or absence of sterol biosynthesis throughout the animal phyla.

The effects of the two compounds on larval growth and metamorphosis of the hornworm may be explained in a number of ways. They could be caused by an accumulation of large quantities of desmosterol in the tissues, in spite of our previous finding that the hornworm grows and develops normally on an artificial diet in which desmosterol is the sole added dietary sterol (*2*). Dietary desmosterol is nearly quantitatively converted to cholesterol by the hornworm (*2*), and in vitro studies indicate that the hornworm intestinal tract is a good source of Δ^{24}-sterol reductase activity. Thus, desmosterol may not gain access to the internal tissues or organs when it is obtained from the diet. Apparently certain sterols (for example, β-sitosterol and desmosterol) are not normally transported across the tissues of the intestinal tract in any quantity, since normally there is little accumulation of β-sitosterol or desmosterol in the tissues. Perhaps the transport of sterols might be altered to permit passage of these two compounds when certain steps in the dealkylation mechanism are blocked.

In addition to an accumulation of desmosterol, three to four times more unchanged β-sitosterol is present in hornworms that have been fed the inhibitors than in the controls, possibly because of the rate-limiting effect of the accumulated desmosterol on dealkylation of β-sitosterol. This increase in β-sitosterol is accompanied by a decrease in the relative content of cholesterol, which may be reduced to less than 10 percent of the content in normal insects. The low titer of cholesterol in these insects could limit the availability of this precursor for the molting hormones (ecdysones) and thus potentially be another factor in growth inhibition. The house fly, *Musca domestica* L., does not dealkylate but can use either β-sitosterol or desmosterol as a sparing sterol to replace more than 99 percent of its total dietary cholesterol requirement (*2, 7, 8*). In the latter insect, a ratio of β-sitosterol or desmosterol to cholesterol as great as 100 to 1 in the tissues still allows normal growth and metamorphosis. However, since the hornworm efficiently dealkylates plant sterols, perhaps it has not evolved a mechanism whereby it may spare its cholesterol requirement by using structurally related sterols as tissue components. Indeed, the plant sterol β-sitosterol or the intermediate desmosterol may actually inhibit growth and metamorphosis of the hornworm when they are incorporated in appreciable quantities into tissues of this phytophagous insect.

Other possible explanations of the inhibitory action of the hypocholesterolemic agents are (i) they may bring about formation and accumulation of minor steroid metabolites (other than desmosterol) which may act as growth inhibitors or (ii) they may affect physiological systems other than dealkylation. Minor steroid metabolites have been detected (*2*); these are currently under study, and the second possibility is being tested by feeding the hypocholesterolemic compounds in diets containing cholesterol.

Insects require a dietary source of sterol for normal growth, metamorphosis, and reproduction, and this essential sterol serves both as a structural component of the tissues and as a precursor of the steroid molting hormones, the ecdysones. Insects that feed on plants must derive most, if not all, of their essential cholesterol through the dealkylation of phytosterols such as β-sitosterol. This study demonstrates that in the tobacco hornworm the conversion of β-sitosterol to cholesterol can readily be blocked and that this interference severely inhibits larval growth and metamorphosis. These results suggest this to be an area worthy of intensive research both for its comparative biochemical and biomedical importance and for its potential in the development of chemicals that might be used to disrupt the development of plant-feeding insects.

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Synaptic Connections between a Transplanted Insect Ganglion and Muscles of the Host

Abstract. *When a metathoracic ganglion from one cockroach* (Periplaneta americana) *is transplanted into the coxa of another cockroach, it innervates only those leg muscles that have been previously denervated. The transplanted ganglion evokes hyperpolarizing synaptic potentials in the host muscles that it innervates. These potentials are correlated with twitching of the host limb.*

The pattern of connections among members of a population of neurons may in large part determine the form of the behavior evoked by that group of cells. To examine the factors controlling the formation of connections between one unit and another, it is desirable to have a small population of cells in which each member can be identified and in which the connections between units can be experimentally manipulated. If the system also yields defined bits of behavior that can be related to the development of specific connections, then one might have a model for examining cellular events responsible for the behavioral capacity of a nervous system. Our study indicates that transplanted central ganglia of insects, and the connections they form with the host cells, have many of the required elements for such a model system.

