insects, however, were the elongated galeae of the maxillae. Since in the normal adult the galae form the proboscis, the strong elongation of the galeae in 6th instar larvae must be regarded as a development in the direction of proboscis differentiation. It is interesting to note that this developmental trend of the galeae could in no case be fully suppressed during L_5/L_6 moults of *Pieris*, not even when high doses of juvenoids were applied at a very early stage (during or shortly before the preceding L_4/L_5 moult) nor when the head capsule was shed normally. Thus perfect L_6 of *Pieris* have never been obtained. They all died of starvation, because their abnormally shaped maxillae prevented proper feeding.

A number of the L_6 who could not get rid of the old head capsules showed, at the extremities of the galeae, heavily sclerotized mandible-like structures (Figure 2). Thus the appendages of the maxillary segment had produced structures which normally are restricted to the mandibular segment. This phenomenon must be regarded as a case of homoeösis and involves transdetermination of the cells situated in the apical region of the galeae. It indicates that the juvenoid Ro 20–3600, if applied at the right time and in proper concentration, can activate the genes responsible for mandibular differentiation in cells in which these genes normally would never be activated.

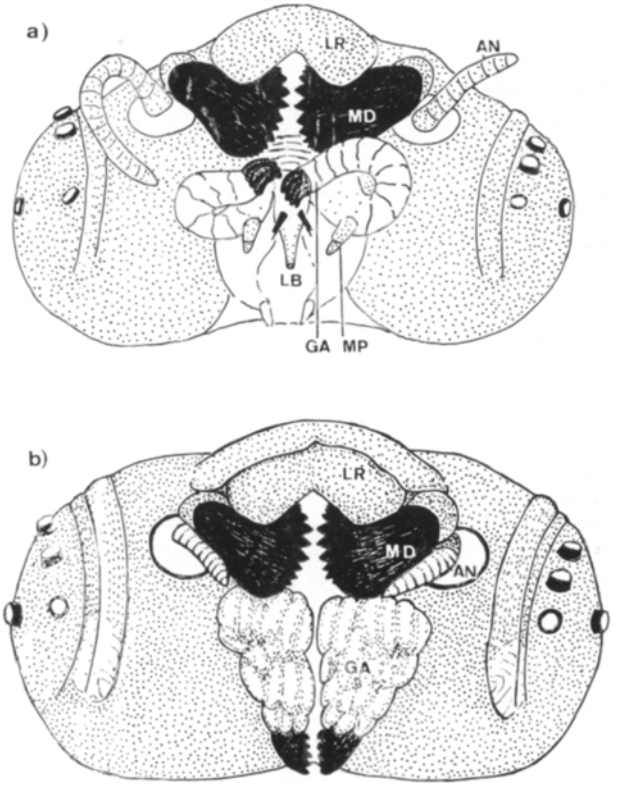

Fig. 2. Two examples of L_6 similar to the one shown in Figure 1b, but with less strongly developed galeae bearing mandible-like structures at their extremities. Same symbols as in Figure 1.

It is probable that the effect is not specific for Ro 20–3600, but that the application of the juvenoid leads to an abnormal state of relatively high concentration of juvenile hormone. In normal ontogenesis, the titer of juvenile hormone becomes low in the 5th larval instar. Under this condition the mandibles disintegrate and the maxillae, especially the galeae, elongate during the next moulting process. The larvae with abortive L_5/L_6 ecdysis demonstrate that it is easy to prevent the loss of the mandibles, and that it is difficult to prevent the pupal outgrowth of the galeae by the topically applied juvenoid. This may indicate that a relatively low titer of juvenile hormone is sufficient to maintain the formation of mandibular structures, but that a high titer of the hormone is needed in last instar larvae in order to maintain the larval character of the maxillae during a further moult. In the few individuals in which the differentiation of mandibular structures on the maxillae had been induced, the juvenoid was obviously present in an intermediate concentration, i.e. a concentration high enough to maintain mandibular structures, but not high enough fully to prevent differentiation of the galeae in pupal direction, yet sufficiently high to induce the genes in the cells of the apical region of the galeae to furnish the information needed to produce strongly sclerotized organs of mandible-like shape. This probably indicates that juvenile hormone, if acting during a period in which it would normally be absent or present at a lower titer, can activate certain genes which would not become active in normal development and that a specific titer of juvenile hormone is crucial for the realization of each step and each stage of differentiation.

