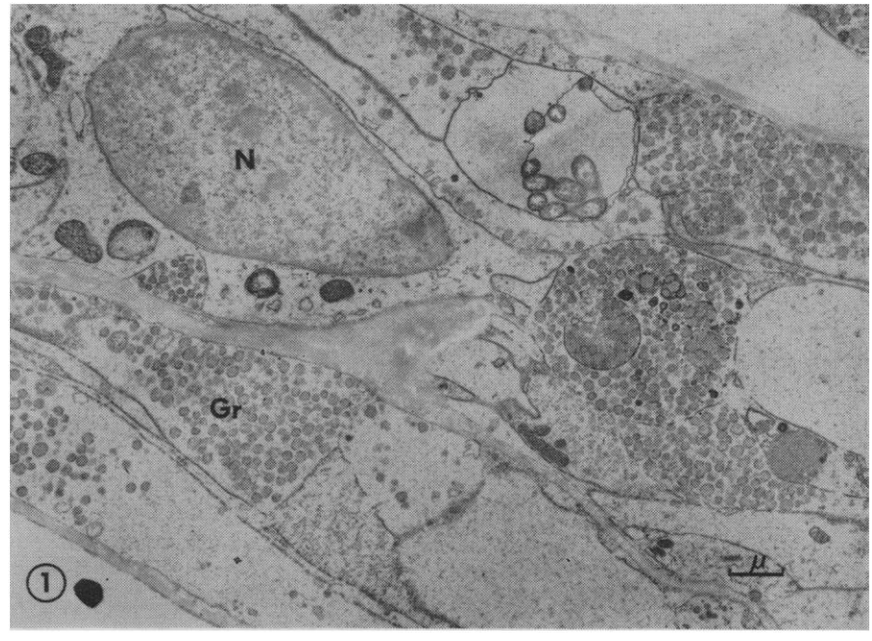

Fig. 1. Oblique section through the aortal nerve of *Periplaneta americana*. The nerve was fixed in osmium and embedded in Epon. The section was stained with lead hydroxide. The axons contain numerous dense, membrane-limited granules (*Gr*), about 1500 Å in diameter. The nucleus (*N*) is part of a glial cell.

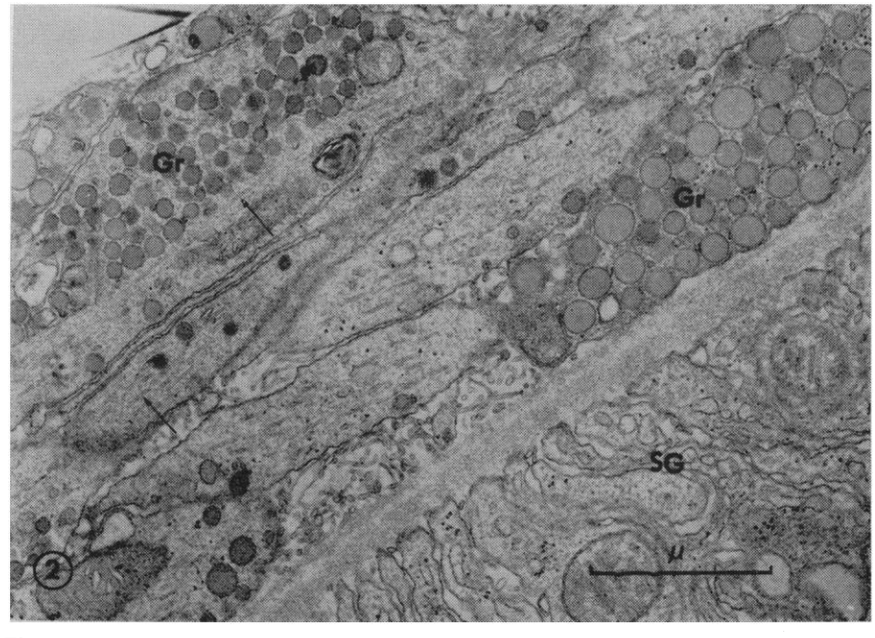

Fig. 2. Section through a lateral nerve from the corpus cardiacum of *Myzus persicae*. The nerve was fixed in glutaraldehyde, post-fixed in osmium, and embedded in Epon. The section was stained with lead hydroxide. In this region the nerve is closely applied to a lobe of the salivary gland (*SG*). The axons contain large populations of granules (*Gr*) which vary in diameter between 1300 and 2200 Å. The granules appear to be membrane limited. Neurotubules (arrows) can be seen in the axons.

cardiaca along axons to their target organs. An electron micrograph by Meyer and Pflugfelder (*10*) showing axon terminals close to the nucleus of a cell in the corpus cardiacum of *Carausius,* and the presence of neurosecretory-type granules in the cytoplasm of intrinsic corpus cardiacum cells of *Carausius* (*10*), *Leucophaea* (*11*, *12*), and *Myzus persicae* (*13*) lend some support to this theory. The finding that materials are transported from the corpora cardiaca along nerve axons does not necessarily indicate that there is no release of neurohormones (*14*) from these organs into the blood; this may still occur, especially in view of evidence from a recent electron microscope study (*12*). But before accepting as fact such release into the blood in intact insects, it would be desirable to have more direct evidence than is at present available (*15*).

