retention characteristics on both Carbowax 20M and SE 30 SCOT GC columns was *(E,E)*-10,12-hexadecadienal (figure). The presence of an *E,E* conjugated diene system was also indicated by the observed reaction of the natural pheromone component with TCNE, since, on similar treatment of synthetic hexadecadienals, the *E,E* isomers reacted but the *Z,E*, *E,Z* and *Z,Z* isomers were unchanged[8,10].

Synthetic *(E,E)*-10,12-hexadecadienal elicited an EAG response from the male moth comparable to that to the natural pheromone component, and it has been shown to attract male *E. insulana* moths to traps in the field[15,16]. *(E,E)*-10,12-Hexadecadienal has not been found previously as a moth sex pheromone component, although the corresponding alcohol and the *E,Z* isomer of the aldehyde have recently been detected in extracts of the pheromone glands of female silkworm moths, *Bombyx mori*[17].

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Methanic fermentation in the digestive tract of a xylophagous insect: *Oryctes nasicornis* L. larva (Coleoptera; Scarabaeidae)

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Summary. Strict anaerobic conditions and the production of methane have been demonstrated in the proctodeum in larvae of *Oryctes nasicornis* L., a xylophagous coleopteran. In ruminants, the breakdown of cellulose by extracellular symbiotic organisms is complete and leads to the formation of by-products which may act as substrates for methanogenic bacteria.

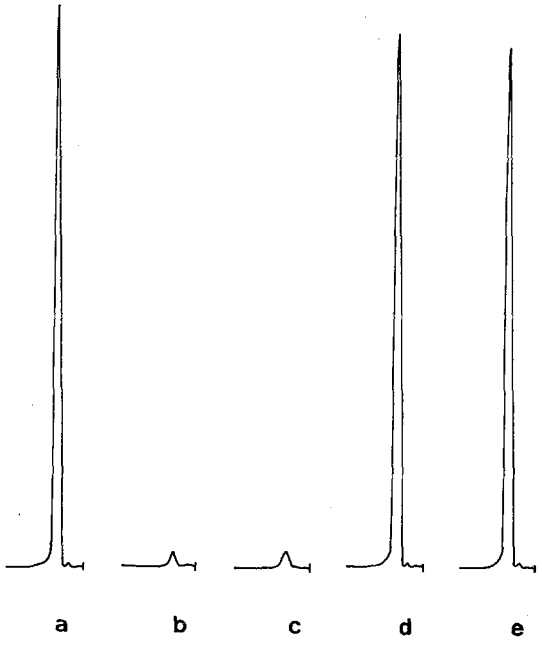
Recently there has been a large amount of research on methanogenic bacteria because of their economic importance and also because of their very characteristic metabolism[1]. They are found in anaerobic conditions in the soil and in the flora of the gastrointestinal tract of herbivorous mammals, where they are capable of producing methane. Coleoptera Scarabaeidae larvae, which feed on lignin and cellulose litter, have a proctodeal dilatation inhabited by numerous bacteria. We demonstrate here that anaerobic conditions and production of methane occur in this particular intestinal segment of *Oryctes nasicornis* L. larvae.

Material and methods. The insects were reared in La Minière (Versailles INRA[2]). 30 larvae were fed on their natural food (decomposing sawdust) at 28 °C, the optimum growth temperature. The experiment was repeated using 30 larvae which had been fed on pure α-cellulose (Sigma) for 3 weeks.

The midgut and the hindgut were dissected and the electric potential of the contents was readily measured using a potentiometer with a platinum electrode and a fixed calomel reference electrode.

Methane was investigated by gas chromatography. The gas chromatography analyses were carried out using 2.5 m × 2.5 mm columns packed with porapak Q (80–100 mesh). The peaks were obtained using a Perkin-Elmer chromatograph (model 881) with a nitrogen flow of 25 ml/mn (at room temperature 25 °C, detector at 250 °C). These results were verified using a mass spectrometer CH_5 Varian M.A.T. connected with a chromatograph Girdel 3000. The column and the conditions were the same as above, but the carrier gas was helium (20 ml/mn). An attempt was made to detect methane in the midgut and proctodeal dilatation of the larvae. The study was made on 1 group of 15 larvae fed on their natural food and a 2nd group fed on pure α-cellulose. Samples were taken as follows: the digestive tract was rapidly exposed by dissection of the larvae and the mesenteron and the proctodeal dilatation was removed. Each was placed in a 15 ml air-tight bottle. With the aid of a needle pushed through the rubber stopper the intestinal segment was opened to liberate its contents and an aliquot of the atmosphere of the bottle was analyzed. The quantity of methane present was calculated from the peak obtained by gas chromatography read against a reference curve of different air and methane mixtures.

