Summary. Differential predation on tadpoles by newts (*Triturus*). 4 European *Triturus* species (*T. alpestris, T. cristatus, T. helveticus, T. vulgaris*) feed upon the tadpoles of the following Anura species which occur in sympatry with the newts: *Rana esculenta, R. temporaria* and *Bombina variegata*. Only *Triturus cristatus* prey also upon the tadpoles of *Bufo bufo* and *B. calamita*. *Triturus alpestris, T. helveticus* and *T. vulgaris* do not eat any *Bufo* tadpoles. Even larvae of *Triturus alpestris* prey upon tadpoles of *Rana esculenta, R. ridibunda* and *Bombina variegata* but avoid tadpoles of *Bufo calamita*.

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Pseudojuvenilizing Effect of Methanol on the Pupation Processes of *Cerura vinula* L. (Lepidoptera)

The problem. There is increasing evidence that juvenile hormone activity (JH) is mimicked by a variety of compounds which are chemically unrelated to the usual juvenile hormone substances, the terpene derivatives. For example, colchicin and oleic acid were shown to have juvenilizing effects after application or injection on *Pyrrhocoris apterus* and *Galleria mellonella*[1]. The same holds for the insecticide synergists[2,3] sesoxane and piperonyl butoxide. The present paper shows that also methanol is able to mimic JH activity.

The last instar larvae of *Cerura vinula* are well-suited for an examination of the prepupal processes, because these are characterized by impressive changes: a drastic colour change occurs in the lateral integument[4]. At the same time, the larvae travel restlessly around. Thereafter they begin to spin the cocoon for pupation. These processes are probably controlled by some balance of the hormones of the prothoracic glands, ecdysone, and of the juvenile hormone of the corpora allata. At the beginning of these processes both glands are active[5,6]. Earlier experiments[7] showed, on the one hand, that it is possible to induce the colour change process precociously by injection of ecdysone, but, on the other hand, new test series showed that JH-analogues are able to prevent this[8]. In connexion, with the latter experiments, an interesting phenomenon was found: the solvent for the JH-substances that was first used turned out to be a juvenile hormone-mimicking substance in the control larvae. This finding will be analyzed in the following experiments in detail.

Methods. The larvae were kept at a constant temperature of 25 °C in which the prepupal processes normally start within $4^1/_2$ to 5 days after the last ecdysis. Therefore, the tests began on the 3rd or 4th day after this ecdysis. Two methods for assaying the effect of the substance were used. In the first instance methanol was applied to the surface of the cuticle; in the second pure methanol was injected directly into the thorax after anaesthesia with CO_2.

Results and discussion. Both methods showed exactly the same effect. The various concentrations of methanol ordinarily produced certain patterns of inhibition which enables one to classify the intensities of the reactions into the following categories: 1. The treatment started on the 4th day of the last instar.

a) A single dose of 0.4% (based on the body weight) was injected or a small dose was distributed uniformly over the lateral integument. The larvae transformed into normal pupae without delay.

b) A dose of 0.8%, either injected or applied to the integument, resulted in a delay of the colour change process of the wandering phase and of cocoon spinning of nearly 1–2 days. Besides this, defined plaques in the ventro-lateral epidermis remained unpigmented (Fig-

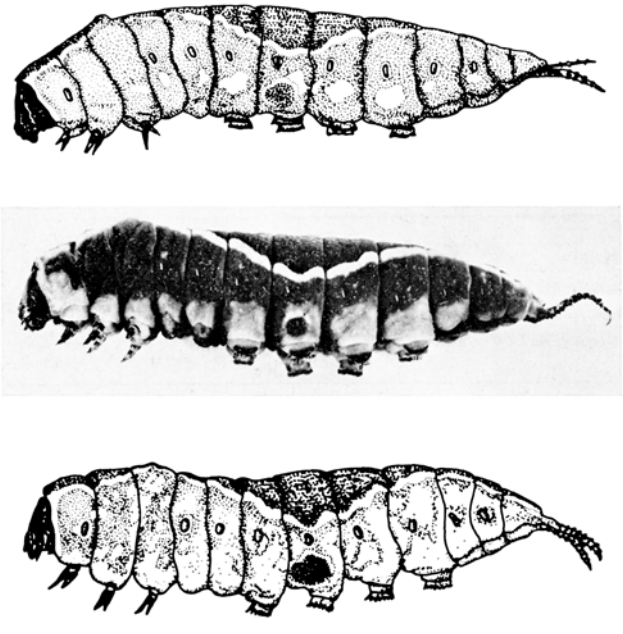

Fig. 1. Effect of increasing doses of methanol on the larvae.

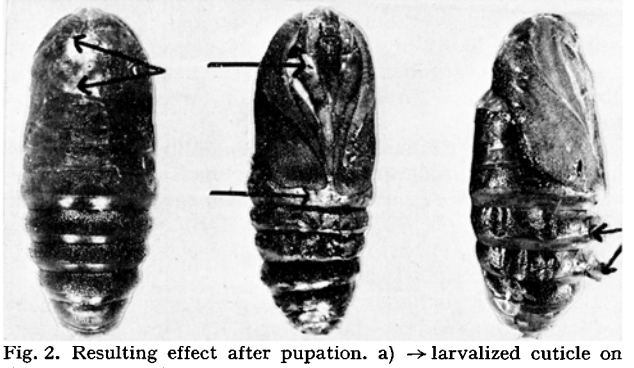

Fig. 2. Resulting effect after pupation. a) → larvalized cuticle on the dorsal part of the pupal thorax. b) → shortened wings and the thoracic legs are partly absent, thereabout cuticle is transparent. c) → vestigial larval legs.

