pre- and postsynaptic events. Whether one substance can have multiple, operationally distinct actions as transmitter, modulator, or hormone, depending on engagement of specific receptors, remains to be determined. Our observations indicate only that one action of [Leu^5]enkephalin falls outside two other previously described functional classes of communication in nervous systems.

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21 October 1977; revised 11 January 1978

Cellulose Digestion in the Midgut of the Fungus-Growing Termite *Macrotermes natalensis*: The Role of Acquired Digestive Enzymes

Abstract. *The midguts of adult workers of the higher termite species* Macrotermes natalensis *contain the entire set of digestive enzymes required for the digestion of native cellulose. The C_x-cellulases and the β-glucosidases are produced, at least in part, by the termite's own midgut epithelium and salivary glands. The C_1-cellulases, on the other hand, are acquired by the termites when they feed on a fungus that grows in their nests. We propose that the involvement of acquired digestive enzymes could serve as the basis for a general strategy of resource utilization and further suggest that the acquisition of digestive enzymes may be a widespread phenomenon among mycophagous invertebrates.*

The nutritive regime of termites is based upon the exploitation of cellulosic materials. In the lower termites (*1*) the digestion of cellulose is brought about by flagellate protozoa resident in the paunch, an enlarged region of the gut posterior to the midgut (*2*). The higher termites lack this assemblage of xylophagous protozoa, and the mechanism by which ingested cellulose is degraded remains a matter of speculation. It has been suggested that, in some species, paunch bacteria have taken over the role of the protozoa, but evidence in support of this notion is meager (*3*). "Cellulases" have been reported from the guts of several higher termites, but in only one species has the full set of enzymes required to digest native cellulose been demonstrated (*4*). It is also possible that some species simply restrict their diets to wood that has already experienced extensive fungal decay.

Termites of the higher termite subfamily Macrotermitinae (*5*), common in Africa and Asia, have long intrigued biologists because of their symbiotic association with fungi that grow in their nests on structures referred to as "fungus combs." The combs, which are spongelike in appearance and corklike in texture, are derived from chewed but undigested plant fragments (*6–8*). The surface of the comb is covered with a sparse growth of mycelium and numerous small white spheres or nodules, 0.5 to 2 mm in diameter (see Fig. 1). These nodules, or synemata, are the conidia or conidiophores of a fungus, *Termitomyces* sp., believed to be restricted to Macrotermitinae nests (*9*). The mycelium is a mixture of *Termitomyces* sp. and various xylariaceous species (*10*).

The termites eat both the comb material (*11*) and the nodules (*12*). It is not known whether in nature they also eat the various cellulosic materials they collect (*6*), or whether these materials are all first incorporated into the fungus combs. The material from which the comb is constructed contains plant fragments with intact cells and cell walls, while extensive deterioration of cellular structure is evident in the contents of the midgut, paunch, and rectum (*8*), suggesting that the digestion of cell wall constituents occurs in the termite's gut. However, laboratory studies have shown that the termites starve if provided only with cellulosic materials, such as sound wood or filter paper, whereas they survive for extended periods of time if provided with fragments of fungus comb, complete with the nodules (*13, 14*).

Our investigation was directed at the question of how the fungus-growing termites digest cellulose and what the basis is for the dependence of the termites on their symbiotic fungal partner.

The enzymatic degradation of cellulose requires the concerted action of several enzymes, the C_1 enzymes (active against crystalline cellulose), the C_x enzymes (active against noncrystalline cellulose and soluble derivatives or degradation products of cellulose), and a β-glucosidase (active against cellobiose) (*15*). We have found that the entire set of enzymes required for cellulolysis is present in the midguts of adult *Macrotermes*

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natalensis workers (Table 1), thus implicating the midgut as a major site of cellulose digestion in this species. Although the enzymes are also present in the paunch and rectum, the activities there are much lower.

It is possible a priori to envision several plausible origins for the midgut cellulolytic enzymes. One obvious possibility is that they are secreted by the termite's midgut epithelium or salivary glands, and indeed we have found that homogenates of both of these tissues do exhibit significant C_x activity, and low but significant β-glucosidase activity (Table 1). However, the absence of any detectable C_1 activity in the midgut tissue, and its presence at levels of dubious significance in the salivary glands (Table 1), leaves unexplained the high levels of C_1 activity in the midgut. A second possibility is that enzymes produced by bacteria in the paunch are transferred to the midgut. However, the very low level of C_1 activity in the paunch and the fact that the enteric valve prevents the reflux of material from the paunch forward into the midgut (*16*) argue against this possibility. Finally, the very low level of C_1 activity in the rectum makes the acquisition of the midgut C_1 enzymes through coprophagy (*17*) highly doubtful.