Zusammenfassung. Topicale Behandlung des letzten Larvenstadiums von *Pieris brassicae* mit einem Juvenoid[1] ergibt Raupen, die eine abortive zusätzliche Larvenhäutung zum L_6 durchmachen, wobei sie die alte Kopfkapsel nicht abstreifen können, weil die Antennen und besonders die Galeae der Maxillen stark verlängert werden. In einigen Individuen wurden an den Enden der Galeae mandibelartige Strukturen ausdifferenziert. Es wird angenommen, dass diese homöotische Transdetermination der apicalen Galeazellen durch den abnormen, intermediären Juvenilhormonspiegel bewirkt wurde.

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The Mode of Action of Four Anthelmintics

From pharmacological experiments with isolated tissue, Van Neuten[1] concluded that the broad spectrum anthelmintic levamisole (1-2, 3, 5, 6-tetrahydro-6-phenylimidazo[2,1-b] thiazole hydrochloride) causes contraction of mammalian muscle by nerve ganglion stimulation. He suggested that the contraction of *Ascaris lumbricoides* caused by levamisole could also be due to ganglion stimulation as the contraction was less pronounced, but not inhibited, in the presence of hexamethonium, and because it has been reported that levamisole causes a reduced membrane potential of *A. lumbricoides* muscle cell bellies[2].

[1] J. Van Neuten, in *Comparative Biochemistry of Parasites* (Ed. H. Van den Bossche; Academic Press, New York 1972), p. 101.
[2] J. Aceves, D. Erlij and R. Martinez-Maranon, Br. J. Pharmac. *38*, 602 (1970).

To study the pharmacology of levamisole on nematodes further, freshly collected whole *A. lumbricoides* were held horizontally in an open Perspex bath containing Tyrode at 37 °C and connected by rubber collars to an isotonic recorder. Solutions in Tyrode were injected into the worms (0.1 ml solution into a 3 g worm). Although tubocurarine, and atropine (all tested up to 20 μg/g) failed to prevent contractions caused by levamisole (0.3 μg/g), both the ganglion blockers mecamylamine and pempidine (5 μg/g) blocked levamisole induced contractions. We consider that these results confirm the suggestion of VAN NEUTEN[1] that levamisole is acting as a ganglion stimulant in nematodes.

In contrast to the report that paralysis of *A. lumbricoides* by levamisole is irreversible[1], our experiments have shown reversible paralysis with 3 species of nematodes, *A. lumbricoides*, *Nippostrongylus brasiliensis*, and *Nematospiroides dubius* when they were continuously maintained in levamisole. Adult *N. brasiliensis* maintained in levamisole solutions in Morgan, Morton and Parker saline with antibiotics (NaCl, 6.8 g/l; KCl, 0.4 g/l; $CaCl_2$, 0.2 g/l; $NaHCO_3$, 2.2 g/l; $MgSO_4 \cdot 7H_2O$, 0.2 g/l; $Fe(NO_3)_3$ $9H_2O$, 0.1 g/l; $NaH_2PO \cdot H_2O$, 0.14 g/l; glucose, 1 g/l; penicillin, 400,000 U/l; dihydrostreptomycin, 0.4 g/l; mycostatin, 100,000 U/l) regained motility. Both the rate of paralysis and de-paralysis was dependent upon the concentration of levamisole. Using approximately 20 worms per 10 ml in 100 ppm of levamisole, all worms paralysed within 10 min and all resumed active movements within 8 h; most worms kept in levamisole maintained these movements for 72 h. The reversibility of paralysis was not however observed with free living infective larvae of *N. brasiliensis*. Similar experiments with *A. lumbricoides* maintained in Tyrode (1 worm/50 ml) containing 100 ppm of levamisole demonstrated that some worms resumed movement after paralysis, though the movements were less vigorous than in normal worms. Both the time taken for recovery and the percentage of *A. lumbricoides* resuming active movements varied widely from batch to batch of worms. The suggestion that levamisole may act by inhibition of fumarate reductase[3] in addition to its function as a ganglion stimulant is difficult to reconcile with the reversible nature of levamisole paralysis.

