*E. coli*-RNA could be detected only if the nuclei were not washed before the transcription assay. Otherwise hybridization with *E. coli*-DNA was negligible, as in the controls not treated with *E. coli*-DNA. This result is consistent with a transcription on the surface of the nuclei or in the surrounding medium. Several repetitions gave the same results.

The control experiments permit us to exclude the participation of bacteria. The bacterial DNA was purified in a CsCl density gradient. Therefore its contamination with bacterial RNA-polymerases seems highly improbable. Additionally the transcription experiments were performed in the presence of rifamycin, a potent inhibitor of bacterial RNA-polymerases. Rifamycin was without any effect on the synthesis of bacterial RNA (figure 2), thus indicating that only the *Petunia*-RNA-polymerases were at work. Similar experiments were performed with Col $E_1$-DNA. The RNA formed was hybridized with single stranded *Petunia*-DNA and Col $E_1$-DNA[12]. The *Petunia*-DNA was transcribed as before. A transcription of the plasmid DNA, however, could not be detected.

The experiments demonstrate that plant polymerases are capable of transcribing bacterial linear DNA. In the case of supercoiled DNA, however, difficulties arise. There are several possibilities to account for the above results: either the plant RNA-polymerases are unable to recognize the initiation sites in the Col $E_1$-DNA, or the differences are part of the conformation of DNA (supercoiled, complete native Col $E_1$-DNA – linear, conditional native *E. coli*-DNA). Further experiments to clarify these questions are in progress. Anyway, plant nuclei prepared from protoplasts offer a simple system for testing the efficacy of plant RNA-polymerases.

12 W. Goebel and W. Schiess, Molec. gen. Genet. *138*, 213 (1975).

## Uptake of ferritin from the medium by *Tokophrya infusionum*[1]

Maria A. Rudzinska[2]

*The Rockefeller University, 1230 York Avenue, New York (New York 10021, USA), 24 May 1977*

*Summary. Tokophrya infusionum*, a suctorian, is deprived of a mouth opening. The uptake of ferritin from the medium is accomplished through pits, invaginations of the plasma membrane which are permanent structures. From the pits containing ferritin, flat vesicles are pinched off transporting the ferritin to the cytoplasm.

*Tokophrya*, as all Suctoria, does not have a cytostome and its feeding apparatus consists of numerous tentacles, long slender tubes extending to the outside of the body and also deep into the cytoplasm[3–5]. At the distal end, the tentacle terminates into a rounded knob covered by the plasma membrane[3]; inside the cytoplasm the proximal end of the tentacle remains open[5]. Each of the tentacles is capable of changing into a mouth when a living ciliate gets attached to the knob of one or more tentacles[4]. The attachment is followed by the rupture of the plasma membrane of the prey which merges with the membrane covering the tentacle and the 2 organisms become united by a continuous common membrane enveloping prey and predator[6]. Immediately thereafter the cytoplasm of the still living prey begins to stream down through the attached tentacle into the predator where it become enclosed into food vacuoles[4]. It was assumed that this way of feeding supplies the predator with all the necessary food and that the medium plays a negligible role as a source of nutrients. However, observations indicated that also the medium influences the life span, the reproduction rate and the survival of clones. Although cultures of *Tokophrya* could grow for some time in a well balanced inorganic medium, or even in distilled water, the conditions for longevity, multiplication and duration of clones improved greatly in a medium containing yeast extract. Apparently for normal growth *Tokophrya* needs some supplements deriving from the medium in addition to the cytoplasm of the prey. A diluted yeast medium has been routinely used for the maintenance of cultures[7]. The mechanism by which nutrients and particularly large molecules enter from the medium to the body of *Tokophrya* was so far unknown and to solve this problem a thorough study of structures covering the organism and experiments with tracers were necessary. The following is an account of such a study.

*Tokophrya* is covered by 3 unit membranes (figure 2, insert) and beneath them lies the epiplasm, a dense homogeneous layer about 70 nm thick (figure 2). To the outside of the external membrane *Tokophrya* is surrounded by an amorphous coat of low density about 300 nm thick (figures 1 and 2). It seemed therefore that the organism is well shielded and as if isolated from the surrounding medium. A more detailed analysis disclosed however the existance of gaps in some of the membranes and layers. Only the external membrane is continuous and at uneven intervals dips down into the cytoplasm (figures 1 and 2) forming small saccules, so-called 'pits'[6,8]. The pits disrupt the 2 other unit membranes and the dense epiplasm. The pits are about 390 nm deep and their opening is over 100 nm in diameter; the part of the pit located in the cytoplasm is covered by a single membrane (figure 2) and it is about 250 nm wide, bulbous in shape but often irregular forming outpocketings.