These circumstances led us to consider the possibility that the termites might acquire their C_1 enzymes when they consume their fungus, and indeed we found that an extract of the fungus nodules, but not of the comb material, exhibits high C_1 activity in addition to high C_x activity and β-glucosidase activity (Table 1).

The likely identity of the midgut and nodule C_1 enzymes was indicated by a comparison of their isoelectric points (*p*I) (*18*). Both midgut and nodule extracts contain two C_1 enzymes. The major enzyme from both sources was distributed over several gel segments which encompassed a *p*H range of 3.60 to 4.05. The activity maximum for the major enzyme from both sources occurred in a gel segment encompassing a *p*H range of 3.90 to 4.05. The minor enzyme in both midgut and nodule homogenates has a *p*I in the range 4.20 to 4.35.

Further experiments confirmed our hypothesis that the fungus nodules are the source of the midgut C_1 enzymes. Newly moulted adult workers that had not yet fed (*19*) had only 14 percent of the C_1 activity of normal workers, while the C_x activity was 64 percent of the normal level, a result consistent with the suggestion that C_1 enzymes are acquired by feeding, whereas C_x enzymes are derived from both internal and external sources.

In another set of experiments, termites were prevented from consuming nodules, either by providing them with no

Table 1. Cellulolytic enzymes of *M. natalensis* workers and of their fungus gardens. Each value represents the mean ± the standard error for the number of determinations indicated in parentheses. A unit of activity [per termite or per milligram of fungus material (dry weight)] is the amount of enzyme required to liberate 1 μmole of reducing equivalents (for the C_1 and C_x enzymes) or *p*-nitrophenol (for the β-glucosidase) per minute under the conditions of the assay (*23, 24*).

Tissue	Units of activity ($\times 10^3$)		
	C_1*	C_x*	β-Glucosidase*
Midgut (tissue + contents)	7.6 ± 0.8 (7)	31.9 ± 2.7 (6)	23.1 ± 0.7 (3)
Paunch (tissue + contents)	0.6 ± 0.2 (5)	10.3 ± 1.4 (5)	0.6 ± 0.2 (3)
Rectum (tissue + contents)	0.8 ± 0.3 (3)	8.4 ± 2.8 (3)	
Midgut tissue	0.0 ± 0.0 (3)	5.5 ± 2.4 (3)	0.2 ± 0.05 (2)
Salivary glands	0.2 ± 0.1 (5)	12.0 ± 2.3 (7)	0.1 ± 0.05 (3)
Fungus nodules	8.8 ± 0.9 (4)	26.6 ± 7.6 (6)	3.1 ± 1.4 (2)
Comb material	0.1 ± 0.1 (3)	0.6 ± 0.5 (3)	

*0.1 to 0.2 $\times 10^{-3}$ unit of C_1 or C_x activity, and 0.01 to 0.02 $\times 10^{-3}$ unit of β-glucosidase activity would have been detected under the conditions of the assay.

Table 2. Midgut C_1 and C_x activities in *M. natalensis* workers maintained on deficient diets for 72 hours. The termites were kept at 22° to 26°C in plastic boxes containing moist soil from their nest. A normal diet consisted of fungus comb which had an abundant coverage of nodules. The starved group received nothing. Another group received barren comb from which the nodules had been removed, and another simply a collection of nodules. Nodules were consumed by those termites provided with them. At the end of the test period, the midguts of the normal group and those supplied with barren comb contained solid material with an appearance similar to that in termites collected directly from a nest. The guts of the starved group were filled with a clear fluid, and those of the group supplied only with nodules contained a clear fluid with occasional suspended soild fragments, which appeared to be pieces of nodules.

