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Role of Microorganisms in the Metabolism of Termites

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Abstract

This article reviews the current knowledge of the role of symbiotic microorganisms in the metabolism of termites. The symbiotic microorganisms, comprising bacteria (higher and lower termites) and protozoa (lower termites) are in the hindgut. Cellulose digestion in higher termites appears to be mediated solely by cellulolytic enzymes secreted by the termites. In the lower termites, cellulose is digested by enzymes secreted both by the termites and by the protozoa. The end products of protozoal metabolism (acetate and butyrate) are thought to be used by termites as energy sources. Another method of cellulose digestion is that of the Macrotermitinae which have a symbiotic relationship with fungi of the genus *Termitomyces*. The termites acquire cellulase from the fungus. The bacteria found in the hindgut are usually facultative even though the hindgut is anaerobic. Some of these bacteria appear to be involved in dinitrogen-fixation in the gut, but do not play any part in cellulose metabolism. Some evidence suggests that termites may be able to re-utilize the nitrogen in the uric acid stores in their fat body using hindgut bacteria. While there is evidence that lignin can be degraded in termite tissues it is difficult to assess the role of the hindgut microbiota in this process.

Introduction

Termites (or 'white ants') are highly developed social insects which comprise the order Isoptera. Within this order, there are six families, of which five are classed as the lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae and Serritermitidae). The lower termites have unique genera and species of oxymonad, trichomonad and hypermastigote flagellates, which are capable of ingesting wood and living as symbionts in the paunch of the hindgut. The other family of termites (Termitidae), comprising some 75% of all species, are the higher termites. Protozoal populations in these termites (when they occur) are low and do not include xylophagous species (Honigberg 1970).

The termites present in a colony consist of several castes, which are morphologically and functionally distinct and have been described in detail by Miller (1969) and Noirot (1969*b*). The castes may be divided into two broad groupings, reproductive and sterile. The most important of the sterile castes are the soldiers and the workers, with the latter being the most numerous and also being responsible for building the nest and for all foraging activity. In addition, the workers care for the eggs and feed the larvae (newly hatched individuals), the soldiers and the queen, all of whom are incapable of feeding themselves. Most biochemical and microbiological studies of termites have been carried out on the worker caste because they easily constitute

the most numerous individuals in the nest and because of their importance in feeding the other castes.

Three groups of microorganisms are usually associated with the termites in a symbiotic existence. Two of these are the bacteria and protozoa which live within the hindgut of the insect. The third group are the fungi which some termites cultivate as 'fungus gardens' or 'fungus combs' (Sands 1969). Most termites live in the tropical, subtropical and warmer temperate zones of the world and subsist on a diet rich in cellulose, which may be in the form of living or dead wood, woody tissue of plants, humus or dung (Lee and Wood 1971, p. 19). Though it has been often stated that termites rely primarily on symbiotic protozoa to digest cellulose, this statement ignores the fact that most termites have no such protozoa. Most biological work which has been carried out on termites has been concerned with the lower termites and the results from the few species studied have produced generalizations which do not necessarily apply to the whole order.

This article describes the role of symbiotic microorganisms in the metabolism of termites and is divided into two main parts. The first part describes the conditions in the termite gut, an essential prerequisite for an understanding of the metabolic processes occurring in the hindgut, and the attendant gut microorganisms. The second part defines the separate and sometimes overlapping roles of the termite itself and its microorganisms in carbohydrate, nitrogen, and lignin metabolism.

The Gut and its Microorganisms

Conditions in the Gut

The digestive system of termites (Fig. 1*a*) has been described in detail by Noirot and Noirot-Timothée (1969). In summary, it consists of the foregut (consisting of the crop and the gizzard), the midgut and the hindgut. The hindgut may be divided into five successive segments, the first proctodeal segment, the second segment called the enteric valve, which controls the entrance into the third segment known as the paunch and in which the symbiotic microorganisms are abundant. The last two segments are the colon and the rectum. An important function of the enteric valve is to prevent the return of the paunch contents to the midgut or foregut (Noirot and Noirot-Timothée 1969). The Malpighian tubules enter the gut at the junction of the midgut and the first proctodeal segment, just in front of the proctodeal valve which is a simple circular swelling at the beginning of the first proctodeal segment. In many species of higher termites, the mesenteron is an elongation of the midgut formed as a prolongation on one of the faces of the intestinal tube, forming a 'mixed segment', so-called because the intestinal lumen is limited on one side by the mesenteron and on the other by the proctodeum (Fig. 1*b*).

The termite hindgut has generally been assumed to be anaerobic. Several pieces of evidence have led to this concept: for example the sensitivity of termite protozoa to oxygen (Cleveland 1925*b*, 1925*c*; Trager 1934; Hungate 1939; Mauldin *et al.* 1972), studies on the metabolism of cellulose by termites in which acetate and hydrogen were reported as end products (Hungate 1939, 1943; Cook 1943) and the *in situ* formation of methane by hindgut bacteria (Breznak 1975). The redox state of the gut of nine species of termites, one from the Mastotermitidae, four from the Kalotermitidae, two from the Hodotermitidae and one each from the Rhinotermitidae and the Termitidae, was measured by Veivers *et al.* (1980) using redox dye-feeding

techniques. The foregut and midgut of all species were aerobic with an E'_0 in excess of +100 mV. The paunch and colon were anaerobic with an E'_0 of about −230 to −270 mV, except in *Coptotermes lacteus* and *Nasutitermes exitiosus* whose colons were aerobic with an E'_0 of −50 to −125 mV. In four species (*Incisitermes barretti*, *Glyptotermes brevicornis*, *Stolotermes victoriensis* and *C. lacteus*) the rectum was aerobic (E'_0 about +60 mV), whereas the rectum of the other species (*Mastotermes darwiniensis*, *Neotermes insularis*, *Ceratokalotermes spoilator*, *Porotermes adamsoni* and *N. exitiosus*) was anaerobic (E'_0 about −125 to −270 mV).

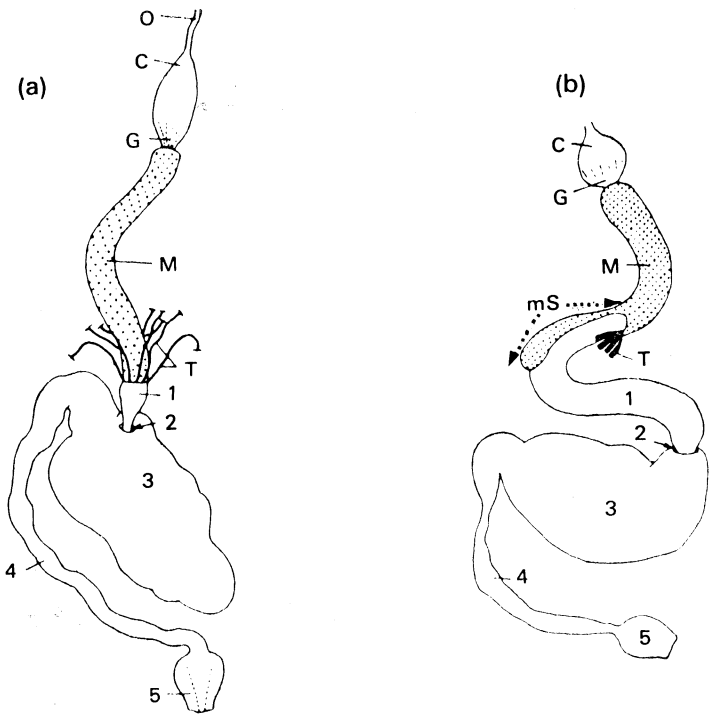

Fig. 1. (*a*) Gut of the lower termite *Kalotermes flavicollis*. (*b*) Gut of the higher termite *Nasutitermes arborum*. *C*, crop; *G*, gizzard; *M*, midgut; *mS*, mixed segment; *O*, oesophagous; *T*, Malpighian tubules. *1–5*, the five segments of the hindgut: *1*, the first proctodeal segment; *2*, enteric valve; *3*, paunch; *4*, colon; *5*, rectum. (After Noirot and Noirot-Timothée 1969.) (Reproduced by permission of Academic Press, Inc.)

