the ultrastructural studies, the embryo and the mycetomes of various stages were removed from the eggs and prepared for electron microscopy[8].

Results and discussion. During embryogenesis of the host, the paired symbiont organs develop in its abdomen. Following the basic investigations of the embryonic development of *Euscelis plebejus* by SANDER[6,9], the single steps between the infection mass of the symbionts and the mature mycetome could be studied[7]. The three main results of the above studies can be defined as follows: 1. Both types of symbionts are incorporated at specific times by distinct cells of the embryo. 2. Translocation experiments of the symbiont mass within the egg suggested that other cell types are not capable of incorporating the symbionts[10]. 3. When the symbionts were experimentally eliminated from the egg, embryogenesis as well as development of the (symbiont free) mycetome proceeded just as in the control eggs[11].

Electron microscopic examinations of the two symbionts during various stages of mycetome development show that each of both symbionts occurs in two forms which differ in their morphology. One of these forms, which appears during the entrance of the symbiont into the prospective mycetocytes, is called the 'infection form'[8]. The second form, called the 'vegetative form', is present during the remaining time of the host's embryogenesis.

The infection form of the *a*-symbiont often shows binary fission stages and appears extremely electron-dense. In contrast, the vegetative form of the *a*-symbiont exhibits lower electron density and is probably unable to divide. Enzymatic digestion experiments and the large number of ribosomes suggest that the infection form of the *a*-symbiont has an increased protein synthesis[8].

The corresponding forms of the *t*-symbiont, which show only slight variation in their electron density, can easily be distinguished by their characteristic morphology: the infection form of the *t*-symbiont appears more spherical, whereas the vegetative form has lobed contours[12].

BUCHNER[1] named the *a*- and *t*-symbionts which enter into the ovaries, 'transmission form' or 'infection form'. Infection forms and vegetative forms of the adult mycetome of the female correspond in their morphology to the infection forms and the vegetative forms in the embryonic mycetome. Thus, *a*- and *t*-symbionts develop a specific infection form, which serves not only for the transmission to the following host generations but also for invasion of the prospective mycetocytes of the embryonic mycetome. In analogy to a parasite-host-cycle, a simplified symbiont-host-cycle can be postulated in which a specific form of the symbiont is correlated with a certain developmental stage of the mycetome and of the host (Figure).

A similar pleomorphism to that mentioned above, has been described for numerous intracellular symbiotic bacteria. The symbionts of coleopterous families such as Nosodendridae, Chrysomelidae, Curculionidae, Silvanidae, Lyctidae, as well as of the Trypetidae (Diptera) for instance, develop an infection form designed to continue the symbiotic relationship[13]. In these groups investigations concerned with the behaviour of the symbionts during embryogenesis of the host are very rare up to now.

A remarkable feature of the *a*- and *t*-symbiont of *Euscelis plebejus* is that during the ovarial transmission, as well as during the development of the mycetome, the symbiotic microorganisms pass an extracellular stage before they are incorporated de novo by specialized host cells.

[8] H. K. KÖRNER, Z. Parasitenk. *40*, 203 (1972).
[9] K. SANDER, W.Roux' Arch. EntwMech. Org. *151*, 660 (1960).
[10] K. SANDER, Devel. Biol. *17*, 16 (1968).
[11] H. K. KÖRNER, Experientia *25*, 767 (1969).
[12] H. K. KÖRNER, Z. Parasitenk. *44*, 149 (1974).
[13] P. BUCHNER, *Endosymbiosis of Animals with Plant Microorganisms* (Wiley, New York 1965), p. 909.

In vitro Attachment of Trypanosomes to Plastic

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Summary. Description of an in vitro system for the study of the attachment of trypanosomes to polystyrene flasks by means of hemidesmosomes. This type of attachment, whose significance is so far unknown, reproduces a natural stage in the life cycle of medically important trypanosomatid flagellates in their vector.

Recently, several workers have studied the ultrastructure of trypanosomatid flagellates during their development in their insect hosts. The results so far have shown that all members of the Family Trypanosomatidae (of which many are of medical or veterinary importance) present developmental stages ('haptomonads') which are attached by their flagellar tips ot the cuticular lining of the gut wall by means of 'hemidesmosomes'. This general pattern has been found so far in the *Trypanosoma* subgenera *Herpetosoma*[2], *Duttonella*[3], *Trypanozoon*[4], *Megatrypanum*[5], in the insect flagellate genera *Crithidia*[6] and *Herpetomonas*[7] and in the genus *Leishmania*[8].