BRUCE JOHNSON *
BLAIR BOWERS
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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* Present address: Waite Agricultural Research Institute, University of Adelaide, South Australia.

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Genetic Control of Differential Heat Tolerance in Two Strains of the Nematode Caenorhabditis elegans

Abstract. *Two strains (Bergerac and Bristol) of the nematode* Caenorhabditis elegans, *with different temperature tolerances, were reared axenically at 23° to 25°C. The Bergerac strain is heat sensitive (that is, it is sterile at maturity), whereas the Bristol strain is heat resistant (that is, it matures and reproduces normally). Hybrid hermaphrodites (F_1), produced by crossing Bristol males and Bergerac hermaphrodites, are heat tolerant. Heat sensitivity segregates as a simple Mendelian recessive in the F_2 and F_3 generations.*

Caenorhabditis elegans, (Maupas, 1900) Dougherty, 1953 (family Rhabditidae), has been maintained in continuous axenic cultivation (that is, in the absence of other living species) on various complex media since 1956 (*1*). This nematode can also be readily maintained monoxenically (that is, with one other species only—in this instance, *Escherichia coli*) on Difco nutrient agar slants. Populations of the organism, grown in the laboratory between 13° and 20°C, consist almost entirely of self-fertilizing protandrous hermaphrodites (*2*). Males are encountered occasionally and, under certain conditions, can be crossed with hermaphrodites. Typically the mated hermaphrodites produce progeny of mixed uniparental and biparental origin; consequently there is a considerable increase in the percentage of males. We have exploited this reproductive pattern in a study of the inheritance of heat tolerance as opposed to heat sensitivity in two strains of *C. elegans*, named according to their geographic origin—namely, Bergerac (France) (*3*) and Bristol (England) (*4*).

Reproduction of the Bergerac strain by self-fertilization under axenic conditions on a suitable medium is a function of temperature. When both it and the morphologically identical, but physiologically different, Bristol strain are grown in a number of organic media at temperatures between 10° and 25°C, the Bergerac strain matures and reproduces normally only up to about 18°C, whereas the Bristol strain matures and reproduces consistently. At 20°C reproduction of the Bergerac strain is variable; and at 23°C the worms grow to adult size but do not reproduce. This difference in temperature tolerance provides a basis for studying genetic control of reproduction in the two strains.

For the studies reported here, techniques of axenic cultivation have been largely those described by Dougherty *et al.* (*5*). A significant departure from work hitherto reported (*1*, *3–5*) was in the composition of the medium; ours is crudely organic, and contains a mixture of 50 percent, by volume, of Oxoid liver extract solution (*6*) at 45 mg per milliliter, 1 percent chick-embryo extract (*4*), and 49 percent glass-distilled water.

An initial difficulty encountered when attempts were made to obtain hybrids between the Bergerac and the Bristol strains was failure, despite a number of trials, to obtain successful matings under axenic conditions. This obstacle was overcome by crossing Bristol males with Bergerac hermaphrodites in monoxenic culture with *E. coli* on a petri plate containing Difco nutrient agar. (The reciprocal mating, Bergerac males with Bristol hermaphrodites, has so far been unsuccessful.) The males are left with the hermaphrodites for 24 hours at 20°C; then the mated hermaphrodites are rendered free of other organisms by being placed for 1 hour in an antibiotic solution, containing 1000 units of potassium penicillin G and 100 μg of streptomycin sulfate per milliliter. At the end of the hour the worms are washed through four changes of 0.067*M* potassium phosphate buffer (*p*H 7) to reduce the concentration of antibiotic to a negligible amount; they are then left in a fifth change of buffer (0.5 ml in a "Bureau of Plant Industry" watch glass) for 24 hours at 20°C to lay eggs. The larvae produced during this period are free of living bacteria. Cultures are then initiated in small test tubes (10 mm outside diameter), each containing 0.2 ml of medium; only one larva is placed in each tube and all of the tubes are incubated at 23°C.

Three types of F_1 progeny have been observed: (i) nonreproducing hermaphrodites (usually very few in successful matings), assumed to be the product of self-fertilization by Bergerac hermaphrodites; (ii) reproducing hermaphrodites, originating from cross-fertilization of Bristol males with Bergerac hermaphrodites; and (iii) males, also originating from cross-fertilization. The number of males has been approximately equal to the number of reproducing hermaphrodites. From the results of the study of the F_1 progeny we may assume that the presumptive gene (or genes) for heat tolerance, as ex-