The level of oxygenation of the gut was studied in a different manner by Bignell and Anderson (1980). They showed that when the guts of four species of termite (*Nasutitermes costalis*, *Cubitermes severus*, *Microcerotermes arboreus* and *Zootermopsis nevadensis*) were homogenized with air-saturated Ringers solution, the dissolved oxygen content of the Ringers solution was reduced, which they attributed to an oxygen deficit *in situ* in the gut and the consumption of oxygen in a chemical reaction.

Table 1 shows the pH of the different sections of the gut of 16 termite species. The hindgut (including the paunch and colon) of most species has a pH in the range of 6–7·5, except for the soil-feeding species (*C. severus* and *Procubitermes aburiensis*) where conditions are more alkaline. Thus the conditions in the hindgut are favourable

Table 1. pH of the guts of termites

Species	Crop	Foregut	Midgut	Mixed segment	Hindgut	Paunch	Colon	Rectum
Kalotermes flavicollis[A]		5·2–5·4	6·8–7·5		5·0–7·5			
Anacanthotermes ahngerianus[B]		7·7	6·9		7·9			
Anacanthotermes turkestanicus[B]					7·7			
Zootermopsis angusticollis[C]		5·2–6·8	5·2		3·0–6·8			
Zootermopsis nevadensis[D]	6·5–7·0		7·0–7·5			7·0–7·5		7·0–7·5
Reticulitermes lucifugus[D]	5·5–6·0		6·5–7·0			6·0–6·5		6·5–7·0
Reticulitermes lucifugus		5·6[I]	6·8–7·2[I]		7·8[B]	7·4[I]		
Reticulitermes sp.[E]		5·5			5·0			
Coptotermes lacteus[F]		3·8–4·4	6·8–7·4			6·8–7·4	6·8–7·4	2·8–3·2
Microcerotermes edentatus[G]		8·8–9·6	8·8–9·6	>9·6	6·0–9·6	7·2–7·6	6·8–7·2	6·0–6·8
Microcerotermes arboreus[D]	6·5–7·5		7·0–8·0	7·5–8·0		6·4–7·0	6·5–7·0	6·5–7·0
Microcerotermes sp.[B]					8·3			
Cubitermes severus[D]	6·0–6·8		6·5–7·5	8·0–9·5		9·0–10·0	7·5–9·0	7·0–8·0
Procubitermes aburiensis[D]	6·0–6·5		6·5–7·5	8·0–9·0		9·0–9·5	7·5–8·5	7·0–8·0
Nasutitermes costalis[D]	5·5–6·0		5·5–6·0	8·0–8·5		5·5–6·5	6·5–7·0	6·5–7·0
Nasutitermes exitiosus[F]		2·0–2·8	6·8–7·5	7·0–7·5		about 6·8	about 6·8	2·8–3·8
Trinervitermes trinervoides[H]			5·6					

[A] Noirot and Noirot-Timothée (1969). [B] Korovkina (1971, cited by La Fage and Nutting 1977). [C] Randall and Doody (1934, cited by Noirot and Noirot-Timothée 1969). [D] Bignell and Anderson (1980). [E] Brown and Smith (1954, cited by La Fage and Nutting 1977). [F] McEwen *et al.* (1980). [G] Kovoor (1967*b*). [H] Potts and Hewitt (1974). [I] Grassé and Noirot (1945, cited by Noirot and Noirot-Timothée 1969).

for the growth of many microorganisms which are usually tolerant of pH values ranging from 6 to 9. The midgut, in which enzyme secretion occurs, also has a pH around neutrality.

Hindgut Bacteria

Bacteria are found in the hindgut and the mixed segment. Although bacteria have been observed in the mixed segment of species of five genera of termites no attempt has been made to identify these bacterial species, even though the mixed segment contains a large number of bacteria which have the appearance of a pure culture and do not mix with the alimentary bolus (Noirot and Noirot-Timothée 1969). One species of termite, *Mastotermes darwiniensis*, has symbiotic bacteria in mycetocytes in its fat body (Jucci 1952), but the bacteria have not been identified or cultured and their function is unknown.

There are some 2000 termite species, but few have been examined to determine their gut flora. A high proportion of the bacteria so far isolated are facultative heterotrophs belonging to the genera *Streptococcus*, *Staphylococcus*, *Enterobacter* and *Citrobacter*, despite the fact that the hindgut is anaerobic (Krasil'nikov and Satdykov 1970; French *et al.* 1976; Schultz and Breznak 1978; Eutick *et al.* 1978*a*). These organisms have been found in concentrations of 10^5–10^9 per gut (Krasil'nikov and Satdykov 1969, 1970; Orlova 1972; Eutick *et al.* 1978*a*; Schultz and Breznak 1978), or 10^5–10^9 per millilitre of gut contents (To *et al.* 1980). Schultz and Breznak (1978) have estimated that their recovery of bacteria was only about 13 % of the total present. Obligate anaerobic bacteria, but not obligate aerobic bacteria, have been isolated by Schultz and Breznak (1978), whereas Eutick *et al.* (1978*a*) failed to find obligate anaerobes. The complex nature of the association of the flora and fauna in the gut, with bacteria adhering to the epithelium of the paunch or to other bacteria or protozoa (Breznak and Pankratz 1977; To *et al.* 1980; Bignell *et al.* 1980*b*) would make it difficult to recover all of the bacteria present in the gut.

Thayer (1976) has isolated *Bacillus cereus*, *Arthrobacter* sp., *Alcaligenes* sp. and *Serratia marcescens* from *Reticulitermes hesperus*. An examination of the hindgut of the humus-feeding termites *Procubitermes aburiensis* and *Cubitermes severus* by electron microscopy showed that actinomycete-type bacteria were the main microbial flora, with non-filamentous bacteria, mainly rods, colonizing the walls of the first proctodeal segment, the colon, and the third proctodeal segment (Bignell *et al.* 1979, 1980*a*).

The studies of Krasil'nikov and Satdykov (1970), Thayer (1976), Schultz and Breznak (1978) and Eutick *et al.* (1978*a*) have shown that the same species of termites collected from different locations possessed a similar microbiota, indicating that some degree of selection operates within the hindgut and influences the bacteria allowed to reside there. The anaerobic nature of the hindgut implies that the strictly aerobic bacteria found in termites are not part of the autochthonous flora, but are, probably, transient organisms ingested by the termite or are contaminants from the outside surface of the termite.

A possible symbiosis between *Streptococcus lactis* and a species of *Bacteroides* isolated from the hindgut of *Reticulitermes flavipes* has been studied by Schultz and Breznak (1979). *S. lactis*, when grown alone, ferments glucose to lactate as a major end-product with small amounts of formate, acetate, ethanol and carbon dioxide.