The molecular basis of the attachment, as well as the role played by such a mechanism in the life cycle of these parasites is still unknown, but these results suggest that attachment is an indispensable step for the establishment of infection and subsequent transmission. This communication reports the development of an in vitro model of the haptomonad attachment.

Material and methods. The in vitro culture of trypanosomes was carried out as previously described[9] in a 25 cm² Falcon flask in the presence of BHK-cells, an overlay of R.P.M.I. 1640 and 10% foetal bovine serum.

[1] We wish to thank Professor W. PETERS and Dr. D. H. MOLYNEUX for their advice and encouragement.
[2] D. H. MOLYNEUX, Parasitology *59*, 55 (1969).
[3] K. VICKERMAN, J. Protozool. *20*, 394 (1973).
[4] R. F. STEIGER, Acta tropica *30*, 64 (1973).
[5] D. H. MOLYNEUX, Acta tropica *32*, 65 (1975).
[6] B. E. BROOKER, Protoplasma *73*, 191 (1971).
[7] R. BRUN, Acta tropica *31*, 219 (1974).
[8] R. KILLICK-KENDRICK, D. H. MOLYNEUX and R. W. ASHFORD, Proc. R. Soc. Lond., B *187*, 409 (1974).
[9] M. HOMMEL, Trans. R. Soc. trop. Med. Hyg. *69*, 3 (1975).

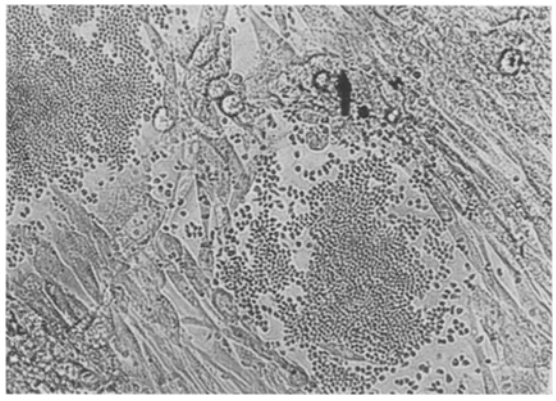

Fig. 1. Culture of *Trypanosoma blanchardi* in the presence of BHK-cells. (Living material observed on Zeiss inverted microscope) × 160.

As trypanosomes are well known to be affected by a change of pH[10], we limited these changes by use of a zwitterionic buffer (25 m*M* HEPES). The culture was kept at 26 °C in the same flask, in which half the medium was changed at intervals of 2 or 3 days (according to the relative acidification indicated by the change in colour of the phenol red in the medium.)

A mouse-adapted strain of *Trypanosoma blanchardi*[11] was used in this experiment. The monolayer was processed in situ for electron-microscopy by the routine glutaraldehyde-osmium fixation[5] and embedded in Araldite. After hardening, 3 mm square pieces were cut out and re-embedded vertically including the plastic[12]. Sections were cut on an LKB ultratome MK III, stained with uranyl acetate and lead citrate and examined on an AEK EM6B electron microscope. (The holes in the polystyrene are artefacts of embedding.)

Results and discussion. After 2 weeks in culture the trypanosomes attach to the bottom of the culture flasks, and start dividing, forming 'plaques' of numerous attached haptomonads in spaces between cells (Figure 1). These plaques progressively colonise the flask, and after 2–3 months form a monolayer of attached trypanosomes. On Giemsa-stained preparations the parasites look like 'extracellular amastigotes'. The study of the ultrastructure of haptomonads in situ (Figures 2 and 3), shows that they are attached by their flagellar tips to the plastic surface by means of hemidesmosomes. The expanded flagellar membrane comes in close contact with the bottom of the flask, with a distance of 20–30 Å between the plastic and the outer leaflet of the plasma membrane. Lying beneath the inner leaflet is an electron dense area 300–400 Å thick, from which a large number of filaments appear to arise, converging into a bundle towards an area situated near the end of the axoneme *para*-axial rod complex. From the junctional area emerge a number of pinocytic vesicles of various sizes. The BHK-cells did not show any attachment by hemidesmosomes.

In vitro attachment of flagellates to each other or to cellular debris had been observed by early workers. Brooker[13], for example, by ultrastructural studies proved that the attachment of *Crithidia fasciculata* to inert substrates (Millipore filters) was produced through a hemidesmosomal junction and discussed the non-specificity of the mechanism. However, in our system a consistant 'attachment' could only be obtained with a few species of Herpetosoma[9,11], while *T. brucei*, *L. mexicana mexicana*, *Herpetosomas* sp. and even *T. lewisi* never showed, in our hands, any sign of attachment, although all have been found to be able to produce a hemidesmosomal attachment with the cuticle of their respective invertebrate host.

