AMP-stimulating agents was much more potent in reducing the cell number of melanoma cells in culture than that produced by the individual agent. This was due primarily to an extensive cell death as evidenced by the increased number of floater cells in the medium. The most effective combination was when the cells were treated with either sodium butyrate and RO20-1724 or sodium butyrate and theophylline. On the removal of drug 6 days after treatment the cells renewed cell division and eventually became confluent irrespective of treatment.

The treatment of human amelanotic melanoma cells in culture with sodium butyrate and cyclic AMP-stimulating agents produced varying degrees of morphological alterations. Control cells were large with one or more cytoplasmic processes. Their cellular boundaries were difficult to identify during the growth period and finally became completely fused with each other at confluency. Theophylline and RO20-1724 caused a marked morphological change as evidenced by the increased length of cytoplasmic processes. The entire cell appeared elongated. However, papaverine did not produce such a change; PGE_1 did induce morphological alterations in some cells, but the shape of most cells remains similar to that observed in control culture. Sodium butyrate caused flattening of cells, but the cytoplasmic processes were similar to those seen in control culture.

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A response to monoamines in *Peripatopsis moseleyi* (Onychophora)

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Summary. Preparations of longitudinal muscle respond to catecholamines if the ventral nerve cords are present, and also to 5-hydroxytryptamine. The response to 5HT is complex if the central nervous system is present, but muscle alone is probably inhibited.

The Onychophora are primitive terrestrial arthropods, placed together with myriapods and insects in the phylum Uniramia[3,4]. Physiological knowledge of their nervous system[5,6] and distinctive smooth muscle[7-9] is incomplete. Preparations of longitudinal muscle react to acetylcholine but not to glutamate, gamma-amino butyric acid, picrotoxin or noradrenaline[10,11], suggesting that the motor innervation is cholinergic. In these pharmacological properties Onychophora resemble annelids rather than arthropods. We wished to test further the effects of monoamines.

Materials and methods. Etherized *Peripatopsis moseleyi* were dissected longitudinally. 7 ventral preparations were made of body wall longitudinal muscle including the central nervous system, and 3 with dorsal longitudinal muscle only. A preparation was suspended in 30 ml of Ringer solution[12,13] of pH 7.3, at room temperature (19.5–21 °C), and aerated with 95% O_2, 5% CO_2. Tension was 100–400 mg and contractions were recorded via a Washington T1 isotonic transducer.

The majority (6/7) of ventral preparations showed slow variations in baseline tone with irregular rhythmical contractions (3–8/min) superimposed. The latter are referred to as spontaneous or rhythmical activity. Dorsal preparations without the nerve cords had a stable baseline tone and showed little spontaneous activity.

Test drug solutions were added to the bath by syringe (0.1–0.5 ml). A 15–60 sec contact time was used for acetylcholine responses and 2–5 min for the monoamines. The preparations were washed by overflow and at least 5 min were allowed between doses.

Results. All preparations responded to acetylcholine (ACh: $3\text{–}30\times10^{-6}$ g/ml) with a single smooth contraction. Eserine ($0.8\text{–}1.5\times10^{-6}$ g/ml, 5 applications) evoked similar but longer-lasting contractions. ACh responses were markedly potentiated (mean greater than 200 times) when tested immediately after recovery of previous baseline tone following an eserine response.

Ventral preparations responded to noradrenaline (NA) and dopamine (DA). NA ($5\text{–}80\times10^{-6}$ g/ml, 13 applications in 5 preparations) markedly increased tone, and this continued so long as the drug was in the bath (up to 5 min). DA ($40\text{–}80\times10^{-6}$ g/ml, 13 applications in 6 preparations) raised the tone to a smaller extent but induced repeated or rhythmical contractions. The DA responses tended to reverse within 3–5 min, before the drug had been removed. Dorsal preparations did not respond to NA (up to 16×10^{-5} g/ml, 4 applications) or to DA (up to 24×10^{-5} g/ml, 4 applications).

With ventral preparations 5-hydroxytryptamine (5HT, $10\text{–}100\times10^{-6}$ g/ml) caused a response resembling that to DA in 8 applications, but in 2 applications the existing tone and spontaneous activity were reduced. In 6 other applications both kinds of effect were combined: after an initial increase in tone and spontaneity, both declined. 5HT affected only one of the 3 dorsal preparations and the response consisted of a reduction in tone.

