Figure 2 shows that whereas the acid and the alcohol at a concentration of 10^{-4} *M* caused an extensive rapid lysis of lysosomes suspended in 0.25 *M* sucrose, the ester exhibited its lytic effect only after an incubation for a period longer than 15 min. The remarkable lag period in the lytic effect of the ester could possibly reflect the need for its hydrolysis within the membrane domain by lysosomal lipases or non-specific esterases prior to exerting organelle damage. If this is the case, then high local concentration of free fatty acid will be the cause of lysosomal rupture. Under conditions where spontaneous lysis of lysosomes was recorded (37 °C, prolonged incubation, 0.25 *M*), it is plausible that membrane phospholipid hydrolysis which brings about the release of free fatty acids, may be the initiator for the damage to organelle intactness. Lysolecithin, the second product of phospholipid hydrolysis, has also been shown to have a biphasic effect on lysosomal integrity. The biphasic effect was observed when lysosomes were exposed to lysolecithin under the same conditions as applied for testing the effect of oleic acid (Figure 1) as well as when lysosomes were exposed to the phospholipid in 0.25 *M* sucrose for 2 h at 37 °C.

De Duve et al.[6] suggested that compounds causing lysis to lysosomes may act by initiating a sequence of events involving altered permeability to solutes, osmotic swelling and rupture. Induction of changes in membrane permeability may lead to the penetration of low molecular weight substrates rendering them available to lysosomal enzymes, while a high molecular weight solute may still be impermeable. Such differential permeability changes have been shown by Badenoch-Jones and Baum[13], who studied the effect of progesterone on the available and free acid phosphatase activity of lysosomes suspended either in sucrose or in polyethylene glycol (PEG, m.w. 1000).

We have tested the possibility of differential permeability changes in lysosomes exposed to 10^{-4} *M* of oleic acid. Figure 3 shows that within 15 min of exposure to oleic acid, 70% of total acid phosphatase activity was non-particulate while 100% of it was already available. Suspending the organelles in 0.25 *M* PEG (m.w. 1000, Carbide and Carbon Co., N.Y.) led to a reduction in both the available and free enzyme activity. At 15 min of exposure to the acid, about 42% of enzyme activity was non-particulate and 50% was available. Thus lysosomes, suspended in sucrose, reacted to oleic acid in the same pattern exhibited upon exposure to progesterone, but PEG did not seem to afford proection against rupture by oleic acid.

The observation that available activity in sucrose exceeds that in PEG (Figure 4) could derive from an expected difference in the initial properties of lysosomal suspensions in the two media. Alexandrowicz[14] presented evidence that high molecular weight polyethylene glycols, at high concentrations in solution have high osmotic coefficients; i.e. an increase from 1 to 5 was recorded for PEG of 6000 molecular weight. According to his theory, 0.25 *M* solution of PEG of a molecular weight of a 1000 would have a higher osmotic pressure than that of 0.25 *M* sucrose. The shift in the osmotic fragility curve, observed for lysosomal suspensions in PEG as compared to those in sucrose (Figure 4), is consistent with this interpretation. Thus in 0.25 *M* PEG the lysosomes are expected to shrink to a certain extent and therefore to be less prone to damage by oleic acid. The very small difference in the values of available and free acid phosphatase in PEG solutions implies that the primary interaction with oleic acid suffices to bring about leakage of enzymes and does not involve a change in permeability that allows substrate to permeate while PEG permeation is still retarded.

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Evidence of Mitotic Division of Coelomocytes in the Normal, Wounded and Grafted Earthworm *Eisenia foetida*

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Summary. In the oligochaete *Eisenia foetida* the free amoebocytes, once released into the coelomic fluid were observed to remain mitotically active following the trauma of receiving a body wall graft or wound.

There is general agreement[2–5] that the prime source of earthworm coelomocytes is the lining of the coelomic cavity. However, disagreement exists as to whether once free these coelomocytes are capable of dividing; several authorities[3, 6–8] have indicated that under stress there is an increase in the mitotic activity in the worm. However only one author[3] reported observations of mitotic figures in free coelomocytes, others[6, 7] reported this increase to be associated with the coelomocyte stem cells only. Several papers[7, 8] have indicated that cells in the cicatricial tissue which is regenerating and differentiating following trauma also exhibit mitotic activity. Contrary to these reports, Liebmann[2] observed no mitotic activity in the free coelomocytes and believed that they never undergo division. This latter view was recently supported by Cooper[9] who agreed that mitotic figures were never seen in coelomocytes.

In an attempt to resolve this problem, I examined coelomocytes for mitotic figures, in earthworms which had been subjected to wounding and a variety of graft combinations.