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ure 1a). After a delay of 5 to 7 days these larvae transformed into normal pupae.

c) Injections of 1.2% methanol, all at once or in 2 parts or a forceful local treatment, caused a delay of the colour change of nearly 3–4 days and large defined plaques in the ventro-lateral epidermis remained unpigmented including the thoracic and abdominal legs (Figure 1b). It is important to emphasize once more that these defined plaques resulted from both methods. Here, too, the wandering-phase and the cocoon spinning was delayed.

d) An injected dose of 1.6% methanol and a moderate, repeated, local application caused a delay of the colour change process up to 5 days and only a few parts of the dorsolateral epidermis were pigmented (Figure 1c). These animals, at first, were unable to spin a cocoon but began to spin 7 days after the termination of the treatment.

e) Higher doses proved to be toxic. The larvae died 1 to 2 days after the treatment.

2. The treatment started on the 3rd day in the last instar.

a) The very small dose of 0.8% methanol resulted in the same effect as described in 1, c.

b) However, after application of a dose of 1.6% methanol, the larvae failed to change colour and were not able to spin a cocoon. Moreover, all these larvae were destined to die sooner or later, i.e. up to 2 weeks after treatment. Histological examination directly after death showed that the prothoracic glands were degenerated.

The larvae of the experiments 1c, d and 2a transformed into pupae on an average of 21 to 28 days after the first indication of the colour change, which means the pupal moult was greatly delayed. These pupae showed the following larval characteristics: 1. There was a localized area near the site of injection dorsally at the thorax; the cuticle had larval plaques, being unsclerotized and unpigmented (Figure 2a). These plaques were smooth and somewhat transparent. Towards the margins of the patch the larval cuticle usually passes over abruptly into pupal cuticle. Methanol may accumulate at the injection point and thereby cause the formation of larvalized cuticle. 2. The wings were slightly shortened but well formed (Figure 2b). 3. The prothoracic and sometimes the mesothoracic legs could be absent or vestigial and their cuticle was unpigmented and transparent (Figure 2b). 4. The transformed pupae showed vestigial larval legs on the abdomen, which were surrounded by pupal cuticle and therefore inflexible, in contrast to normal mummy-like pupae without abdominal legs (2c).

The results show that the ventrolateral epidermal areas are more sensitive to the methanol than the dorsolateral epidermal areas, in which the initial processes which are critical for the prepupal processes, for example the colour change, occur sooner. This seems to be in keeping with the pro- and mesothoracic legs in contrast to the other extremities of the thorax and the head. This means that a gradient determines the beginning of the colour change process in the lateral epidermis and the differentiation of the thorax and head extremities. The effects were caused by differences in dose absorbed combined with individual differences in physiological sensibility. Large doses of the methanol applied on the third day after the last ecdysis, just when the prothoracic glands are less active[6], caused a definite inhibition of the prepupal processes and the larvae died as so-called Dauerlarvae. But, on the 4th day, when the prothoracic glands are highly active, some tissues could no longer be prevented from metamorphosing by application of high doses. The epidermal structures, wings, and legs, could still be influenced on the 3rd day and sometimes at the beginning of the 4th day but even this sensibility was rapidly lost to the end of this day and, thereafter, treatment was without effect. This means that the response of the tissue is reduced between the 3rd and 4th day, being dependent upon the increasing ecdysone titre.

The biological effects produced by methanol resemble those of synthetic JH substances[5,8]. It may perhaps act either directly on the cells or it may give rise to an active compound. Alternatively it may act by bringing about a change in the cells so that their response to a future hormonal environment, which would normally be signalling the pupation processes, is impaired[9].

Zusammenfassung. Niedrige Dosen von Methanolapplikationen haben auf die Raupen des letzten Larvenstadiums juvenilisierende Wirkung. Differenzierungsprozesse können teilweise oder ganz ausfallen.

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Ingestion of *Acanthamoeba* by *Entamoeba invadens*

Entamoeba are known to ingest various types of food particles which include red blood cells, starch grains, epithelial cells, bacteria and other micro-organisms. However, up till now there has been no report of *Entamoeba* ingesting trophozoites of another species of amoeba. During this study *Entamoeba invadens* (polyxenic strain) was grown in JONES' medium[1] and *Acanthamoeba* sp. (axenic strain) was grown in 4% (w/v) Mycological peptone (Oxoid). The incubation temperature for both species was 25 °C. *Entamoeba* were harvested in about 7–8 days time when the starch content in the medium was very low, and most of the trophozoites were free of starch grains. The concentrated suspension of *Entamoeba* was then transferred to a small glass tube to which were added a few drops of 4% Mycological peptone containing a heavy suspension of *Acanthamoeba*. The mixture was gently shaken and examined after 1 min, 10 min and 20 min. It was observed that the ingestion of *Acanthamoeba* by *Entamoeba* took place rapidly. Within 1 min a few trophozoites of *Entamoeba* were found containing *Acanthamoeba* in the cytoplasm. After 10–20 min most of the trophozoites of *Entamoeba* had ingested *Acanthamoeba*. The process of ingestion was next studied under a phase-contrast microscope and the