Diet	Activity (percentage of normal)	
	C_1	C_x
Starved	45	89
Barren comb	54	85
Nodules	106	147

Fig. 1. Adult workers of *M. natalensis* on a fragment of their fungus garden. The small white nodules are the conidia or conidiophores of the symbiotic fungus *Termitomyces* sp. The termites consume both the nodules and the supporting comb material. The scale bar represents 1 cm. [Photograph by R. Crewe, Department of Zoology, University of the Witwatersrand]

food at all, or else by providing them with comb from which the nodules had been removed; it was noted that midgut C_1 activity declined with time while midgut C_x activity did not (Table 2). In contrast, both C_1 and C_x activity remained high when the termites were provided with nodules taken from the comb. Indeed, with respect to midgut C_1 and C_x activity levels, termites supplied with barren comb are hardly different from those that had been starved, whereas those provided solely with fungus nodules have enzyme levels even higher than the normal group.

Our research has established that the fungus gardens make a major contribution to the nutrition of the Macrotermitinae (*20*). The fungus nodules contribute a critical enzyme required for the digestion of cellulose. Only after this enzyme is acquired by ingestion of the fungus nodules do the termites possess the appropriate biochemical machinery to digest sound wood or other cellulosic materials that have not undergone prior microbial decay. This conclusion clarifies the previously puzzling observation of Sands (*14*) that *Odontotermes badius* workers, which survive well in culture if provided with fungus garden, rapidly starve if provided with a garden that has been sterilized. Sterilization would denature the nodule enzymes which must continue to function in the termite's gut if cellulose digestion is to occur. Sand's observation is inconsistent with the widely held, but unsubstantiated view that the fungus comb is simply a site where cellulose is partially digested prior to being consumed by the termites, since sterilization would not alter the degree to which cellulose digestion had already occurred, and hence would not render the comb valueless as a nutrient source (*21*).

We suspect that a strategy of resource utilization, based on the acquisition from external sources of digestive enzymes that expand the range of natural substrates suitable for exploitation as nutrient sources, may be a general one (*22*). We suggest that there exist two distinct strategies by which an organism can exploit a potential nutrient source that its own digestive machinery is unable to process efficiently: (i) by maintaining a culture of endosymbionts capable of digesting the potential resource or (ii) by acquiring the requisite enzymes by consuming another organism or substrate that contains them. The first strategy involves a complex coevolved mutualism which probably has its evolutionary antecedents in a condition of mild parasitism, while the latter could evolve directly from polyphagy. Because of the ability of fungi to produce stable digestive enzymes active against a wide spectrum of natural substrates, such as cellulose, chitin, and lignin, we predict that acquired digestive enzymes will be found to play a particularly important role in the biology of fungus-feeding invertebrates, and in food chains based upon litter, detritus, and dead wood.

