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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[9] This investigation was supported by Deutsche Forschungsgemeinschaft.

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

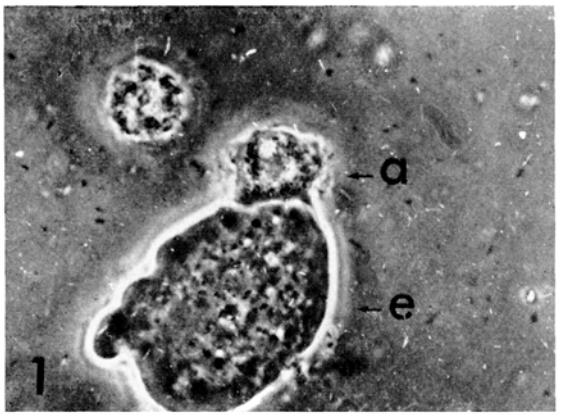

Fig. 1. Cell membranes of *Entamoeba* and *Acanthamoeba* lying in close proximity to each other, prior to the ingestion of *Acanthamoeba*. a, *Acanthamoeba*; e, *Entamoeba*. Phase-contrast ×1000.

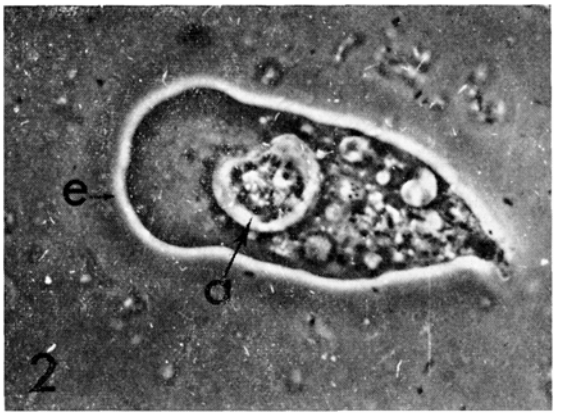

Fig. 2. Fully ingested *Acanthamoeba* lying in a food vacuole in *Entamoeba*. a, *Acanthamoeba*; e, *Entamoeba*. Phase-contrast ×1000.

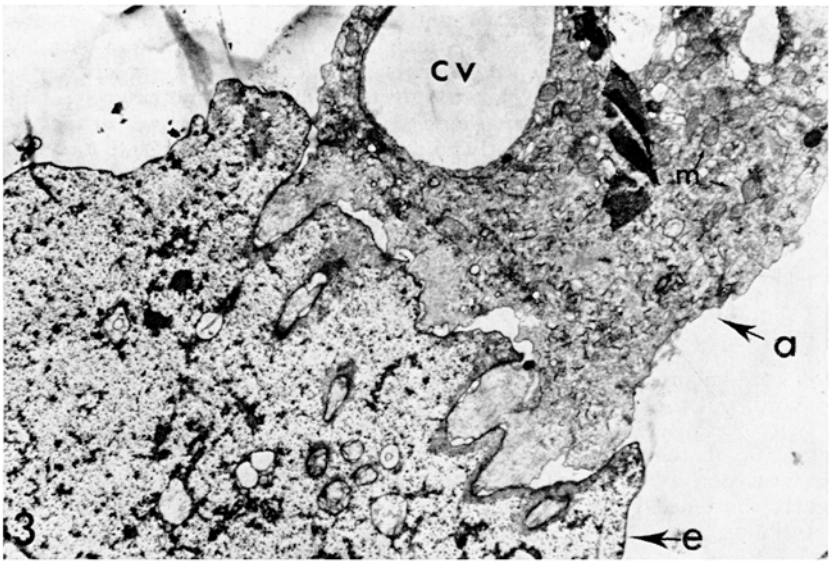

Fig. 3. Interlocking of the cell membranes of *Acanthamoeba* and *Entamoeba*. *Acanthamoeba* can be recognized by the presence of the contractile vacuole and mitochondria. a, *Acanthamoeba*; e, *Entamoeba*; m, mitochondria; cv, contractile vacuole. Electron-micrograph ×8000.

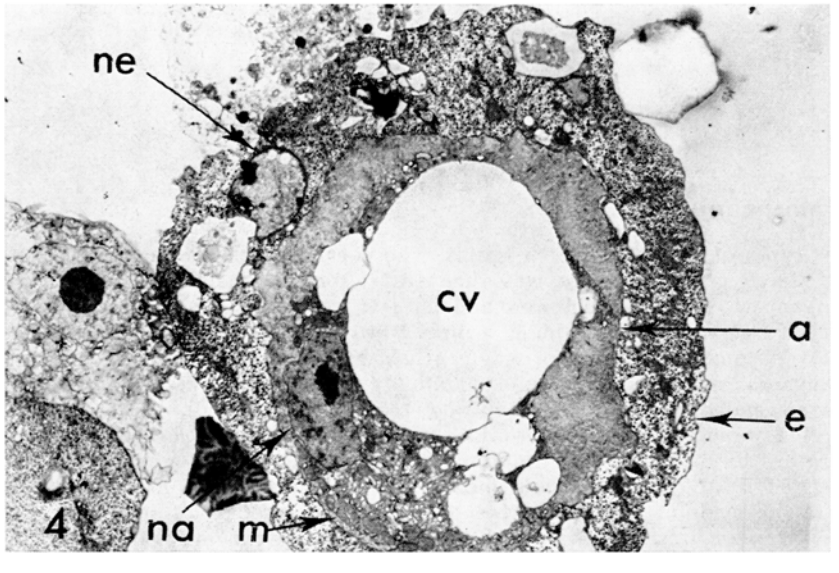

Fig. 4. Complete ingestion of *Acanthamoeba* by *Entamoeba*. The nuclei of both the parasites are visible. a, *Acanthamoeba*; e, *Entamoeba*; cv, contractile vacuole; m, mitochondria; na, nucleus of *Acanthamoeba*; ne, nucleus of *Entamoeba*.

sequence of events recorded photographically with an electronic flash. During this process, the *Entamoeba* moved rapidly and came in contact with *Acanthamoeba*, and when this happened the plasmalema of the 2 amoebae became interlocked with each other (Figure 1). The *Entamoeba* then gradually started to draw in the *Acanthamoeba* into its cytoplasm. Sometimes the whole amoeba was taken in completely intact and could be seen lying inside the food vacuole in *Entamoeba* (Figure 2).