Bodenstein (*1*) demonstrated that the thoracic ganglion of the cockroach *Periplaneta americana* can be transplanted into the coxa of a host cockroach and that it survives. He reported that the transplant causes fibrillation of the host coxal muscles, presumably by

forming connections with them. We know which motor neurons normally innervate a given leg muscle in *Periplaneta* (*2*), and we have determined several of the parameters associated with degeneration and regeneration of neuromuscular junctions in this animal (*3*). We therefore proceeded to determine whether the transplanted ganglion does indeed form neuromuscular junctions with the host muscle and to study some of the factors that influence these connections.

A flap of cuticle over the ventral coxal muscles of a metathoracic leg was turned back in the donor animal. A portion of muscle 181B and a transverse block of 181C was cut out to make room for the ganglion transplant (Fig. 1). Both of these muscles are flexors of the trochanter (*4*). By removing a section of muscle 181C, we also cut out a length of nerve 3B since it runs along the dorsal surface of this muscle and is tightly attached to it. Nerve 3B innervates muscle 142, which is located in the femur and extends the tibia (*4*). Therefore, the muscle dissection which prepared the host for the ganglion transplant denervated a part of muscles 181B, all of 181C (flexor trochanteris), and all of 142 (extensor tibia). A metathoracic ganglion was removed from a donor animal and placed in the prepared coxa of the host (Fig. 1). The transplanted ganglion was oriented in the host with the anterior connectives pointing anteriorly and the dorsal surface of the ganglion facing toward the dorsal surface of the coxa. The cuticular flap on the host coxa was replaced and sealed with a wax having a low melting point. During all surgery the animals were lightly anesthetized with CO_2. Using young adult male cockroaches (*Periplaneta americana*) as host and donor animals, we made five preparations in this manner. All preparations showed evidence of connections being established between the donor ganglion and leg muscles of the host.

After the transplant, the host used the operated leg in routine locomotor activity. Flexion of the trochanter was impaired, and tibial extension was absent, as expected, because the transplantation operation interfered specifically with muscles and nerves used in these movements. Approximately 30 days after implantation of the ganglion, spontaneous clonic twitching movements appeared in the host leg. These movements were confined to extension of the tibia and flexion of the trochanter (Fig. 1, inset) and were not related to the locomotor activity of the animal.