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References and Notes

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3. Cellulolytic species have been identified among the paunch bacteria of certain termites, but there has been no measure of the extent to which they actually contribute to the digestion of dietary cellulose [K. E. Lee and T. G. Wood, *Termites and Soils* (Academic Press, New York, 1971), pp. 1–251].
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5. The Macrotermitinae constitute one of the four subfamilies which make up the higher termite family, Termitidae.
6. The Macrotermitinae forage in seasoned structural timber, fence posts, sound dead wood (both standing and prone), stumps, grass, and the dung of herbivorous mammals [W. G. H. Coaton and J. L. Sheasby, *Cimbebasia Ser. A* **2**, 1 (1972)].
7. There has been controversy concerning the nature of the comb material. W. A. Sands [*Insectes Soc.* **7**, 251 (1960)] has observed the construction of comb from feces by *Ancistrotermes guineensis*. However, in studies involving the genera *Macrotermes* and *Odontotermes*, the weight of evidence favors the interpretation that comb is constructed primarily of chewed but undigested plant material [P. P. Grassé, in *Traité de Zoologie 9*, P. P. Grassé, Ed. (Masson, Paris, 1949), p. 408]. Not only is the comb material more similar chemically to fresh plant material than to termite fecal material [G. Becker and K. Seifert, *Insectes Soc.* **9**, 273 (1962); S. H. W. Cmeliak and C. C. Douglas, *Comp. Biochem. Physiol.* **36**, 493 (1970)], but also intact cells and cell walls, which are clearly evident in comb material, are not present in fecal material (*8*).
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9. W. A. Sands [in *Biology of Termites*, K. Krishna and F. M Weesner, Eds. (Academic Press, New York, 1969), vol. 1, p. 495] has reviewed the literature dealing with the identity of the fungi growing on the comb.
10. It is generally asserted that the *Termitomyces* species is a symbiotic partner of the termites, whereas the xylariaceous species are merely saprophytic commensals. Although the xylariaceous species are known from sources other than Macroterminae nests, there is no information which precludes a symbiotic status for them when they are associated with the Macrotermitinae.
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16. C. Noirot and C. Noirot-Timothée, in *Biology of Termites*, K. Krishna and F. M. Weesner, Eds. (Academic Press, New York, 1969), vol. 1, p. 49; J. Kovoor, *Experientia* **23**, 820 (1967); *Insectes Soc.* **18**, 49 (1971).
17. Although proctodeal food exchange does not occur in the higher termites, coprophagy has been reported [E. A. McMahan, in *Biology of Termites*, K. Krishna and F. M. Weesner, Eds. (Academic Press, New York, 1969), vol. 1, p. 387].
18. Isoelectric focusing was carried out at 5°C for 4 hours (initial constant current, 2 mA per tube; maximum voltage, 350 V) on 60-mm, 5 percent acrylamide gels with the use of an ampholyte with a nominal *p*H range of 3.5 to 10.0. The gels were sliced into 2- or 3-mm segments and eluted with 1.5 ml of water for 24 hours. The *p*H of the eluent was determined, and the C_1 assay was performed as described in (*23*).
19. Such workers are characterized by a sclerotized head and a white abdomen in which the guts are devoid of the usual dark, solid contents typical of adult workers. Of the 14 workers of this type collected during one excavation, two had a few solid fragments in their midguts, indicating that they had fed. Regrettably, these two individuals were included in the experiment described, and we suspect that they were the source of the limited C_1 activity detected.
20. Numerous suggestions have been advanced to explain the contribution of the fungus gardens to the nutrition of the termites, but only in a few cases have supporting data accompanied speculation. It has been proposed that the fungus is a source of vitamins [P. P. Grassé, *Ann. Sci. Nat. Zool. Biol. Anim.* **6**, 97 (1944)], that it degrades lignin thereby facilitating cellulose digestion by the paunch bacteria [P. P. Grassé and C. Noirot, *ibid.* **20**, 113 (1958)], and that it degrades cellulose in the comb [G. W. H. Coaton, *Afr. Wildl.* **15**, 39 (1961)]. Rohrmann (*8*) has shown that critical nutrients, such as nitrogen, potassium, and phosphorus, are concentrated in the nodules. M. Lüscher [*Nature (London)* **167**, 34 (1951)] and G. Rohrmann [*Sociobiology* **2**, 283 (1977)] have demonstrated that the fungus comb influences the temperature and humidity of the mound.
21. It is interesting that P. P. Grassé [*Nature (Paris)* (1959), p. 385] kept a colony of *Macrotermes* sp. alive for 18 months on rotten wood while depriving it of fungus comb. Not only would C_1 enzymes probably be present in rotten wood, but in addition, the cellulose present would already have undergone extensive degradation.
22. P. Büchner [*Holznahrung und Symbiose* (Springer-Verlag, Berlin, 1928), pp. 1–64] appears to have anticipated a role for acquired enzymes in his discussion of the siricid woodwasps, but subsequent research has neither confirmed nor contradicted his speculative comments.
23. Termite tissues, in groups of 10 to 50, were homogenized and centrifuged. Fungus materials were homogenized, centrifuged, and dialyzed against water. The C_1 and C_x activities were assayed by adapting methods described by D. R. Whitaker [in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 3, 1971), vol. 5, p. 273]. The rate of liberation of reducing sugars (maltose equivalents) was determined during incubation of a portion of extract with 50 mg of powdered cellulose or 5.0 ml of a 0.5 percent solution of carboxymethylcellulose, respectively, in acetate buffer (*p*H 5.0) at 37°C. β-Glucosidase activity was assayed by adapting the method of S. A. Kuby and H. A. Lardy [*J. Am. Chem. Soc.* **75**, 890 (1953)]. The rate of liberation of *p*-nitrophenol during incubation of a portion of extract with 1.0 ml of a 1 percent solution of *p*-nitrophenyl-β-D-glucoside in acetate buffer (*p*H 5.0) at 37°C was measured.
24. M. M. Martin and J. S. Martin, in preparation.
25. M.M.M. was supported by a science faculty fellowship from National Science Foundation (Washington, D.C.) during a sabbatical leave. We thank C.S.I.R. (Pretoria) for grant 398/77/Z1/RE1, and Professor H. Paterson, Dr. R. Crewe, and E. Vaccaro of the University of the Witwatersrand, for their help.
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18 August 1977; revised 28 December 1977