[10] G. A. M. Cross and T. C. Manning, Parasitology *67*, 315 (1973).
[11] M. Hommel and F. Miltgen, Protistologica *10*, 17 (1974).
[12] B. K. Nelson and B. A. Flaxman, Stain Techn. *47*, 261 (1972).
[13] B. E. Brooker, Protoplasma *72*, 19 (1971).

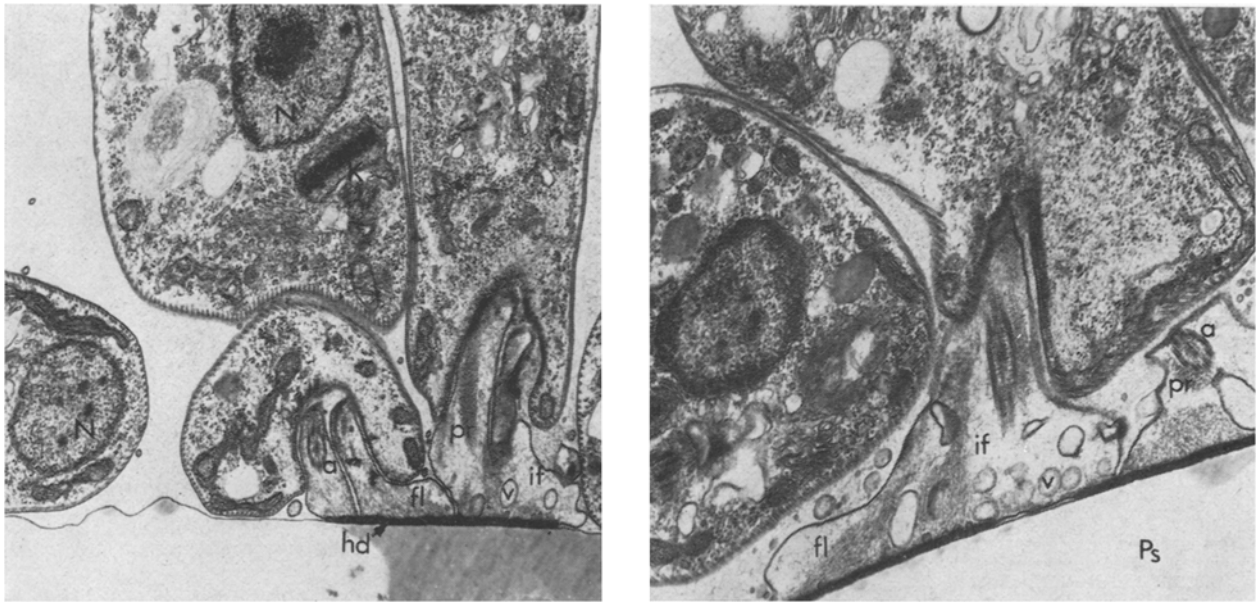

Fig. 2 and 3. Ultrastructure of haptomonads attached to polystyrene flasks. (Fig. 2 × 15,000; Fig. 3 × 20,000). Abbrevations: N, nucleus; K, kinetoplast; a, axoneme; pr, *para*-axial rod; Ps, plastic surface; hd, hemidesmosomes; if, enlarged intraflagellar space; v, vesicles; fl, filaments in relation with the structure of the hemidesmosomal junction.

In our model, we believe that anchorage is an important step for in vitro growth of trypanosomes of the subgenus *Herpetosoma* for the following reasons: a) Division only starts after 'dedifferentiation' of adult bloodstream trypomastigotes into attached epimastigotes; b) by their solid attachment the haptomonads maintain the infection in the flasks throughout the subsequent changes of medium; c) dividing haptomonads, while invading progressively the bottom of the culture flask, release in the medium intermediate forms which rapidly transform into trypomastigotes or small 'metatrypomastigotes'; d) haptomonads seem to be able to resist better than 'free' trypanosomes to variations of pH (the zwitterionic buffer indispensable for the development of attachment, can be left out afterwards).

These observations suggest that further studies with this model on the mechanism of attachment might enable us to understand the significance of this particular type of differentiation in the life cycle of Trypanosomatidae.

Nematicidal Activity of Secondary and Tertiary Alkyl Amides and Amines

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Summary. Several C_{11} to C_{15} amides and amines that disrupt growth in certain insects showed high nematicidal activity in direct contact tests. Two amides and 9 amines killed *Panagrellus* at 5–10 ppm. Of these, 1 amide and 3 amines killed *Meloidogyne* larvae at 20 ppm.