Autografts using *Eisenia foetida* var *unicolor*, allografts using *E. foetida* var *typica* and var *unicolor* and xenografts using *Lumbricus terrestris* as the donor and *E. foetida*

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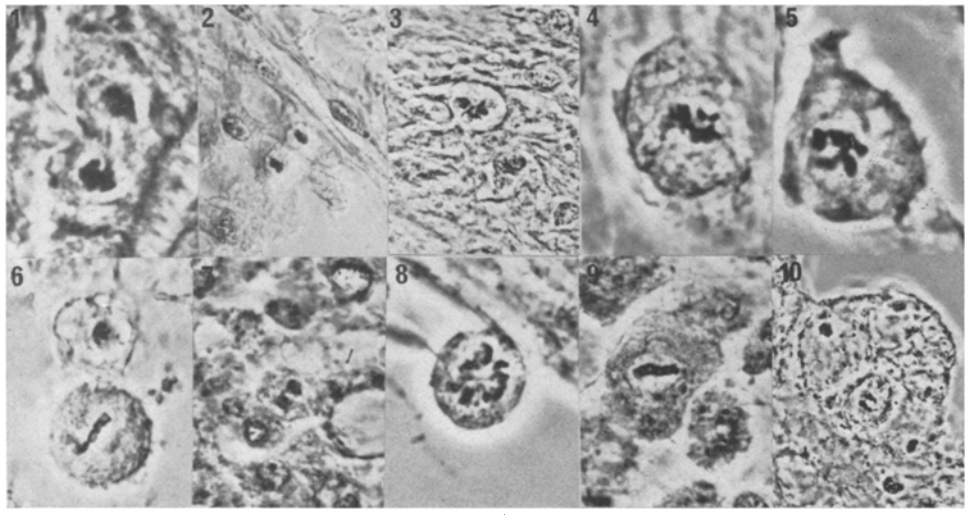
Distribution of mitotically active coelomocytes in lumbricids

Graft type	Time post grafting at which fixation took place	Amoebocytes					Eleocytes
		Septa and peritoneum	Free Amoebocytes in PVC	Cicatricial tissue	Host muscle	Other locations	
Control	—	—	—	—	—	—	—
Wounded EU worms	5 hours	—	—	+	—	—	—
	17 hours	—	—	—	—	—	—
	120 hours	—	—	+	+	—	—
	15 hours	—	—	—	—	—	—
Autograft EU worm grafted onto itself	5 hours	—	—	—	—	—	—
	17 hours	—	+	—	—	Muscle of graft	—
	120 hours	+	+	+	—	—	—
	15 days	—	+	—	—	—	—
	20 days	—	—	—	—	Muscle of graft	—
Allograft ET donor on EU host	5 hours	—	—	—	—	—	—
	17 hours	—	—	—	—	—	—
	72 hours	—	—	—	—	—	—
	16 days	—	—	—	—	—	—
Xenograft LT donor on EU host	5 hours	+	+	—	—	—	—
	17 hours	+	—	+	—	—	—
	120 hours	+	+	—	+	—	—
	16 oays	+	—	+	—	—	—
	40 days	+	+	+	—	—	—
	80 days	+	+	+	—	—	—
	Post 2nd graft						
Second xenograft DV or LT donor on EU host	3 days	+	+	—	—	Nephridial wall	—
	10 days	+	+	+	—	—	—
	45 days	—	+	+	—	—	—

ET, *Eisenia foetida typica;* EU, *Eisenia foetida unicolor;* LT, *Lumbricus terrestris;* DV, *Dendrobaena veneta;* PVC, Peri visceral cavity. —, No mitotically active cells found; +, Mitotically active cells found. Amoebocytes, which are phagocytic cells that produce pseudopodia, were found to be mitotically active. No eleocytes were found to be mitotically active. Eleocytes are spherical cells which have a characteristic yellow color and a high lipid content. They are believed to be a type of 'trophocyte'. They are normally found free in the PVC. Their origin is believed to be the gut wall.



Figs. 1–10. 1. 72 h allografted worm – nephridial wall. 2. 120 h xenografted worm – beneath graft. 3. 40-day xenografted worm – cicatricial tissue. 4. 120 h autografted worm – intersegmental septa. 5. 120 h autografted worm – free amoebocyte. 6. 120 h xenografted worm – free amoebocyte. 7. 3 day 2nd xenografted worm – beneath first graft. 8. 3 day 2nd xenografted worm – free amoebocyte. 9. 3 day 2nd xenografted worm – free amoebocyte. 10. 3 day 2nd xenografted worm – somatopleural peritoneal cell.

var *unicolor* as the host, were made according to a previously reported method[10]. 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 Feulgens stain others in Ehrlichs 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 comprized 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[3] 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.

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

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Summary. The axons of motorneurons 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 motorneurons is duplicated about the mid-line.

Larval and adult cockroaches regenerate appropriate connections between thoracic motorneurons and leg muscles when the nerves to the legs are cut[3–7]. Motorneurons of the mesothorax will connect with homologous metathoracic muscles when offered a metathoracic limb in which to grow[8], indicating that the classification of cells on which the orderly connection of muscles and motorneurons 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 motorneurons will connect with homologous muscles of the opposite side when offered a contralateral leg in which to grow.

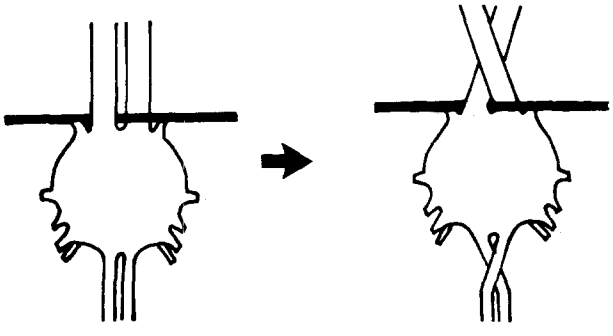

Fig. 1. Rotation of ganglia.

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[9]. The results given here are for the metathorax but are similar in each of the three segments.

[1] I thank NAN COWAN for her expert technical assistance.

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