When *S. lactis* was grown in co-culture with the *Bacteroides* sp. it grew faster than the *Bacteroides* sp. and rapidly consumed the glucose, producing lactate. Growth of the *Bacteroides* sp. thereafter increased and the lactate gradually disappeared from the medium with a concomitant increase in propionate, acetate and carbon dioxide well in excess of that measured in cultures of *S. lactis* growing alone. The two organisms might exist in a commensal relationship in which cross-feeding is important, but as yet there is no evidence to indicate that this occurs *in vivo* in the termite. Lactate is not a major product of cellulose metabolism by *R. flavipes*, but acetate, formate, propionate, and butyrate have been detected (Schultz and Breznak 1978).

Spirochaetes, which inhabit the hindgut of a large number of higher and lower termites (Breznak 1973; To *et al.* 1978), are conspicuous when the gut is viewed microscopically, existing as free-swimming organisms or attached to some of the gut protozoa (Cleveland and Grimstone 1964; Smith and Arnott 1974; To *et al.* 1978). Despite reviews on the biology (Breznak 1973), anatomy (Holt 1978), and taxonomy (To *et al.* 1978), our knowledge of the role of the spirochaetes in termites is limited by the fact that they have not been isolated and cultivated in axenic culture. To *et al.* (1978) kept a small number of hollandinas and pillotinas alive for 72 hours in mixed culture only by excluding oxygen from the medium and carefully maintaining anaerobic conditions when removing the organisms from the gut of the termite.

Cleveland (1928) claimed that removing the spirochaetes from the gut of a termite (species not named, but probably *Zootermopsis*) did not have adverse affects on the termite. However, Eutick *et al.* (1978*b*) found that when the spirochaetes in *Nasutitermes exitiosus* were killed by feeding metronidazole or by exposure to pure oxygen the life span of the termite was reduced from 250 days to 13 days. The other bacteria present in the gut of *N. exitiosus* are facultative anaerobes (Eutick *et al.* 1978*a*) and are not killed by metronidazole. Since *Zootermopsis* sp., but not *N. exitiosus*, has symbiotic flagellates, it may indicate that the spirochaetes are important for metabolism where the flagellates are absent. To date, the only function attributed to the spirochaetes is one of providing a propulsion mechanism for some species of the gut protozoa (Cleveland and Grimstone 1964; Smith and Arnott 1974; To *et al.* 1978). The elucidation of their role in the termite gut would be an important step in understanding the role of bacteria in termite nutrition.

Fungi

Fungi are found in the gut contents of termites but apparently do not contribute significantly to the digestion of cellulose (Hungate 1936). Eutick *et al.* (1978*b*) found that feeding *Nasutitermes exitiosus* with the fungicide pimaricin had no effect on the life span of the termite. Fungi in the gut, therefore, may be transient and present only as a result of ingestion by the termite.

Protozoa

Most lower termites possess several species of protozoa (Honigberg 1970) and those found in some termite species have been listed by Yamin (1979). Because exposure of termites to pure oxygen leads to the death of the protozoa it may be inferred that they are strict anaerobes (Cleveland 1925*a*, 1925*b*; Hungate 1939; Mauldin *et al.* 1972; McEwen *et al.* 1980). Protozoa have been found in the gut of some species of higher termites, but of the 1200 species of Termitidae only the

guts of some 4% have been examined for the presence of protozoa. The role of the protozoa in these higher termites is unknown (Honigberg 1970). Their role in the digestion of cellulose by the lower termites is discussed below.

Cellulose Metabolism

The enzymatic hydrolysis of cellulose involves the action of two types of cellulase [1,4-(1,3;1,4)-β-D-glucan-4-glucanohydrolase, EC 3.2.1.4], namely C_1 and C_x. The C_1 enzyme is active against crystalline or native cellulose and appears to be an exoglucanase, whereas the C_x cellulase hydrolyses non-crystalline cellulose or soluble cellulose derivatives (for example, carboxymethylcellulose) and appears to be an endoglucanase. A third enzyme, cellobiase or β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21), which hydrolyses cellobiose to glucose, completes the hydrolysis of cellulose (Wood and McCrae 1979).

Role of the Gut Protozoa

Most studies of cellulose metabolism by termites have been directed towards determining the role of the gut bacteria and protozoa in this process. Cleveland (1924, 1925*a*, 1925*b*, 1925*c*, 1928) established that the gut protozoa are involved in cellulose digestion in the lower termites (see review by Honigberg 1970). Trager (1932) found cellulase and cellobiase activity in extracts of the intestines of *Zootermopsis angusticollis* and *Reticulitermes* sp.; no activity was found in the defaunated termites. Cellulase was found in extracts of one of the flagellates (*Trichomonas termopsidis*) from *Z. angusticollis* which Trager (1934) had succeeded in cultivating in the presence of a bacterium, itself devoid of cellulase activity. Another protozoan (*Trichonympha*) from *Z. angusticollis* was shown by Gutierrez (1956) to metabolize cellulose and cellobiose. Hungate (1938) also found that the protozoa from the hindgut of a species of *Zootermopsis* produced cellulase. Subsequent experiments using fresh suspensions of hindgut protozoa showed that they fermented 70–75% of the cellulose to acetate, carbon dioxide and hydrogen (Hungate 1938, 1943). In addition, Hungate (1939, 1943) showed that acetate production occurred in the hindgut and this acetate was presumed to be metabolized by the termite for its energy needs. However, the symbiosis between the termite and its protozoa was more complex than simply the production of acetate by the protozoa for their host, since defaunated *Z. angusticollis* did not survive when fed on acetate (Cook 1943; Hungate 1946*a*).

A study of carbohydrate utilization by *Z. angusticollis* (Cook 1943) showed that hydrogen was one of the products of cellulose metabolism by the hindgut protozoa, since it was produced by normal, but not by defaunated, termites. However, when the normal termites were fed on starch no hydrogen was produced indicating that the protozoa could not metabolize starch. Gilmour (1940) has shown that *Z. nevadensis* normally produced carbon dioxide and hydrogen but that defaunated termites ceased to produce hydrogen and produced more carbon dioxide. This supports the conclusion that the protozoa have an anaerobic metabolism.

Orlova (1974*a*) studied several species of lower termites and by selective removal of the protozoa by starvation deduced their role in carbohydrate digestion. Thus in *Anacanthotermes ahngerianus* C_1-cellulase activity was associated with the protozoa *Trichonympha turkestanica*, *Microspirotrichonympha* sp. and *Spirotrichonympha*

flagellata; C_x-cellulase was produced by the protozoa *Holomastigota elongatum* and *H. magnium*, whereas *Trichomonas* and *Eutrichomastix* spp. produced β-glucosidase.

Other evidence for the role of protozoa in metabolizing cellulose has emerged from the work of Mauldin and his colleagues (1972, 1977). Mauldin *et al.* (1972) showed that in *Coptotermes formosanus* the radioactivity from [U-^{14}C]cellulose was incorporated into triglycerides, predominantly into oleic acid. If the termites were starved the triglyceride stores were mobilized and the total lipids decreased. Partially defaunated termites lost most of their cellulolytic activity and also the ability to synthesize lipids. The major protozoan removed from the hindgut was *Pseudotrichonympha grassii* and by implication it was presumed to be responsible for cellulose digestion in *C. formosanus*. Similar results were obtained with partially defaunated *Reticulitermes flavipes*, though when *Trichonympha agilis* was removed, the termites recovered some cellulolytic activity and the ability to synthesize lipid was recovered after 11–18 days. It was presumed that the recovery of the cellulolytic activity and the ability to synthesize lipid were due to the termite itself or to the gut bacteria (Mauldin 1977).