The extensor tibia muscle and most

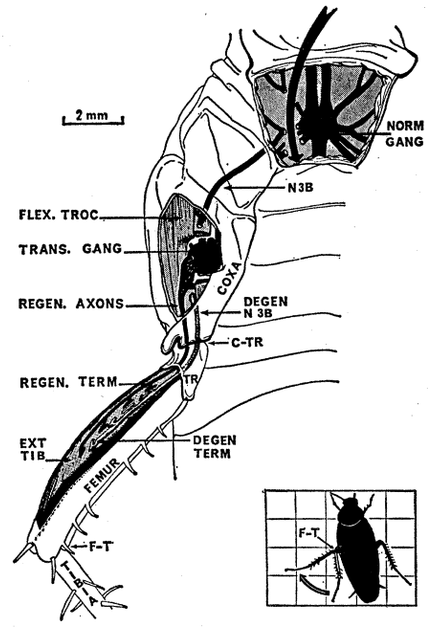

Fig. 1. Diagram of the ventral surface of the cockroach metathorax and the right metathoracic leg. Cuticle over the metathoracic ganglion (*norm gang*) and the coxa was removed. Portions of the flexor trochanteris muscle (*flex troc*) were removed, and a metathoracic ganglion from a donor cockroach (*trans gang*) was transplanted into the space provided. A length of nerve 3B (*N 3B*) was removed in preparation for the implant. This caused motor fibers in the distal stump of N 3B (*degen N 3B*) and their terminals (*degen term*) on the extensor tibia muscle (*ext tib*) to degenerate within 5 days. Regenerating axons (*regen axons*) grow from the transplanted ganglion through the coxal-trochanter (*c-tr*) joint to form functional neuromuscular junctions (*regen term*) with the extensor tibia muscle. Within 30 days after implantation of the ganglion, clonic extensions of the tibia were observed. At this time all the peripheral nerve trunks to the operated leg were cut close to the normal ganglion as indicated by the sweeping black arrow. The clonic extensions of the tibia continued. Inset: superimposed tracings from frames of a moving film showing the nature of the clonic extensions driven by the implanted ganglion; dorsal view; ganglion implanted in left metathoracic leg; right metathoracic leg shown in a resting position. The hatched position of the left leg is the rest position when all normal innervation to this leg is cut. During the clonic extension movements the tibia moved forward at the femural-tibial (*f-t*) joint to the solid position and then returned to the resting position. The grid squares are 1 cm. The ganglia are slightly enlarged in the diagram for better illustration.

of the flexor trochanteris were denervated when the donor ganglion was implanted into the host coxa. The spontaneous flexions of the trochanter and extensions of the tibia in the host limb might have been caused by regeneration from the nerve trunks of the host that were cut during implantation of the ganglion. To control for this possibility, we cut all peripheral nerve trunks to the twitching host limb approximately 0.5 mm from the host metathoracic ganglion 40 to 70 days after the implantation (Fig. 1). The spontaneous twitching of the trochanter and tibia continued after this operation. All other movements of the limb ceased, and it was no longer used in the normal locomotion of the animal. The spontaneous twitching movements were followed up to 16 days after all the host leg nerves were cut. Cut motor axons in the distal nerve stump degenerate within 5 days after injury in *Periplaneta* (*3*). The persistence of these clonic movements for as long as 16 days after the host motor nerves were cut indicates that they were not evoked by neurons from the host ganglion.

From 30 to 40 days after implantation of the ganglion, nerve fibers could be seen streaming toward the distal end of the coxa from all the nerve stumps of the implant (Fig. 1). This finding suggests that regenerating fibers from the implanted ganglion might have reinnervated the extensor tibia of the host and might have been responsible for the clonic twitching of the tibia. To examine this possibility, 40 to 60 days after implanting the ganglion we took intracellular electrical recordings from muscle 142 (extensor tibia) of the implanted leg while the spontaneous clonic extensions of the tibia were taking place. All normal innervation of the implanted leg was cut 5 to 15 days before the recording to allow time for the host motor fibers to degenerate (*3*). Under these conditions, depolarizing and hyperpolarizing junctional potentials were readily recorded from the muscle (Fig. 2). Summation of these junctional potentials resulted in large depolarizations which could be visually correlated with the rapid spontaneous clonic extensions of the tibia. The extensor tibia of the host limb was denervated when the ganglion was implanted. Regeneration of motor axons to this muscle from the normal host ganglion was excluded. We conclude, therefore, that the junctional potentials seen in the extensor tibia under these condi-

tions were evoked by regenerating axons from the donor ganglion which formed new synaptic junctions with this muscle.

A further indication of reinnervation of the extensor tibia muscle is that normal resting potentials of approximately 60 mv can be consistently recorded under the conditions just described. The return of normal resting potential levels in previously denervated *Periplaneta* muscle is associated with reinnervation (*3*). The recorded junctional activity differs from the normal by the frequent occurrence of hyperpolarizing potentials. Hyperpolarizing potentials have not been reported for normal muscle of *Periplaneta*, although they have been observed in normal muscle of *Blaberus* and some Orthoptera (*5*). We have previously seen these hyperpolarizing potentials in reinnervated muscle of *Periplaneta* (*2*) and take this as additional evidence that the extensor tibia of the host leg has been reinnervated by axons from the implanted ganglion.