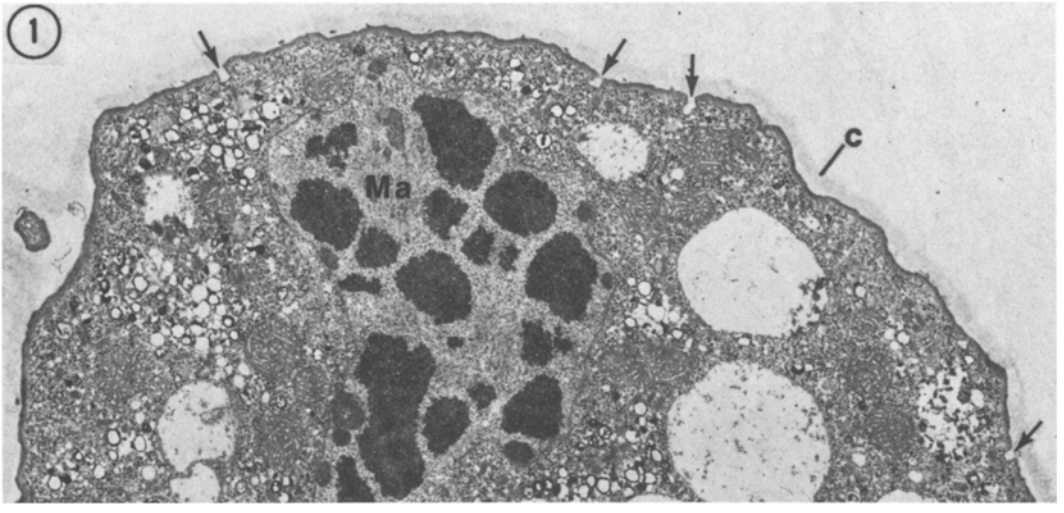
The role and function of the pits has been and still is of great interest. They are found throughout the Subphylum Ciliophora to which Suctoria belong[9]. Experiments with tracers performed on ciliates[10] could not give conclusive results because these organisms possess an oral cavity

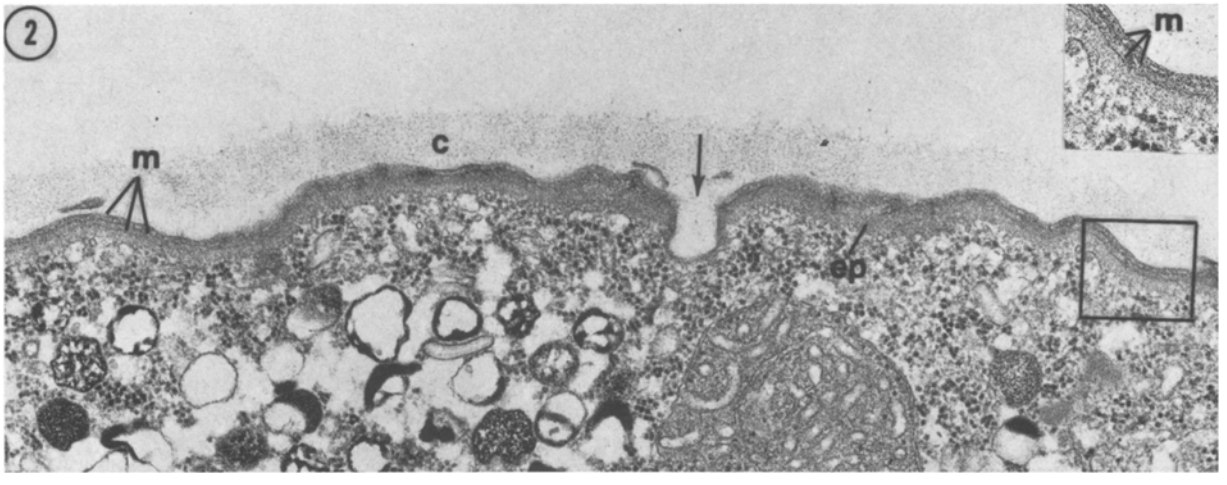
1 Supported by grant AI-08989 US Public Health Service.
2 The author acknowledges gratefully the excellent technical assistance of Mrs Sondra Lewengrub.
3 M. A. Rudzinska, J. Cell. Biol. *25*, 459 (1965).
4 M. A. Rudzinska, Biol. Sci. *23*, 87 (1973).
5 M. A. Rudzinska, J. Protozool. *21*, 721 (1974).
6 M. A. Rudzinska, Trans. N.Y. Acad. Sci., series II, *29*, 512 (1967).
7 M. A. Rudzinska, J. biophys. biochem. Cytol. *4*, 195 (1958).
8 L. L. Millecchia and M. A. Rudzinska, J. Protozool. *19*, 473 (1972).
9 Committee on Taxonomy and Taxonomic Problems, Society of Protozoologists, J. Protozool. *11*, 7 (1964).
10 C. Noirot-Timothee, C. r. Acad. Sci. Paris *267*, 2334 (1968).