Reticulitermes lucifugus and *R. speratus* were completely defaunated by feeding them on arabinose or xylose, and were partially defaunated by feeding them on glucose, mannose, sucrose or maltose (Orlova 1974*b*). This represents an alternative approach to defaunation by heat, starvation or oxygen treatment and could well prove a useful technique for studying the role of the different protozoa in termite metabolism.

Studies on *C. lacteus* in which the protozoa were killed by starvation or oxygen treatment caused a 60–100% decrease in cellulase and cellobiase activity in the hindgut (O'Brien *et al.* 1979; McEwen *et al.* 1980). Similar results were obtained with *Mastotermes darwiniensis* (Veivers *et al.* 1981). Extracts prepared from isolated mixed protozoa from both species had a high cellulase activity which accounted for most of that found in the hindgut extracts (McEwen *et al.* 1980; Veivers *et al.* 1981).

A major breakthrough in studying the role of protozoa in cellulose metabolism has been successful axenic culture of several protozoa by Yamin (1978*a*, 1978*b*, 1981). As yet, only preliminary biochemical experiments have been done, but with the techniques for axenic culture established, progress in the study of protozoal metabolism should proceed rapidly. The culture of *Trichomitopsis* (syn. *Trichomonas*) *termopsidis* from *Zootermopsis angusticollis* was first achieved by Trager (1934) and it was shown to possess cellulase. However, the culture was not axenic since a bacterium was always present. Yamin (1978*a*) has now established *Trichomitopsis termopsidis* in axenic culture and found that it could only be maintained on a medium containing cellulose, indicating its ability to produce a cellulase. The cellulose could not be replaced by glucose, cellobiose or other carbohydrates. A study of [^{14}C]cellulose metabolism by *T. termopsidis* showed that the cellulose was converted to $^{14}CO_2$ (25–30%) and [^{14}C]acetate (55–60%) in equimolar ratios. No neutral volatile compounds were produced and the remaining end-products of cellulose metabolism were not identified. Hydrogen was also a product of cellulose catabolism and, presumably, arose from the metabolism of pyruvate since extracts of the protozoan contained pyruvate-ferredoxin oxidoreductase [pyruvate : ferredoxin oxidoreductase (CoA-acetylating), EC 1.2.7.1] and hydrogenase (ferredoxin : H^+ oxidoreductase, EC 1.18.3.1) (Yamin 1980). Cellulase has been detected in extracts of *T. termopsidis* (Yamin and Trager 1979).

A second flagellate, *Trichonympha sphaerica*, from *Zootermopsis* sp., also has been obtained in axenic culture. It too required cellulose for growth and produced the same products from [^{14}C]cellulose as did *Trichomitopsis termopsidis* (Yamin 1981). *Trichonympha agilis* in *Reticulitermes speratus* selectively ingests cellulose when the cellulose is mixed with other materials (Yamaoka 1979). Yamin (1978*b*) has also axenically cultured the flagellate *Tricercomitus divergens* from *Cryptotermes cavifrons*. This flagellate does not require cellulose for growth and can be maintained on a medium containing foetal calf serum. *Trichomitopsis* sp. from *Reticulitermes flavipes* has been cultured under anaerobic conditions in a medium containing *Serratia marcescens* and *Enterobacter* sp. (Huntenburg *et al.* 1979).

Wood particles ingested by the symbiotic flagellates from *Reticulitermes lucifugus* are present in the cytoplasm and are not found in vacuoles (Lavette 1966). This raises the possibility that the bacteria found in all xylophagous flagellates, and not the flagellates themselves, could be responsible for producing the cellulolytic enzymes. However, the successful axenic cultivation of xylophagous flagellates in which the endobiotic bacteria have been removed, without affecting the ability of the flagellate to digest cellulose, implies that the endobiotic bacteria are not responsible for cellulose metabolism by the protozoan (Yamin 1981).

Role of Gut Bacteria

Isolation of cellulose-degrading bacteria from termites has been the object of many investigations, most of which have met with mixed success. Mannesmann (1972) isolated cellulose-degrading bacteria from *Reticulitermes virginicus* and *Coptotermes formosanus*. Thayer (1976) isolated *Bacillus cereus*, *Serratia marcescens* and *Arthrobacter* sp. from *Reticulitermes hesperus*; all the bacteria were capable of growing on cellulose–salts agar. The *Bacillus* and *Serratia* species were shown to produce an enzyme which could hydrolyse carboxymethylcellulose to reducing sugars (Thayer 1978). Krelinova *et al.* (1977*a*, 1977*b*) have also reported the isolation of Gram-negative, cellulose-degrading bacteria from several species of termite.

Many attempts to isolate cellulose-degrading bacteria from termites have been unsuccessful (Cleveland 1924; Dickman 1931; Hungate 1936; Eutick *et al.* 1978*a*; Schultz and Breznak 1978) and other claims of successful isolation of this type of bacteria need to be viewed with some caution since the methods used for the surface sterilization of the termites were either not described or not evaluated (French 1975; Rohrmann and Rossman 1980; for references to earlier work see Eutick *et al.* 1978*a*). In most cases where positive results were obtained the bacteria were not identified and no attempt was made to count them. The digestion of cellulose by the isolated bacteria was a slow process requiring days or even months to be achieved (Beckwith and Rose 1929; Hungate 1946*b*; Mannesmann 1972; Krelinova *et al.* 1977*b*) and in the case of the isolates obtained by Beckwith and Rose (1929) and Mannesmann (1972), growth of the organisms on cellulose was better under aerobic than under anaerobic conditions. The removal of the gut bacteria from *Nasutitermes exitiosus* and *Coptotermes lacteus* by feeding tetracycline had little effect on the cellulase activity of whole termites, indicating that bacteria play little part in cellulose digestion in these termites (O'Brien *et al.* 1979).

The presence of acetate, propionate and some butyrate in the hindgut of *Microcerotermes edentatus* was established by paper chromatography (Kovoor 1967*a*) and implies that the bacteria in the higher termites may have a role similar to that

of the protozoa in the lower termites, i.e. the formation of volatile fatty acids from the digestion products of cellulose which, in turn, may be used as an energy source by the termite.

Role of Enzymes Secreted by Termite Gut Cells

Higher termites

In a study of the C_x-cellulase activity of *Trinervitermes trinervoides*, Potts and Hewitt (1973) found that about 70% of the total activity was in the midgut and that about 40% of this activity was associated with the midgut wall. In one experiment the hindgut was shown to possess about 15% of the cellulase activity, whereas in another experiment a value of about 50% was obtained. Potts and Hewitt (1973) were unable to remove the gut flora of *T. trinervoides* with antibiotics, but still concluded that the cellulase was produced by the termite itself since no bacteria were found in the foregut and midgut. An examination of the chemical and physical properties of the partially purified cellulase from whole *T. trinervoides* indicated that a single enzyme was present (Potts and Hewitt 1974) which seems to preclude the possibility of production of different cellulases by both the termite and the gut bacteria (Potts and Hewitt 1973). The partially purified cellulase showed both C_1 and C_x characteristics since it was capable of hydrolysing crystalline cellulose and carboxymethylcellulose.