The transplanted ganglia were fixed in place in the host leg 30 to 70 days after implantation, sectioned transversely, and stained with pyronine-malachite green (*6*). The histological appearance of the individual nerve cell bodies in the transplanted ganglion was normal. Many cells had eccentric nuclei, an indication of a high level of synthetic activity typical of regenerating neurons (*7*). The spatial distribution of the nerve cell bodies in the transplanted ganglion was somewhat altered thus making difficult comparison of individual cell bodies with the motor neuron maps we have prepared from normal ganglia (*2*). These results show that the regenerating axons grow from the nerve stumps of the ganglion transplant, but they leave in doubt the identification of the specific neurons that have reinnervated the extensor tibia muscle of the host.

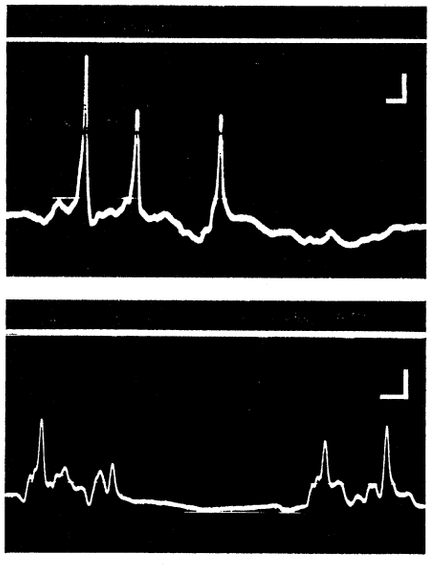

Fig. 2. Intracellular records of hyperpolarizing and depolarizing synaptic activity in the extensor tibia muscle driven by spontaneous activity of a ganglion implanted in the coxa. All normal innervation to this muscle has degenerated. The larger fast-rising depolarizations are correlated with a twitch extension of the tibia. The lower record shows periodic bursts of electrical activity associated with clonic extensions of the tibia. Summation of the small junctional potentials frequently occurs. The upper line in each record is the zero potential level. Scales: 10 msec, 10 mv.

Intracellular recordings were taken from flexors of the tibia in host legs containing an implanted ganglion. These legs were showing the spontaneous clonic tibial extensions after the normal motor innervation to the leg had been cut. In the five preparations examined, no electrical activity could be recorded from the tibial flexors under these conditions. Only muscles that were previously denervated during the implantation operation had electrical activity that could be correlated with regenerated fibers from the implanted ganglion. This finding indicates that only denervated muscles will accept regenerating fibers from the implanted ganglion and establish functional neuromuscular junctions with them.

The transplanted ganglion apparently has periods of intrinsic activity when isolated in the coxa of a host animal. These periods are indicated by the bursts of electrical activity and evoked movements in host muscles innervated by the transplant. We have seen some evidence of reflex activity from the transplanted ganglion indicating that some regenerating sensory fibers may have been captured by the transplant, as indicated by others (*8*). We can specify which muscles the transplant will innervate by selective denervation of the host muscle because only denervated muscles will form connections with the regeneration axons. The transplanted ganglion provides an "in vivo" tissue culture preparation isolated from all other central nervous influences. It offers the intriguing possibility of constructing an integrated excitable system where the connections can be specified and related to overt behavioral acts. Such a system seems ideally suitable for answering some of the questions about the factors that control connections between excitable cells and the relation of these connective patterns to behavior.

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Nerve Regeneration: Correlation of Electrical, Histological, and Behavioral Events

Abstract. *Within 5 days after the leg nerves of a cockroach are injured, miniature end-plate potentials have disappeared, and the muscle is unresponsive to electrical stimulation. The soma of the injured neuron has a dense perinuclear ring of RNA. By 40 days after the injury, locomotor activity has returned, and the miniature end-plate potentials and evoked electrical responses have reappeared in the muscle. The RNA ring has disappeared, and the nucleus of the regenerating neuron has shifted to an eccentric position.*

Integrated behavior arises from groups of excitable cells related to one another by specific patterns of connections. A suitable preparation for the investigation of the factors controlling the connections between excitable units and for relating the patterns of connection to behavior should satisfy at least three conditions: (i) the individual units within the system must be recognizable from one animal to another and must be available for analysis at several different levels of organization, (ii) the connections between units must be open