

var *unicolor* as the host, were made according to a previously reported method<sup>10</sup>. Other *E. foetida* var *unicolor* worms were wounded by removing a 2 mm × 1 mm portion of dorsal body wall. The combinations made, and the time intervals, post grafting, at which individuals were fixed are recorded in the Table. The hosts were all killed in Bouins fixative between 09.00 h and 10.00 h. This was to eliminate any variation in observations which may have occurred as a result of possible mitotic rhythms. Normal untreated clitellate worms of a similar size (50–60 mm) and 120 days old were used as controls. After embedding in paraffin wax, 10 µm serial sections were cut. Subsequently they were stained, some in Feulgen stain others in Ehrlich's Haematoxylin and Eosin. Sections were viewed under a Zeiss photomicroscope 2 and the location of mitotic figures which were observed are recorded in the Table.

No mitotic figures were observed in normal control worms. In the majority of wounded and grafted worms mitotic figures were found. The coelomocyte population is comprised of amoebocytes and eleocytes. All mitotic figures observed were in amoebocytes, no eleocytes were seen undergoing division. This was found to be the case following observations made using Haematoxylin and Eosin stained sections as this allowed amoebocytes to be discerned from eleocytes by the appearance of their cytoplasm.

Mitotic activity was observed in host tissue only, except in the case of 17-h-old and 20 day autografts in which mitotic figures were found in the circular muscle layer of the graft. This suggests that all transplants, except autografts, do not aid in the process of host graft integration as they remain mitotically inactive.

In worms which received second grafts from the same individual, another individual of the same species or from an individual of another species i.e. *Dendrobaena veneta*; mitotic figures were found in similar locations as those found in worms receiving first grafts only. Consequently the results from these three combinations were tabulated together.

I conclude that earthworms having being subjected to the stress of receiving a graft or a wound proliferate their amoebocytes from the intersegmental septa and somatopleural peritoneum and some proliferation of these cells can occur when they are free in the coelomic cavity. CAMERON<sup>3</sup> also observed mitotic activity in similar sites after injecting particulate matter into the coelomic cavity of *Lumbricus terrestris*. This suggests that the free amoebocytes are not fully differentiated when released from their site of origin, and also this increase in mitotic activity reflects the importance of amoebocytes in the regeneration of new tissue and also in the defence of the organism.

<sup>10</sup> M. PARRY, *Experientia* 31, 117 (1975).

## Nerve Growth in Cockroaches (*Periplaneta americana*) with Rotated Ganglia

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**Summary.** The axons of motoneurons in rotated cockroach ganglia grow into the limb opposite the one they normally innervate and appear to synapse with appropriate muscles. Apparently the classification of motoneurons is duplicated about the mid-line.

Larval and adult cockroaches regenerate appropriate connections between thoracic motoneurons and leg muscles when the nerves to the legs are cut<sup>3–7</sup>. Motoneurons of the mesothorax will connect with homologous metathoracic muscles when offered a metathoracic limb in which to grow<sup>8</sup>, indicating that the classification of cells on which the orderly connection of muscles and motoneurons depends is repeated in each of the three thoracic segments. In this paper I report preliminary experiments to establish whether the classification is also duplicated about the mid-line, that is whether motoneurons will connect with homologous muscles of the opposite side when offered a contralateral leg in which to grow.

Freshly moulted nymphal cockroaches (*Periplaneta americana*) of various instars were lightly anaesthetised with carbon dioxide and held down on their backs with plasticine. A small flap of cuticle was raised over either pro-, meso-, or metathoracic ganglion and all peripheral nerve trunks of the exposed ganglion were severed, leaving the longitudinal connectives intact. The ganglion was then inverted, with a hair placed between the anterior connectives to prevent re-rotation. The operation is shown diagrammatically in Figure 1. The operated animals were kept at 27°C for 3 months before examination and during this time they underwent at least 2 moults.

The effect of the operation on the regrowth of axons into the legs was assayed by retrograde iontophoresis of cobalt chloride into the main nerve (nerve 5) of one of the legs in the operated segment using conventional techniques<sup>9</sup>. The results given here are for the metathorax but are similar in each of the three segments.

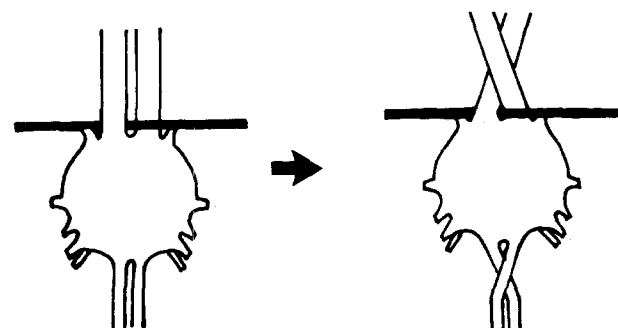


Fig. 1. Rotation of ganglia.

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<sup>3</sup> D. BODENSTEIN, *J. exp. Zool.* 136, 89 (1957).

<sup>4</sup> D. M. GUTHRIE, *J. Insect. Physiol.* 8, 79 (1962).

<sup>5</sup> D. M. GUTHRIE, *J. Insect. Physiol.* 13, 1593 (1967).

<sup>6</sup> J. W. JACKLET and M. J. COHEN, *Science* 156, 1640 (1967).

<sup>7</sup> K. G. PEARSON and A. B. BRADLEY, *Brain Res.* 47, 492 (1972).

<sup>8</sup> D. YOUNG, *J. exp. Biol.* 57, 305 (1972).

<sup>9</sup> S. B. KATER and C. NICHOLSON, *Intracellular Staining in Neurobiology* (Springer Verlag, New York 1973).