In *Microcerotermes edentatus*, most of the C_x-cellulase activity of the worker termites was in the midgut. However, there was considerable variation among the colonies of termites tested (Kovoor 1970). The cellulase activity of *Termes* (*Cyclotermes*) *obesus* resided wholly in the hindgut (Misra and Ranganathan 1954). These authors isolated cellulose-degrading bacteria (not identified) which, however, did not increase the rate of cellulose digestion when mixed with gut extracts.

O'Brien *et al.* (1979) found that the C_x-cellulase of *Nasutitermes exitiosus*, first reported by Tracey and Youatt (1958), was distributed in the foregut (19%), midgut (59%), the mixed segment (14%), and the hindgut (8%). Removal of the gut flora by feeding the termite with tetracycline or by starving the termite did not affect the activity of the total cellulase, indicating that the termite secreted its own enzyme(s). The presence of C_x-cellulases in the gut of *Macrotermes natalensis* (Martin and Martin 1978, 1979) and *M. subhyalinus* (Abo-Khatwa 1978) is discussed below under the heading of *Role of Acquired Enzymes.* Cellulase has been detected in *Amitermes rhizopus* and *A. vilis*, but no attempt was made to determine in which sections of the gut the enzyme was located (Zhuzhikov and Korovkina 1972; Orlova 1974).

A major product of the hydrolysis of cellulose by cellulase is the disaccharide cellobiose, which in turn can be hydrolysed to glucose by cellobiase or β-glucosidase. Cellobiase was evenly distributed between the midgut and the hindgut paunch of *Trinervitermes trinervoides*; the cellobiase activity in the hindgut was attributed to the hindgut bacteria (Potts and Hewitt 1973). An aryl β-glucosidase is present largely in the heads of *T. trinervoides* (Potts and Hewitt 1972), but as yet its function is not known. Kovoor (1970) found considerable variation in cellobiase activity in *Microcerotermes edentatus* with workers from one nest showing activity in the midgut, whereas those from another nest showed activity in both the hindgut and midgut. In *Macrotermes natalensis* β-glucosidase activity was mainly in the midgut (Martin

and Martin 1978, 1979) and in *M. subhyalinus*, the activity was distributed equally between the midgut and the paunch (Abo-Khatwa 1978). In *Termes* (*Cyclotermes*) *obesus* cellobiase was mainly in the hindgut (Misra and Ranganathan 1954). Over 90% of the cellobiase activity of *Nasutitermes exitiosus* and *N. walkeri* was located in the midgut (McEwen *et al.* 1980).

Lower termites

Yokoe (1964) concluded that at least part of the C_x-cellulase of *Reticulitermes* (*Leucotermes*) *speratus* was secreted by the termite itself. The conclusion was based on the finding that defaunated termites and nymphs of the second form (devoid of protozoa) showed cellulase activity. In a later study with the same termite species Yamaoka and Nagatani (1975) found that extracts of salivary glands contained C_1- and C_x-cellulase activities, whereas only C_x-cellulase was detected in extracts of the foregut and midgut. Extracts of the hindgut protozoa contained both C_1 and C_x cellulases. Starvation for 24 h led to about a 40% reduction in the protozoal C_x-cellulase.

C_x-cellulase activity in *Hodotermes mossambicus* was distributed through all sections of the gut of the workers, but was mainly in the first paunch of the larvae and the second paunch of the soldiers (Retieff and Hewitt 1973*b*; Botha and Hewitt 1979). On feeding the termite with green leaves of *Themeda triandra* there was a decline in the number of protozoa in the hindgut, but an increase (some sixfold) in C_x-cellulase activity, particularly in the combined extracts of the foregut and midgut which were essentially free of protozoa. C_x-cellulase was present in the wall fraction prepared from whole guts and increased in activity when the termites were fed on the green leaves. These results indicate that the termites can secrete C_x-cellulase. Secretion of C_x-cellulase occurs in the foregut and midgut of *Coptotermes lacteus* (O'Brien *et al.* 1979) and in the salivary glands and midgut of *Mastotermes darwiniensis* (Veivers *et al.* 1981) and in both species the amount of enzyme secreted is at least half of the total cellulase activity of whole termites. The C_x-cellulase activity in the foregut and midgut of *Coptotermes lacteus* was unaffected by treatments which killed the hindgut protozoa (O'Brien *et al.* 1979; McEwen *et al.* 1980).

β-Glucosidase activity has been detected in the foregut, midgut and paunch of workers of *Hodotermes mossambicus* (Retieff and Hewitt 1973*b*; Botha and Hewitt 1979). The activity of β-glucosidase in combined extracts of the foregut and midgut increased some twofold when the termite was fed green leaves of *Themeda triandra*, whereas activity in the hindgut was little affected, even though the protozoal numbers had declined as a consequence of the feeding regime (Botha and Hewitt 1979). Orlova (1974) has reported β-glucosidase activity in extracts of alates, soldiers, and workers of three species of termites. With *Coptotermes lacteus* McEwen *et al.* (1980) found 78% of the cellobiase in the hindgut and 21% in the midgut. Removal of the gut protozoa and spirochaetes by oxygen treatment did not affect the midgut activity, but resulted in almost the complete loss of the hindgut activity. The protozoa in the hindgut had a high cellobiase activity which accounted for most of the activity of this enzyme found in the hindgut. The cellobiase of *Mastotermes darwiniensis* was present in the salivary glands (37%), midgut (37%) and hindgut (20%) (Veivers *et al.* 1981).

Role of Acquired Enzymes

The Macrotermitinae have a symbiotic relationship with fungi of the genus *Termitomyces*. These termites cultivate 'fungus gardens' by growing fungi on structures known as 'fungus combs' which are derived from chewed, but undigested, plant fragments or are constructed from faecal pellets (Lee and Wood 1971, p. 143). The conidiophores appear as pearly white nodules on the surface of the comb and are eaten, along with the comb material, by the termites. The termites starve if deprived of the fungus comb and the conidiophores (see review by Sands 1969).

The fungus nodules possess both C_1- and C_x-cellulases and studies with *Macrotermes subhyalinus* (Abo-Khatwa 1978) and with *M. natalensis* (Martin and Martin 1978, 1979) have led to the hypothesis that these termites acquire most of their C_1-cellulase by ingesting the conidiophores. The extent to which the conidiophores were digested by the termite was not determined. C_1-cellulase activity in the salivary glands and the paunch of *M. natalensis* was very low [0·2–0·6 μmol of reducing equivalents (measured as maltose) per minute per termite], but was of reasonable activity in the midgut (7·6±0·8 μmol of reducing equivalents per minute per termite), whereas a high activity of C_x-cellulase (about 10–32 μmol of reducing equivalents per minute per termite) was found in the salivary glands, the midgut and the paunch (Martin and Martin 1978, 1979). Further evidence for this hypothesis was shown by newly moulted adult workers of *M. natalensis* having only 14% of the C_1-cellulase activity of normal workers, but 64% of the C_x-cellulase activity. In termites which were either deprived of the nodules or were starved, the activity of the midgut C_1-cellulase declined, whereas the C_x-cellulase remained constant; C_1- and C_x-cellulase activities remained high in those termites fed on the nodules.