which is active in engulfing the surrounding medium. Therefore, tracers can reach the cytoplasm through the mouth opening. To find out about the function of the pits only organisms deprived of a cytostome could be successfully used. Suctoria seemed to be ideal for this purpose. They do not have an oral cavity and whatever enters their cytoplasm from the medium has to pass through the sheaths covering the body. The membranes are apparently not a barrier for small molecules in ciliates according to the work on *Tetrahymena*[11]. It has to be stressed that ciliates are also covered by 3 membranes like *Tokophrya*. The problem becomes however crucial in regard to macromolecules. The pits seemed to be the most likely channels for their uptake.

To check on this assumption the tracer ferritin was used. Ferritin, a large molecule (7–10 nm in diameter) of great density, is easy to identify in electron microscopy. Cationized ferritin (Research Div., Miles Lab., Kankakee, 111. 60901) prepared according to Danon et al.[12] was added to cultures of *Tokophrya* starved for 24–48 h and incubated at room temperature for 1–16 h. In some experiments the organisms were exposed to 0.1–0.5% trypsin prior to incubation in ferritin to facilitate the penetration of ferritin through the external coat. The cultures were fixed and processed for electron microscopy as described in previous papers[5].

11 L. Rasmussen, Carlsberg Res. Commun. *41*, 144 (1976).
12 D. Danon, L. Goldstein, Y. Marikovsky and E. Skutelsky, J. Ultrastruct. Res. *38*, 500 (1972).




Figures 1 and 2. Electron micrographs of untreated *Tokophrya*. Fig. 1. Low magnification of a section of *Tokophrya* to show the coat (c) and the uneven distribution of the pits (arrows). Ma, macronucleus. ×8800. Fig. 2. Section through the periphery of *Tokophrya* to show the 3 unit membranes (m) covering the organism. A higher magnification of the 3 membranes (m) is seen in the insert at the upper right. The figure also shows a pit (arrow) surrounded only by the most external membrane; the epiplasm (ep), a dense layer adjacent closely to the most internal of the 3 membranes; and the coat (c) located at the outside of the cell. ×37,500; insert, ×60,000.

Figures 3–6. Electron micrographs of *Tokophrya* treated with cationized ferritin. Fig. 3. *Tokophrya* incubated for 4 h in ferritin. In addition to the coat (c) the organism is surrounded by fringes (fr) visualized by ferritin. However, no ferritin is present in the coat (c) nor close to the outer membrane except for one small area indicated by f. Several pits (arrows) are distributed at random and in one of them (long arrow) ferritin can be detected. ×12,250. Fig. 4. Section through *Tokophrya* incubated in ferritin for 16 h. The coat (c) is slightly peppered with ferritin and along the outer membrane ferritin (f) is present only in some areas; one of the 2 pits (long arrow) contains ferritin (f); fringes are seen at fr. ×37,500.

Fig. 5. Electron micrograph of organism treated with 0.1% trypsin before incubation for 3 h in ferritin. Most of the vesicles in this pile of flat vesicles (pfv) contain ferritin (f). ×47,000. →