For further study of this phenomenon, a mixture of *Entamoeba* plus *Acanthamoeba* which had been kept together for 10 min was processed for electron microscopy. These cells were fixed in buffered glutaraldehyde (pH 7.2) and post fixed in Osmium tetroxide. Following acetone dehydration, they were embedded in araldite, sectioned and stained with lead citrate and uranyl acetate according to the standard technique. Sections were examined on formvar coated grids in a Hitachi HS8 microscope.

The observations made in electron microscope confirmed the findings of the light microscope. Figure 3 shows the interlocking of the 2 amoebae before the ingestion of *Acanthamoeba*. The cell membrane of *Entamoeba* appears more electron dense than *Acanthamoeba* and at this stage it was not possible to say which amoeba will be able to ingest the other. Figure 4 shows *Acanthamoeba* lying inside the cytoplasm of *Entamoeba*. *Acanthamoeba* was easily recognized by the large contractile vacuole, a more homogenous cytoplasm, and the presence of mitochondria. The cytoplasm of *Entamoeba*, on the other hand, was granular, containing a large number of electron-dense particles, and had no mitochondria. The nuclei of both *Acanthamoeba* and *Entamoeba* are visible in the section.

The ultimate fate of the ingested *Acanthamoeba* is not known. Apparently they are broken down by *Entamoeba*, as in a number of cells nuclear remnants of *Acanthamoeba* were seen after a few hours of ingestion[2].

Résumé. L'ingestion de l'*Acanthamoeba* par l'*Entamoeba invadens* est observée pour la première fois. Elle a été étudiée par microscopie lumineuse et électronique. An cours de l'ingestion l'*Acanthamoeba* et l'*Entamoeba* se rapprochent; leurs membranes cellulaires s'entrelacent et l'*Entamoeba* achève l'ingestion de l'*Acanthamoeba*.

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2 The species of *Acanthamoeba* used in this study was obtained through the courtesy of Mrs. K. M. G. Adam, Department of Zoology, University of Edinburgh.

On the Synthesis of Poliovirus RNA at Supraoptimal Temperatures[1]

At certain supraoptimal temperatures, the incorporation of labelled precursors in poliovirus RNA is reduced to insignificant levels and virus multiplication completely prevented[2,3]. According to some authors[4], the phenomenon is due to an inhibition of viral RNA synthesis; according to others[5], it would depend upon an equilibrium between a normal synthesis of viral RNA and its parallel digestion by nucleases activated by supraoptimal temperatures. The question is of interest in that at supraoptimal temperatures, poliovirus proteins are synthesized[6], responsible for the early blockade of cell metabolism and for the late cytopathic effect[7,8]. If these events occur in the absence of viral RNA synthesis, supraoptimal temperatures could be indicated to evaluate the direct effect of antiviral compounds on the viral protein synthesis.

Materials and methods. Actinomycin D and neutral red were obtained from Merck; cristalline insulin from Lilly; peroxide-free phenol and ethyl ether from Mallinkrodt; Sephadex G 100 from Pharmacia; H^3 Uridine (5 T; 24,000 mC/m*M*) and H^3 Leucine (15,200 mC/m*M*) from Amersham. HeLa cells (American type culture collection) were grown in Eagle's MEM plus 10% calf serum, pH 7.3, supplemented with 0.01 U/ml of insulin to prevent a possible antipolio activity of actinomycin D[9]. Poliovirus type 1 (Brunhenders) was used throughout.

Completely confluent cell monolayers in small petri dishes (10^6 cells) were infected with 1 ml of viral suspension (30 plaque forming units – PFU/cell in Hank's BSS) at 4 °C for 1 h, washed 3 times, supplemented with Hank's BSS (3 ml, pH 7.3) and incubated at different temperatures, in water bath. At prefixed time intervals, the entire cultures were frozen and thawed 3 times, the debris removed by centrifugation (5000 × *g*, 5 min) and PFU titrated according to the Dulbecco technique[10]. For the evaluation of the overall viral RNA synthesis, cell monolayers, infected as above, were added with Hank's BSS containing actinomycin D (2 μg/ml) and H^3 Uridine (1 μC/ml) and incubated either at 37 °C or at 41.5 °C (for more details see results). At various time intervals cells were scraped from the glass with rubber policeman, washed 3 times in cold Hank's BSS supplemented with unlabelled uridine (50 μg/ml), precipitated twice at 4 °C in trichloroacetic acid (5% in H_2O) and dried at 37 °C for 20 h. Precipitates were dissolved in Soluene and radioactivity was determined in a Packard scintillation counter (scintillation fluid: 7 g PPO, 0.6 g dimethyl POPOP, 1000 ml Toluene).

RNAase sensitivity of viral RNA was determined in phenol extracts obtained with the technique of Gierer and Schramm[11] modified by Muntoni et al.[12] from cells

1 Work supported by a Grant of Consiglio Nazionale delle Ricerche, Rome.

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