Most of the C_1-cellulase in *M. subhyalinus* was found in the contents of the midgut and only about 3% was associated with the midgut wall, whereas some 33% of C_x-cellulase was present in the midgut wall, indicating that little C_1-cellulase is secreted by the termite (Abo-Khatwa 1978). The distribution of enzymes in the paunch was 19% for C_1-cellulase, and 43% for C_x-cellulase. No C_1-cellulase activity was observed in termites raised on a fungus-free diet, although traces of C_x-cellulase were found. Starvation of the termites caused a 50% decline in C_1-cellulase and a 20% decline in C_x-cellulase (Abo-Khatwa 1978).

The results of Martin and Martin (1978) explain how *Odontotermes badius* could survive in culture if it was provided with a fungus garden, but rapidly starved if it was provided with sterile comb material (Sands 1956). Sterilization would denature the enzymes of the nodules and thus they would be of no value to the termite digestive system. The study by Martin and Martin (1978) disposes of the widely held view that the fungus comb was simply a place for the partial degradation of cellulose prior to its ingestion by the termite.

Comparison of Cellulose Digestion by Lower and Higher Termites

It is evident that protozoa play a major part in cellulose digestion in the lower termites and that bacteria do not seem to be involved to any great extent in this process in either the higher or the lower termites. An important mechanism for cellulose digestion by the higher termites, which are free of xylophagous protozoa, is the secretion of cellulolytic enzymes by the gut cells; higher termites are thus independent of their gut microorganisms. The lower termites also possess this ability,

but their production of cellulolytic enzymes is, apparently, inadequate to fully satisfy their requirements as is shown by the failure of lower termites to survive defaunation (Honigberg 1970). Enzymes found in the salivary glands or midgut are not of microbial origin and cannot come from the hindgut since the enteric valve prevents entry of hindgut contents to the midgut (Noirot and Noirot-Timothée 1969).

In studying cellulase activity in termites most investigators have used some soluble form of cellulose, usually carboxymethylcellulose, as a substrate and thus have measured only C_x-cellulase activity. The use of crystalline cellulose as a substrate has been restricted to a few studies and has indicated low activities of C_1-cellulase in the salivary glands of *Reticulitermes speratus* (Yamaoka and Nagatani 1975), *Macrotermes natalensis* (Martin and Martin 1978, 1979) and *Mastotermes darwiniensis* (Veivers *et al.* 1981) and little or no activity in the midgut wall of *Macrotermes subhyalinus* (Abo-Khatwa 1978) and *M. natalensis* (Martin and Martin 1978, 1979). The activity of the C_1-cellulase is very low when compared to the C_x-cellulase activity found in these termites, but may simply be a reflection of the insoluble nature of the substrate used to measure C_1-cellulase activity. Thus while there is good evidence that termites are able to secrete C_x-cellulases the present data make it difficult to assess whether C_1-cellulases are truly secreted by termites. However, there is now considerable evidence for the synergistic action of C_1- and C_x-cellulases in certain fungi and for C_x-cellulase, and not C_1-cellulase, being responsible for the initial attack on crystalline cellulose (Wood and McCrae 1979). If such a state of affairs pertains to termite cellulases it may provide the reason for the apparent low C_1-cellulase in termites which do not use fungus combs as a source of C_1-cellulase.

Other Enzymes of Carbohydrate Digestion

The ability of termites to catabolize starch and some carbohydrates is implied in the finding of amylase (Montalenti 1932; Hungate 1938; Visintin 1947, cited by Day and Waterhouse 1953; Krishnamoorthy 1960; Noirot 1969*a*; Zhuzhikov and Korovkina 1972; Veivers *et al.* 1981), α-glucosidase (or maltase) (Krishnamoorthy 1960; Retieff and Hewitt 1973*a*; Veivers *et al.* 1981), invertase (Montalenti 1932; Krishnamoorthy 1960; Zhuzhikov and Korovkina 1972; Veivers *et al.* 1981) and lactase (Krishnamoorthy 1960) in a number of termite species. These enzymes were secreted by the salivary glands, the foregut and the midgut and were not of microbial origin.

Nitrogen Metabolism

Dinitrogen (N_2)-fixation

Termites live on a diet that is poor in vitamins, protein and other sources of nitrogen. The experiments of Cleveland (1925*a*), by which he showed that termites live for long periods when fed only on filter paper, led him to postulate that termites obtain their nitrogen requirements via dinitrogen-fixation by their gut symbionts. The attempts to prove this hypothesis (Greene and Breazeale 1937; Hungate 1941; Tóth 1948) were equivocal and it was not until 1973 that evidence was obtained confirming that termites can fix atmospheric nitrogen. Breznak *et al.* (1973) approached the problem by measuring the reduction of acetylene to ethylene by termites. Workers of three species of lower termites (*Coptotermes formosanus*,

Reticulitermes flavipes, *Zootermopsis* sp.) that were fed on wood were able to reduce acetylene; in the soldiers of two of the species this reducing ability was lower than that of the workers. The highest activity was observed in reproductive nymphs (individuals succeeding the larval stages and showing external wing buds) of *Cryptotermes brevis*. Acetylene reduction by *Coptotermes formosanus* was associated solely with the guts of the termites and was repressed by feeding them with some form of nitrogen (NH_4^+, NO_3^-, aspartic acid or autoclaved *Escherichia coli* cells). The repression was reversed when the termites were transferred to a diet low in nitrogen. The ability to reduce acetylene was apparently a property of the hindgut bacteria, for when they (but not the protozoa) were eliminated by feeding antibiotics to the termite, dinitrogen-fixing ability was completely lost. Potrikus and Breznak (1977) isolated two strains of dinitrogen-fixing *Enterobacter agglomerans* from termites. The number of *E. agglomerans* in the gut was insufficient to account for the rate of dinitrogen-fixation by the intact gut and Potrikus and Breznak (1977) attributed this either to the incomplete dispersion of bacterial aggregates during the preparation of the gut homogenates, or to the sensitive nature of the bacterial nitrogenase.

Benemann (1973) found acetylene reduction by workers and soldiers of *Kalotermes minor*, with the workers having the higher activity. Other termites tested (*Cryptotermes brevis*, *Zootermopsis angusticollis*) had only low rates of dinitrogen-fixation equivalent to about 10 μg nitrogen fixed per month per gram wet weight compared to *Kalotermes minor* which gave values ranging from 14 to 25 times greater. French *et al.* (1976) reported acetylene reduction by three species of Australian termites (*Coptotermes lacteus*, *Mastotermes darwiniensis* and *Nasutitermes exitiosus*) with the rate of reduction being lowest in *C. lacteus* and highest in *M. darwiniensis*. An interesting, but unexplained, finding was that *N. exitiosus* failed to reduce acetylene when it was fed on wood, but did so when it was fed on filter paper. French *et al.* (1976) isolated nitrogenase-positive *Citrobacter freundii* from all three species of termites. In a survey of the gut bacteria of nine species of Australian termites [including the species examined by French *et al.* (1976)] Eutick *et al.* (1978*a*) isolated putative dinitrogen-fixing organisms (identified as *Enterobacter* sp.) from seven species, including *Nasutitermes graveolus*, but not from *N. exitiosus* and *N. walkeri*.