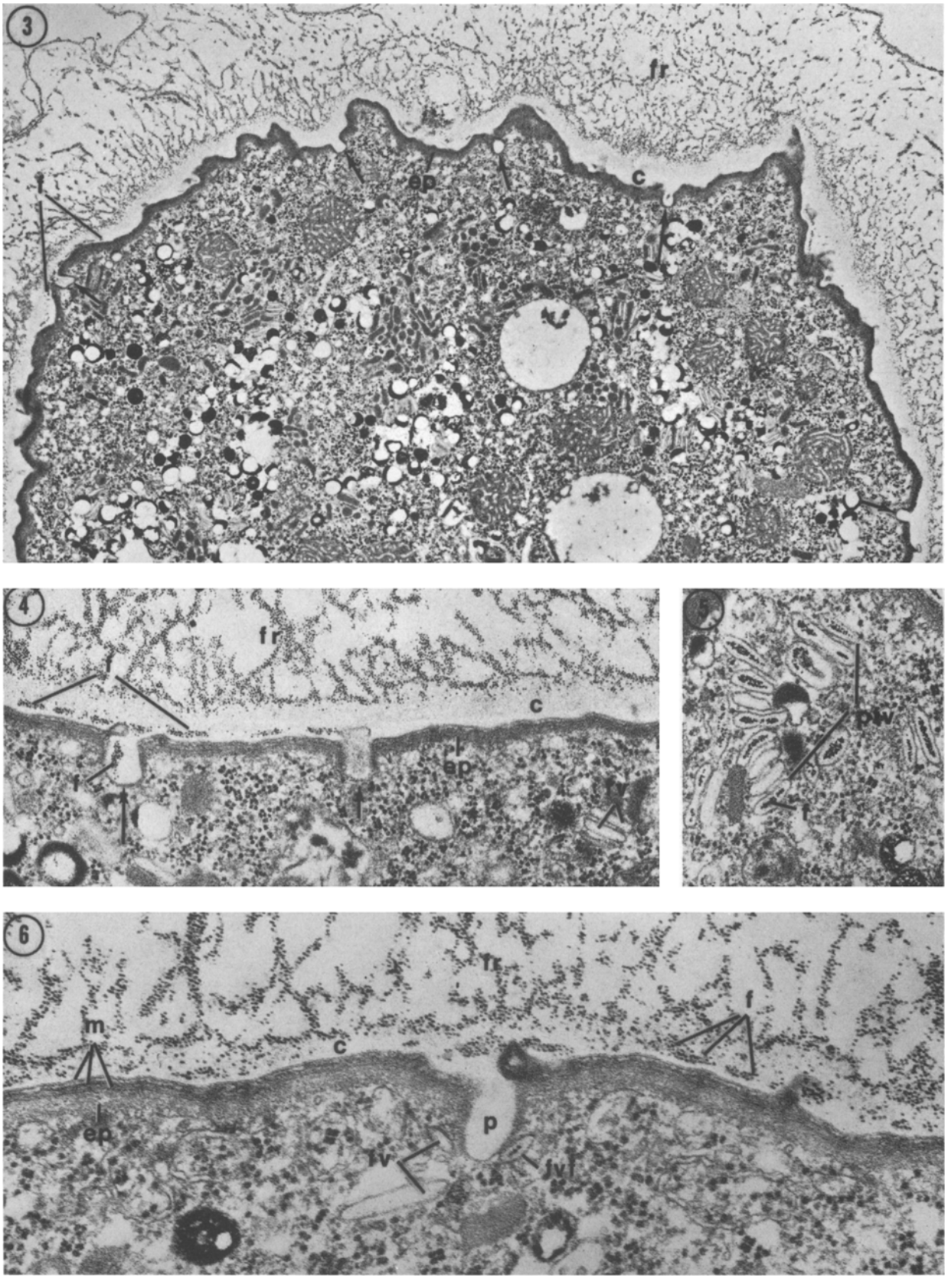



Fig. 6. Section through organism treated in the same way as in figure 5. Closely adjacent to the pit (p) is a flat vesicle with a few grains of ferritin (fvf). The vesicle looks like in the last stage of pinching off from the pit. 2 other flat vesicles (fv) are nearby the pit. Ferritin granules (f) are present in the fringes (fr), in the coat (c) and along the outer membrane. The 3 unit membranes are clearly seen at m. ×63,000.

In all experiments ferritin was found around *Tokophrya* in long fringes (figures 3, 4 and 6) extending above the coat, and also close to the external membrane (figures 4 and 6), as well as in the pits (figure 4) and what is most important inside the organism in flat vesicles measuring 370 × 40 nm (figure 5). The flat vesicles are a structure characteristic of the cytoplasm of *Tokophrya*. They are often assembled in groups of 3–5 (figure 4) or piled up in stacks up to 17 vesicles (figure 5). In previous papers this structure was referred to as a possible primitive Golgi apparatus[5].

No ferritin was ever encountered free in the cytoplasm, or in the epiplasm, or between the membranes covering *Tokophrya*. The tracer was present only in the flattened vesicles, scattered throughout the cytoplasm and often near the pits (figure 6) or sometimes very close to them as if in the process of pinching off (figure 6) strongly suggesting that they derive from the pits. It is assumed that once a vesicle becomes detached from the pit it is carried away and if more are pinched off in succession they remain together forming piles of vesicles.