The connection between the mode of action of levamisole and 2 other anthelmintics, methyridine (2-[2-methoxyethyl]pyridine) and pyrantel (*trans*-1-methyl-2-[2-(2-thienyl)vinyl]-1, 4, 5, 6-tetrahydropyrimidine) was first suggested by experiments with *N. brasiliensis*. Adult worms that had resumed active movements after incubation in 100 ppm of levamisole were washed and transferred to Morgan, Morton and Parker saline or saline plus 100 or 1000 ppm of methyridine. Even after 16 h in 1000 ppm of methyridine, some of the worms which had recovered from levamisole remained active although normal worms in methyridine were inactive. Worms were also incubated in either 100 ppm pyrantel (as tartrate), or 100 ppm levamisole or 100 ppm of pyrantel plus levamisole. Significantly more worms were showing activity in levamisole or levamisole plus pyrantel than in pyrantel on its own.

Both methyridine, bephenium[4] (benzyl dimethyl-2-phenoxyethyl ammonium) and pyrantel[5] cause contraction in normal *A. lumbricoides* muscle. However, when injected into *A. lumbricoides* that had recovered motility after incubation in levamisole, methyridine (30 μg/g), pyrantel (30 μg/g) and 40 μg/g bephenium (as hydroxynaphthoate) failed to cause contractions; acetyl choline (1 μg/g) and choline phenyl ether (0.6 μg/g) however caused contractions. It would appear that levamisole can cause a tachyphylactic type of response at the nerve ganglion receptor site of nematodes permitting muscular activity to resume. The presence of levamisole at the receptor site blocks the activity of certain other anthelmintics, which therefore presumably act at the same or closely adjacent site in the ganglion. The variation in ability of the 4 anthelmintics to paralyse nematodes will depend on both the variation in their affinity for the receptor sites as well as differences in rate of entry into the nematodes.

Résumé. Les *Ascaris* qui se sont «déparalysés» après une incubation prolongée dans 100 ppm de levamisole, ne se contractent pas après injection des anthélminitiques béphenium, méthyridine et pyrantel. Etant donné que la mécamyline et la pempidine bloquent les contractions des *Ascaris* provoquées par le levamisole, il paraît probable que chez les Nématodes ces 4 anthélmintiques sont des stimulateurs des ganglions.

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26 April 1974.

[3] H. VAN DEN BOSSCHE, in *Comparative Biochemistry of Parasites* (Ed. H. VAN DEN BOSSCHE; Academic Press, New York 1972), p. 117.
[4] A. W. J. BROOME, in *Drugs, Parasites and Hosts* (Eds. L. G. GOODWIN and R. H. NIMMO-SMITH; Churchill, London 1962), p. 43.
[5] M. L. AUBRY, P. COWELL, M. J. DAVEY and S. SHEVDE, Br. J. Pharmac. *38*, 332 (1970).
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Reproductive Incompatibility between Similar Genotype Flies (*Drosophila melanogaster*) Raised at Two Different Temperatures

In *Drosophila melanogaster*, the usual growth temperature is 25 °C, although it is possible to get complete development from egg to adult at temperatures ranging from 12° to 32 °C[1]. A biometrical analysis[2] showed important morphological modifications in adults grown at low temperature. Thus, we decided to look for possible correlative physiological differences in such flies. As 13° reared males proved to be sterile[3], 13° females were crossed with normal (25°) males in order to measure egg production and fertility. It was then discovered that in this cross copulation often resulted in premature death of females. Moreover, the survivors produced poorly hatching eggs, suggesting alteration of the fertilization process. From these observations we concluded that a kind of reproductive incompatibility arose between adults of the same genotype raised under different environmental conditions.

[1] J. DAVID and M. F. CLAVEL, C. r. Acad. Sci. Paris *262*, 2159 (1966).
[2] J. DAVID and M. F. CLAVEL, J. Insect Physiol. *13*, 717 (1967).
[3] Y. COHET, C. r. Acad. Sci. Paris *276*, 3343 (1973).