A survey of termites from pastures, secondary forest and primary rain forest in the central Amazon by Sylvester-Bradley *et al.* (1978) produced evidence for dinitrogen-fixation in 21 species, with *Nasutitermes* spp. having the highest nitrogenase rate (more than 100 nmol acetylene reduced per gram of termites per hour). *Amitermes* sp., *Spinitermes* sp. and *Neocapritermes* sp. were free of nitrogenase activity. Schaefer and Whitford (1979) have shown dinitrogen-fixation in the hindguts of the desert termites, *Amitermes wheeleri* and *Reticulitermes tibialis*.

An examination of three species of termite by Rohrmann and Rossman (1980) revealed that *Macrotermes ukuzii*, which maintains a fungus garden, did not reduce acetylene and that a soil-feeding *Cubitermes* sp. reduced only slight amounts of acetylene (0·7 nmol acetylene reduced per gram of termites per hour). *Trinervitermes trinervoides* readily reduced acetylene (about 30 nmol acetylene reduced per gram of termites per hour) and the results indicated that this species could fix 8–12% per year of its total nitrogen content. Rohrmann and Rossman (1980) speculated that the high chitin content of the fungus maintained and eaten by *M. ukuzii*, plus the

presence of chitinase [poly(1,4β-(2-acetamido-2-deoxy-D-glucoside) glycanohydrolase, EC 3.2.1.14)] in the gut of this termite, may provide sufficient nitrogen for the termite and thus repress dinitrogen-fixation in the gut; *Cubitermes* sp. was presumed to obtain its nitrogen requirements from the soil it ingests.

In a study of *Nasutitermes ephratae* and *Rhynchotermes peraramatus* (both higher termites) in a Costa Rican rain forest Prestwich *et al.* (1980) observed that the nitrogenase activity in the soldiers of *N. ephratae* (28·4 μg nitrogen fixed per gram fresh weight per day) was about fourfold greater than in the nest workers or foraging workers. On the other hand, the workers of *R. peraramatus* had a nitrogenase activity (about 3·5 μg nitrogen fixed per gram fresh weight per day) some ten-fold greater than the soldiers. However, the nitrogen content of the soldiers and workers of both species was of the same order. The difference in dinitrogen-fixing ability of the two species appeared to correlate with their feeding habits. Thus *N. ephratae* fed on woody litter low in fixed nitrogen, whereas *R. peraramatus* fed on leaf litter with a higher fixed nitrogen content. The nitrogenase activity was higher in the leaf litter than in the woody litter and was presumed to be due to the indigenous microorganisms. A finding in this study, and one which must be borne in mind in future studies of dinitrogen-fixation in termites, was the rapid loss (within 24 h) of nitrogenase activity in the termites when the colonies were transferred from the field to the laboratory.

Nazarczuk *et al.* (1981) carried out an experiment in which groups of *Coptotermes lacteus* were kept under Ar/O_2 and N_2/O_2 mixtures for 7 weeks. Over this period there was no appreciable difference between the two groups with regard to survival, total nitrogen content or uric acid content, indicating that dinitrogen-fixation is not essential, at least in the short term, and also indicating that the cellular nitrogen was well conserved. The cellulose on which the termites were fed contained 0·2% nitrogen and this may have been sufficient for their needs. Potrikus and Breznak (1980*a*) found that the dinitrogen-fixing activity of *Reticulitermes flavipes* fell by 75% over a period of 9 months in the laboratory. These results, along with the increase in uric acid content of captive termites (discussed below), imply that the metabolism of nitrogen-containing compounds by termites kept in the laboratory may not be a true reflection of that which occurs in field populations.

Amino Acid Metabolism

Several studies have been carried out to determine whether changes in the gut flora and fauna affect the amino acid content of termites. Mauldin and Smythe (1973) found that the amino acid content in the form of protein of *Coptotermes formosanus* changed little when the termites were partially or completely defaunated and concluded that the termites can maintain their protein levels without protozoa, that the dead protozoa are, probably, not a source of nitrogen and that the symbiotic protozoa do not fix dinitrogen. Speck *et al.* (1971) found that removal of the gut bacteria in the lower termite *Reticulitermes santonensis* led to a decrease in the amount of free amino acids when the termites were fed [^{14}C]cellulose, whereas no change was observed in the higher termite *Nasutitermes nigriceps*. On the other hand, Nazarczuk *et al.* (1981) found that the free amino acids of both the lower termite *C. lacteus* and the higher termite *N. exitiosus* were not affected when the flora (and/or the fauna in *C. lacteus*) were removed by antibiotic or oxygen treatment. Attempts by Mauldin

et al. (1978) to determine the fate of the free amino acids of *C. formosanus* in which the gut bacteria had been removed by feeding antibiotics (penicillin plus streptomycin) met with mixed success. After transferring the termites to an antibiotic-free diet there was no consistent pattern in the composition of the free amino acids when compared with untreated termites and after 18 days the free amino acid composition was similar to that of the untreated termites. However, by this time the termites treated with the antibiotics had become colonized by bacteria dissimilar to their original flora. A similar experiment using streptomycin alone showed a decline of some 35% in the free amino acids 18 days after the end of the antibiotic treatment. Again the termites were colonized by a foreign bacterium, but not to the same extent as in the first antibiotic treatment. Measurement of the specific activity of the amino acids present in the cellular protein synthesized from [^{14}C]acetate showed that completely defaunated termites or termites fed antibiotics were unable to synthesize these amino acids as readily as normal termites or partially defaunated termites (which lacked one of their three gut protozoa). Mauldin *et al.* (1978) concluded from this investigation that the normal gut bacteria and two of the protozoa participate in the synthesis of the amino acids found in the cellular protein.

Nazarczuk *et al.* (1981) observed that the free amino acid content of *N. exitiosus* and *C. lacteus*, contrary to expectation, increased on starvation indicating that the amino acids were not mobilized by either the termites or their gut microbiota to provide carbon for growth or maintenance. Mauldin *et al.* (1978) found that the free amino acids of partially defaunated, starved *C. formosanus* increased 11 days after treatment, but declined about 25% below that of controls after 18 days. Other evidence that amino acids are not mobilized during starvation was shown by the failure of the amino acids in the cellular protein of *C. formosanus* to undergo any change after prolonged starvation of the termite (Mauldin and Smythe 1973; Mauldin *et al.* 1978).

Uric Acid Formation and Utilization

An hypothesis to account for Cleveland's (1925*a*) finding that termites could survive on a diet of pure cellulose was put forward, but not tested, by Leach and Granovsky (1938). They suggested that termites may be able to re-utilize the nitrogen present in their uric acid stores, either directly or with the aid of microbial symbionts. The presence of uric acid in termites has been amply demonstrated (Moore 1969; Potrikus and Breznak 1980*a*; Nazarczuk *et al.* 1981). The best evidence has been provided by Potrikus and Breznak (1980*a*) who showed that freshly collected specimens of six species of lower termites contained uric acid up to 5% of their dry weight. Less than 0·04% by weight of uric acid was found in the faeces. In the case of *Reticulitermes flavipes* 93–95% of the uric acid was stored in the fat body. The uric acid content of *R. flavipes* and *C. formosanus* (Potrikus and Breznak 1980*a*) and of *C. lacteus* (Nazarczuk *et al.* 1981) increased when these termites were kept in the laboratory. The increase in uric acid in *R. flavipes* was at the expense of non-uric acid nitrogen present in the termite; some 25% of non-uric acid nitrogen and 41% of non-uric acid carbon were expended during uric acid synthesis over a 15-month period. Starvation led to an increase, rather than a decrease, in the uric acid content of *N. exitiosus* and *C. lacteus* (Nazarczuk *et al.* 1981). As yet no evidence exists as to the fate of this stored uric acid. The mobiliza-

tion of uric acid would presuppose that the termites contained uricase. However, Potrikus and Breznak (1980*a*) failed to detect any uricase activity in their termites.