Application of the monoamine oxidase inhibitor iproniazid to 2 ventral preparations ($2\text{–}5\times10^{-4}$ g/ml) evoked an increase in tone and spontaneous activity whilst no re-

sponse was obtained in 1 dorsal preparation (9×10^{-4} g/ml).

Discussion. The sensitivity of the muscle to ACh and its potentiation by eserine are similar to previous observations[10,11], suggesting that ACh is a neuro-muscular transmitter in Onychophora.

As found by previous authors[10,11] muscle preparations without the central nervous system do not respond to catecholamines. Ventral preparations, however, respond with increases in tone and spontaneous activity. This suggests that these agents act within the ventral nerve cords. The different effects of NA and DA on tone and spontaneous activity suggest possibly that these 2 catecholamines may act by different mechanisms. The action of iproniazid suggests the presence of monoamine oxidase in the nervous system.

5HT probably has an inhibitory effect on the musculature but a mixed excitatory-inhibitory effect on preparations containing the central nervous system. It seems possible that 5HT increases muscle tone and spontaneous activity when it acts on the nervous system, but if acting directly on the muscles as well it may inhibit their activity.

The monoamine responses of *Peripatopsis* are qualitatively similar to those in the annelid *Lumbricus terrestris*[14], except for the inhibitory effect of 5HT on the muscle. Inhibition was observed, however, in preparations from other annelids[15,16]. Our results may be physiologically significant since catecholamines and 5HT are present within the nervous system[17].

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The presence of monoamines in the nervous system of *Peripatopsis* (Onychophora)

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Summary. Preliminary observations of formaldehyde-induced fluorescence support the suggestion that monoamines such as noradrenaline, dopamine and 5-hydroxytryptamine are transmitter agents in the central nervous system of Onychophora.

Experiments with preparations of the body wall of *Peripatopsis moseleyi* show that a response of the longitudinal muscle to noradrenaline and dopamine depends on the presence of the ventral nerve cord[2]. We therefore examined the distribution of formaldehyde-induced fluorescence in the nervous system, following the method of Falck-Hillarp[3].

The results reported here were obtained with a specimen of *Peripatopsis sedgwicki* and have since been confirmed with *P. moseleyi*[4]. The specimen was anaesthetized with ether and cut into pieces which were quenched in isopentane cooled over liquid nitrogen, freeze-dried and treated with formaldehyde, and embedded in paraffin wax. Orientated 10 µm sections were trimmed and mounted in liquid paraffin. They were examined using a Leitz Ortholux microscope equipped with filters BG12 and K490.

As seen in figure 1 there are several brightly fluorescent tracts in the ventral nerve cords. Their green colour is consistent with the presence of noradrenaline or dopamine or related substances. A few green fluorescent nerve cell bodies were seen from time to time in the cortex of the nerve cord. Yellow cells, which probably contain 5-hydroxytryptamine (or similar compounds) were also observed there, and in some sections rather tenuous yellow fluorescent tracts were seen next to green fibre bundles.

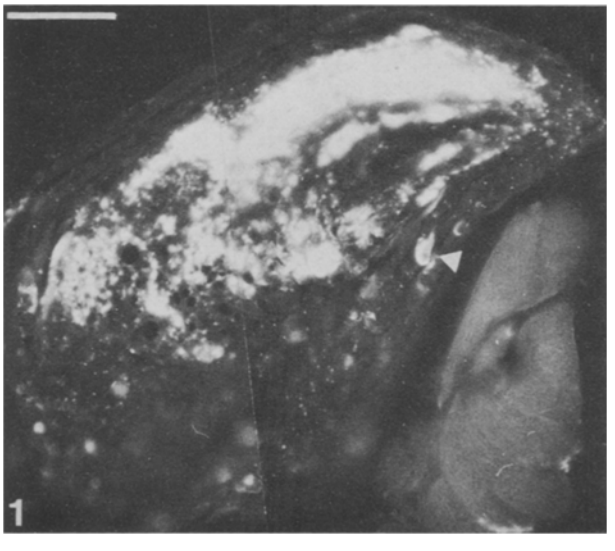

Fig. 1. Right nerve cord of *Peripatopsis sedgwicki* in transverse section, showing green fluorescent tracts. Arrow indicates a small green nerve cell. Bar = 50 µm.