Retrograde filling of the leg nerve cut at the coxal/femoral joint in the metathorax of a normal, unoperated cockroach routinely dyes motoneurons in the ipsilateral anterior ventral quarter of the ganglion, together with median cells which are identified with the common inhibitory neurons described by PEARSON and FOURTNER<sup>10</sup> (Figure 2a). There is no indication in the normal animal that contralateral cells send axons across the ganglion and out of the leg nerve on the opposite side.

Three months after rotation the ganglia appear normal apart from their dorso-ventral inversion, twisted connectives and persistent wound tissues. Peripheral nerve trunks exit to the nearest limb – they do not cross back to their side of origin. If the leg nerve of a rotated ganglion is now filled with dye, the result is the same as in an unoperated ganglion: only neurons ipsilateral to the filled nerve are stained (Figure 2b). However, more cells are filled as other peripheral nerve trunks become closely associated in the wound tissue and take up the dye intended for nerve 5. In a total of 15 assays on rotated metathoracic ganglia there were no signs of contralateral cells being filled by the migrating dye. Clearly left neurons

have sent axons into the right limb and right into left. The appearance of co-ordinated movements in the legs of the operated segment suggests that these ingrowing axons synapse with appropriate muscles previously innervated by equivalent neurons on the opposite side of the ganglion.

Neurons which are displaced often show considerable powers of growth in reaching an appropriate target cell even if the original direction of axon growth is reversed<sup>11</sup>. In these experiments however motoneurons do not grow back to their side of origin when they are disconnected from it and offered the opposite side in which to grow. If the neurons are classified as right or left innervating then the cues by which they distinguish right from left are ineffective at this stage. It is more likely that the leg motoneurons which are the progeny of a bilaterally symmetrical set of mother cells<sup>12</sup> are not distinguished by their side of origin and that they do not discriminate between opposite sides of the mid-line during growth. The duplicate classification of the motoneurons finds its counterpart in the sensory system. The axons of receptor neurons do not respect the mid-line during normal growth<sup>13</sup>. When left and right cerci are exchanged in crickets, the regenerating axons of the sensory neurons enter the ipsilateral half of the terminal abdominal ganglion where they synapse with cells of the opposite side to that which they normally innervate<sup>14</sup>.

The development of coordinated limb movements in the operated animals indicates that the regenerating motoneurons make functional contacts with the muscles of the limb into which they are induced to grow. Further experiments are required to confirm this and to analyse the response of the operated cockroaches to the behavioural problems with which they are confronted.

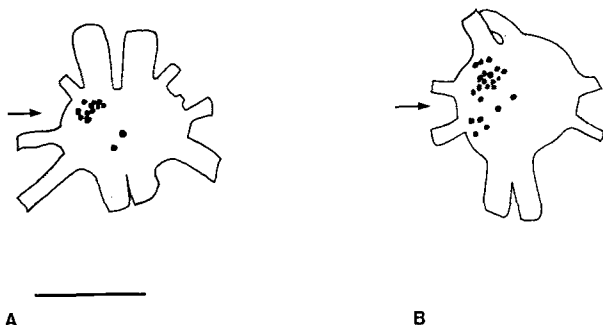


Fig. 2. Neurons stained by retrograde diffusion of cobalt chloride into leg nerve 5 of one side (arrowed).

A) Control ganglion. B) After rotation and regeneration of peripheral nerve trunks. More cells fill in the operated ganglion because nerve trunks associate in wound tissue and take up dye intended for nerve 5. Scale: 1 mm.

<sup>10</sup> K. G. PEARSON and C. R. FOURTNER, *Can. J. Zool.* 51, 859 (1973).

<sup>11</sup> E. HIBBARD, *Expl Neurol.* 13, 289 (1965).

<sup>12</sup> C. M. BATE, *J. Embryol. exp. Morph.* 35, 107 (1976).

<sup>13</sup> P. A. LAWRENCE, in *Cell Patterning* (Ciba Foundation Symposium 1975), vol. 29.

<sup>14</sup> J. PALKA and M. SCHUBIGER, *Proc. natn. Acad. Sci., USA* 72, 966 (1975).

## The Potency of Male Accessory Gland Material in the Mosquito (*Aedes aegypti*)<sup>1</sup>

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**Summary.** Injections of male accessory gland material from *Aedes aegypti* into the hemocoels of virgin female mosquitoes indicate that the potency of the secretion is equivalent to the amount of semen which a male normally places within the female. This estimation is far less than had been previously calculated. It is suggested that the term *matrone* for male accessory gland material is inappropriate since it does not convert a maid into a matron but prevents reinsemination of an impregnated female.

Male *Aedes aegypti* mosquitoes normally inseminate 5 females<sup>2</sup>. Shortly after females are inseminated, virgin males will not force-copulate with them<sup>3</sup>. The male normally ejaculates semen consisting of one-fifth or less of the secretion within a pair of large accessory glands and a relatively small amount of spermatozoa and fluid from the seminal vesicles directly into the seminal bursa of a virgin<sup>2</sup>. Within less than 5 min after being inseminated, the female transfers many spermatozoa into 2 spermathecae<sup>2</sup>, and over the next few days all of the semen within the bursa is resorbed into her hemolymph, leaving

this sac clear and flat like that of a virgin<sup>4</sup>. As soon as the spermatozoa have reached the spermathecae, the female is said to be impregnated<sup>5</sup>, to distinguish this event from the presence of spermatozoa elsewhere within her body.

CRAIG<sup>6</sup> thought that the reason inseminated mosquitoes do not become reinseminated is either because the female avoids copulation or because the male cannot insert his aedeagus into the vagina. SPIELMAN et al.<sup>7</sup> found, however, that inseminated females copulate with additional males. They proposed that these females were reinseminated but quickly ejected the second ejaculate. JONES<sup>4</sup>