Since the pits are the only places on the surface of *Tokophrya* covered by a single membrane they are the natural loci for the uptake of ferritin from the medium. When the tracer enters a pit it apparently induces pinocytosis resulting in the formation of the flat vesicles. Thus the pits in conjunction with the flat vesicles are the vehicles transporting ferritin and probably other macromolecules from the medium to the cytoplasm of *Tokophrya*. Both structures perform an important function in Suctoria, organisms deprived of an oral cavity.

## Juvenile hormone in larval diapause of the codling moth, *Laspeyresia pomonella* L. (Lepidopterae, Tortricidae)

R. Sieber and G. Benz

*Department of Entomology, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich (Switzerland), 3 August 1977*

*Summary.* Whereas in last instar larvae of *L. pomonella* kept under long-day-conditions (LD), the JH-titer is temporarily reduced to zero, it stays relatively high in short-day-conditioned (SD) larvae which enter diapause. Application of JH or a juvenoid to LD-larvae results in diapause, if the treated insects are kept under SD-conditions. From these results it is concluded that in *L. pomonella* diapause is initiated by a relatively high titer of JH during the last larval instar.

When larvae of our laboratory strain of the codling moth, *Laspeyresia pomonella*, are reared under short-day-conditions (SD), i.e. with a photophase of less than 14 h per day, they enter diapause. Under long-day-conditions (LD), i.e. with a photophase of more than 16 h per day, the larvae will pupate and produce adults. In several species of Lepidoptera, it has been demonstrated that larval diapause is caused by a high titer of juvenile hormone (JH). In these cases application of high concentrations of JH mimetica not only induced but also maintained larval diapause, even under LD-conditions[1,2]. Besides this proved mechanism, diapause in other species might be caused by a lack of the moulting hormone, as in pupal diapause, which may be broken by injection of ecdysone[3,4]. The object of the present study was to investigate the relationship between JH and the diapause of the codling moth. Therefore the JH titer during the last instar of LD- and SD-conditioned larvae as well as in diapausing insects was determined. Furthermore the influence of topically applied JH was studied in the last instar of LD-conditioned larvae kept under LD- or SD-conditions after the application.

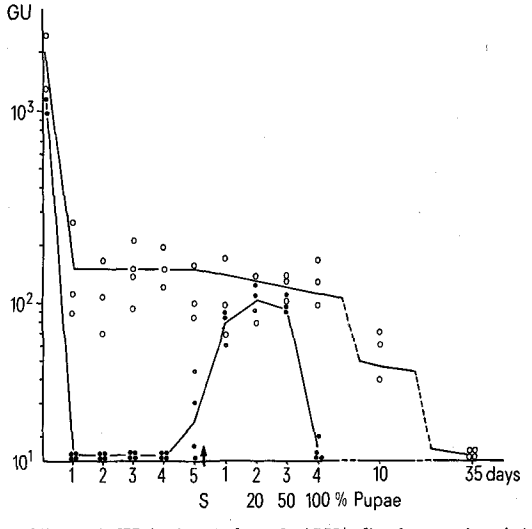



Fig. 1. Titer of JH in haemolymph (GU/ml) of pupating (●) and diapausing (○) *L. pomonella* at different times after last larval moult and spinning of cocoon (S).

*Material and methods.* The larvae of the codling moth were reared individually at 26 °C on a semisynthetic medium in small plastic boxes[5]; 5 days after the last larval moult, the boxes were opened to allow the larvae to spin their cocoons. Under LD-conditions (continuous light) the larvae pupated 2–4 days later. SD conditions resulting in 100% diapausing larvae were 10 h light to 14 h dark.

The titer of JH in the haemolymph of the codling moth (indicated as Galleria units = GU) was determined by a slightly modified Galleria-Wax Test[6,7]; 100–200 µl haemolymph of 5–12 larvae of a given age were collected and extracted by a 6:1 mixture of ethylacetate and

1 S. Yagi and M. Fukaya, Appl. Ent. Zool. *9*, 247 (1974).
2 C. M. Yin and G. M. Chippendale, J. Insect Physiol. *19*, 2403 (1973).
3 C. Hsiao and T. H. Hsiao, Life Sci. *8*, 767 (1969).
4 D. Gibbs, J. Insect Physiol. *22*, 1195 (1976).
5 J. Huber, G. Benz and K. Schmid, Experientia *28*, 1260 (1972).
6 H. A. Schneidermann, A. Krishnakumaran, V. G. Kulkarni and L. Friedman, J. Insect Physiol. *11*, 1641 (1965).
7 J. De Wilde, G. B. Staal, C. A. D. De Kort, A. De Loof and G. Baard, Proc. K. ned. Akad. Wet. Sec. C *71*, 321 (1968).