A series of experiments was conducted to detect any methane given off from a whole animal. 5 larvae, fed on sawdust, were placed in air-tight 500 ml bottles and kept



Gas chromatograms of: *a* Methane-air mixture (1700 nM of methane in a 500 ml air-tight bottle). *b* Ambient air. *c* Atmosphere of a 15 ml air-tight bottle containing 1 opened midgut. *d* Atmosphere of a 15 ml air-tight bottle containing 1 opened proctodeal dilation (animal fed on its natural food). *e* Atmosphere of a 15 ml air-tight bottle containing 1 opened proctodeal dilation (animal fed on *a*-cellulose for 3 weeks).

during 1 h at 28 °C. The experiment was repeated using 5 larvae which had been fed on pure *a*-cellulose. The enclosed atmosphere of the bottles was analyzed by gas chromatography.

Results and discussion. In this insect only the midgut and the proctodeum are well developed. The differences of electric potential of the midgut contents vary between +50 mV and −10 mV. The proctodeum contents vary between −40 mV and −100 mV. Thus the intestinal contents appear to be a reducing medium. In the mesenteron anaerobic conditions are partially facultative, but in the proctodeal dilation the reducing conditions are more intense and there is strict anaerobiosis: soluble organic materials may accumulate and methane may be formed.

No methane can be detected in the mesenteron (figure a–c). However, the chromatograms clearly demonstrate the presence of methane in the proctodeal dilatation (figure a, d and e). The contents of the proctodeum of an animal fed on sawdust weighed 2.4 g (wet wt) on average, and liberated 26.0 to 97.5 nM of methane (an average of 53.9 ± 3.5 nM). The contents from an animal fed on pure *a*-cellulose weighed 2.2 g (wet wt) on average and released 36.0–57.7 nM of methane (an average of 45.5 ± 3.7 nM). These figures are underestimates, as they do not include an unknown quantity of methane, which is dissolved in the complex intestinal contents.

Methane is given off from a whole animal. Animals fed on sawdust released between 308 and 372 nM/h in the enclosed atmosphere of the bottles; that is a rate of release of 34–41 nM $h^{-1}g^{-1}$ (living wt). Animals fed on pure *a*-cellulose released between 343 and 380 nM/h at a rate of release of 38–48 nM $h^{-1}g^{-1}$ (living wt). These results are in the same range as those obtained for the lower termite *Reticulitermes* and the xylophagous cockroach *Cryptocercus*[3]. In ruminants, whose reducing potential is particularly low (−350 mV[4]), the quantities of methane produced are 30 times higher (749.8 nM $h^{-1}g^{-1}$, Wolfe[5]).

These results confirm those which might be expected from studies of the oxidation-reduction potential: the rich bacterial flora present in the proctodeum of *Oryctes* included a population of strictly anaerobic methanogenic bacteria, which find the reducing conditions essential for their development. It is known that anaerobic fermentation of carbohydrates, particularly celluloses, in the soil produces methane. In this respect it is important to realize that the amounts of methane released from the proctodeum of animals fed on sawdust and from those fed on pure *a*-cellulose were not significantly different ($p < 0.1$, Student's t-test). Thus food composed solely of cellulose does not alter the equilibrium of the methanic fermentation: this confirms that cellulose digestion takes place in the digestive tract of the larvae. Digestion of cellulose may start in the midgut, where it is unlikely that cellulases from the animal itself will be active (Marcuzzi and Turchetto Lafisca[6] and personal observations), but where anaerobic conditions facilitate the processes of biodegradation. However, it is in the proctodeal dilatation that cellulose digestion is completed and that the formation of methane can begin.

The symbiosis between insects and extracellular microorganisms has been well studied in lower termites, which have in their proctodeal dilatation protozoan flagellates which degrade cellulose. In those insects there is production of volatile fatty acids[7] and of methane[3], as in ruminants. This functional symbiotic convergence seems to be common to xylophagous insects which have a proctodeal dilatation inhabited by a large bacterial flora. Kovoor demonstrated the presence of volatile fatty acids in higher termites[8]. Volatile fatty acids have also been found and quantified in *Oryctes* larvae (Bayon, in preparation); 3 times as much volatile fatty acid, was found in the midgut as in the proctodeum. Hydrogen and carbon dioxide have been demonstrated in ruminants. The reduction of carbon dioxide during methanic fermentations would lead to the release of methane.

At the present state of knowledge it is difficult to asses the exact role played by methane in the physiology of insect digestion. In ruminants gas is eructed, but the microorganisms in the rumen act as a source of protein for the host. In the insects, where methane is partially or totally released, bacteria do not appear to act as a source of proteins, since the enzyme-producing mesenteron is located before the proctodeal dilatation where the methanic fermentation occur.

It seems that in very different animals, that is in ruminant mammals and xylophagous insects, the breakdown of cellulose by extracellular symbiotic organisms is complete and leads to the formation of byproducts which may act as substrates for methanogenic bacteria.

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