Certain insect hormonal chemicals affect growth, development, and molting in nematodes[3], and extracts from the nematode *Haemonchus contortus* exhibit juvenile and molting hormone activity in insects[4]. These results, plus the recently reported isolation from a parasitic nematode of 20-hydroxyecdysone[5], a major insect steroid molting hormone, suggest that the hormonal control of development and molting in insects and nematodes may be quite similar. For this reason, a number of secondary and tertiary straight and branched chain amides and amines that disrupt the hormone regulated processes of development, molting, and metamorphosis, and block sterol metabolism in certain insects[6] were tested against nematodes. We now report high nematicidal activity for a group of C_{11} to C_{15} alkyl amides and amines in tests against 2 species of nematodes.

Panagrellus redivivus, a saprophytic nematode and a sensitive indicator of nematicidal activity, was exposed for 48 h in water-quartz sand-candidate toxicant mixtures in the standard direct contact test[7] in a range of concentrations for each compound. The compounds were solubilized in a solvent-surfactant-water medium that is non-toxic to nematodes. Approximately 400 nematodes, in all developmental stages, were exposed in each test. Effects were determined during the day immediately after exposure by microscopic examinations[8]. Normal unstressed *Panagrellus* are in continuous rapid motion, and the esophageal areas are hyaline. Exposure to nematicides results in reduced motility, immotility, and death, and esophageal structures in moribund and dead nematodes show disintegration and darkening. Under these test conditions, the LD_{95} for DD (1:1 mixture of 1,2-dichloropropene and 1,3-dichloropropane and related C_3 chlorinated hydrocarbons), a standard commercial nematicide, is 36 ppm, and 40 ppm is lethal.

The results of *Panagrellus* exposures to the amides and amines are presented in Tables I and II and are averages of 4 replications. The most active compounds were the straight chain amides and amines, though fewer of the amides were as active as the amines. Of the amides listed in Table I, the dimethyl amides II and III that have a continuous chain length of 11 and 12 carbons, respectively, were the most active and were lethal at concentrations of 5 to 10 ppm. The other amides were active at 20 to 40 ppm, concentrations which approximate the activity of the nematicide standard used in this study.

Most of the amines listed in Table II were active against *Panagrellus* at 5 to 10 ppm. Of the saturated amines, only compounds XII and XVI required higher concentrations to kill 100% of the test nematodes. The 2 unsaturated amines, N,N-dimethyl- and monoethyl-10-undecenamine, XX and XXI, respectively, were active at 10 to 20 ppm. Certain branched chain amides and amines, such as the N,N-dimethyl-3,7,11-trimethyldodecanamine, were also active against *Panagrellus*.

Table I. Range of concentrations of N-substituted amides required to kill 100% of exposed *Panagrellus redivivus* populations in direct contact tests

Compound		Concentration (ppm)
I	$CH_3(CH_2)_9CON(CH_3)_2$[a]	20–40
II	$CH_3(CH_2)_{10}CON(CH_3)_2$[a]	5–10
III	$CH_3(CH_2)_{11}CON(CH_3)_2$[a]	5–10
IV	$CH_3(CH_2)_{13}CON(CH_3)_2$	20–40
V	$CH_3(CH_2)_9CON(CH_3)C_2H_5$	20–40
VI	$CH_3(CH_2)_{10}CON(CH_3)C_2H_5$	20–40
VII	$CH_3(CH_2)_{11}CON(CH_3)C_2H_5$	20–40

[a] The corresponding mono-N-ethyl amide derivative was not homogeneously dispersed in our test system.

[1] Nematology Laboratory.
[2] Insect Physiology Laboratory.
[3] R. N. JOHNSON and D. R. VIGLIERCHIO, Expl. Parasit. *27*, 301 (1970). – C. S. SHANTA and E. MEEROVITCH, Can. J. Zool. *48*, 617 (1970). – K. G. DAVEY, Int. J. Parasit. *1*, 61 (1971).
[4] W. P. ROGER, Parasitology *67*, 105 (1973).
[5] D. H. S. HORN, J. S. WILKIE and J. A. THOMSON, Experientia *30*, 1109 (1974).
[6] W. E. ROBBINS, M. J. THOMPSON, J. A. SVOBODA, T. J. SHORTINO, C. F. COHEN, S. R. DUTKY and O. J. DUNCAN, III, Lipids, *10*, 353 (1975).
[7] A. L. TAYLOR, J. FELDMESSER and W. A. FEDER, Plant Dis. Reptr. *41*, 527 (1957).
[8] J. FELDMESSER and W. A. FEDER, Proc. Soil Sci. Soc. Fla. *14*, 154 (1954).