Potrikus and Breznak (1980*b*) tested the hypothesis of Leach and Granovsky (1938) by examining the gut of *R. flavipes* for bacteria that were capable of utilizing uric acid both as a carbon and a nitrogen source. They isolated several bacteria capable of growth on uric acid plates (in numbers up to 6×10^4 cells per gut) and identified them as group N *Streptococcus* sp., *Bacteroides termitidis* and *Citrobacter* sp. No bacteria were found in the fat body. They also found that the uric acid-degrading capacity of minced guts was consistent with the density of uricolytic bacteria in the gut. The *Streptococcus* sp. degraded uric acid incompletely, unless formate (or a source of formate, such as fructose) was present, but the *Citrobacter* cell suspensions or cultures failed to degrade uric acid. *B. termitidis* degraded uric acid to CO_2, NH_3 and acetate (Potrikus and Breznak 1980*c*).

The above studies have failed to show how the uric acid, which is stored in the fat body, is mobilized and returned to the gut. This has been partially answered for the lower termite *R. flavipes* by Potrikus and Breznak (1981). They found that $^{14}CO_2$ was evolved by the insects after [2-^{14}C]uric acid was injected into the haemolymph and also that the Malpighian tubules were capable of taking up uric acid. The site of uric acid degradation appeared to be the hindgut since the termite was devoid of uricase (urate : oxygen oxidoreductase, EC 1.7.3.3) activity. They were of the opinion that the injected uric acid was transported to the hindgut via the Malpighian tubules. Degradation of uric acid in the hindgut was effected under anaerobic conditions only by the hindgut bacteria, since their removal with antibiotics, which did not affect the gut protozoa, led to a loss of the ability to degrade uric acid. Feeding of [1,3-^{15}N]uric acid resulted in ^{15}N-labelled material appearing in termite tissue. Uric acid was apparently synthesized in the termite via purine nucleoside phosphorylase (purine-nucleoside : orthophosphate ribosyltransferase, EC 2.4.2.1) and xanthine dehydrogenase (xanthine : NAD^+ oxidoreductase, EC 1.2.1.37) both of which were detected in the termite extracts. It remains to be determined how uric acid is transported to and stored in the fat body and how it is mobilized for transport to the haemolymph. The use of symbionts to degrade uric acid stored in the fat body seems unlikely, except in *Mastotermes darwiniensis*, the only termite known to harbour bacterial symbionts in this tissue (Jucci 1952).

Lignin Metabolism

Lignin decomposition is generally believed to be an aerobic process (Zeikus 1980) and thus it is difficult to explain how it can occur in the anaerobic milieu of the termite hindgut. Lee and Wood (1971, pp. 135–8), in an exhaustive analysis of the published data on lignin degradation, suggested that there may be aerobic sites in the predominantly anaerobic gut, or that the digestion occurs via a different metabolic pathway than that in other lignin-degrading organisms. They suggested alternatively that digestion of lignin occurs in an aerobic portion of the alimentary canal. This is a possibility since the foregut and midgut of a number of termites are aerobic (Veivers *et al.* 1980).

Small amounts of lignin (3–4%) from the wood of two species were apparently decomposed by *Kalotermes flavicollis* (Seifert 1962). The termite faeces, but not the original wood, contained protocatechualdehyde, a breakdown product of lignin.

A more extensive investigation was carried out by Seifert and Becker (1965) using four species of termites and six species of wood. According to their findings from 2 to 83% (average 32%) of the lignin was decomposed. Butler and Buckerfield (1979) have pointed out that passage of the wood through the termites may have made the lignin more acid-soluble without decomposing it. Cold 72% (w/v) sulfuric acid and hot dilute sulfuric acid were used by Seifert and Becker (1965) to remove cellulose from the faeces and this treatment may have also dissolved the lignin, thus leading to an erroneous result. Colour tests indicate that lignin is partially degraded by the protozoa of the kalotermitid *K. flavicollis* and of some rhinotermitids (Lavette 1964). Mishra and Sen-Sarma (1980) have shown the presence of vanillin (4-hydroxy 3-methoxybenzaldehyde) and veratraldehyde (3,4-dimethoxybenzaldehyde) (known degradation products of lignin) in the hindgut of the lower termite, *Neotermes bosii*, and the higher termite, *Speculitermes cyclops*, when the termites were fed on sound wood; these aldehydes were not detected in the foregut or midgut. The failure of Mishra and Sen-Sarma (1980) to find enzymes capable of degrading lignin in the gut tissues, gut contents or salivary glands of *N. bosii*, *S. cyclops* and *Odontotermes distans* (a higher termite) and the absence of vanillin and veratraldehyde in the hindgut of defaunated *N. bosii* led them to the conclusion that the hindgut symbionts are involved in lignin degradation.

French and Bland (1975) prepared ^{14}C-labelled lignin by infusing *Eucalyptus maculata* with [3-^{14}C]cinnamic acid and fed it to *Nasutitermes exitiosus* and *Coptotermes lacteus*. After feeding for 11 h, radioactivity was detected in the body shells (minus the gut) of the termites indicating degradation of the lignin and incorporation of some of the radioactivity into body tissues. However, no information was provided on what proportion of the lignin was degraded. They also assumed that the radioactivity infused into *E. maculata* was incorporated only into lignin.

The ability of *Nasutitermes exitiosus* to digest lignins was studied by Butler and Buckerfield (1979) who fed the termite with natural and synthetic ^{14}C-labelled lignins and related compounds and measured the respired $^{14}CO_2$. They found that release of radioactivity was highest with ring-labelled ferulic acid (64%) and methoxy-labelled maize lignin (63%), but was only of the order of 16–32% with synthetic lignin (methoxy-labelled, C2-labelled or ring-labelled) and 7–17% when ring-labelled sodium phenate was fed. The breakdown commenced immediately on feeding and was linear until the food was consumed; decomposition of lignin did not occur in the faeces and at the end of the experiments the termite bodies contained very little radioactivity. The results implied that *N. exitiosus* can demethylate, depolymerize and degrade the aromatic ring of synthetic lignins and can demethylate and depolymerize natural lignin. The site of lignin degradation was not determined, nor whether the gut flora were involved in the degradation. Butler and Buckerfield (1979) suggest that the lignin may be partially degraded in the gut to smaller polymers or to monomers which could pass through the gut epithelium to an aerobic environment where oxidation could occur.

Conclusion

Over the last few years the mechanism of cellulose metabolism by termites has become clearer and indicates that the higher termites do not rely on their gut

microorganisms as agents for the digestion of cellulose. Honigberg (1970) commented that there is 'no incontestable evidence in support of any particular mechanism' for cellulose digestion among the higher termites. However, he did not exclude the 'possibility that some of these insects produce cellulase' and indeed it is becoming apparent that not only the higher termites, but also the lower termites, secrete their own C_x-cellulase. The evidence indicates that, in the lower termites, the protozoa are necessary to complete the digestion of cellulose and that the end-products of their metabolism are important energy sources for the host termite. In the higher termites the end-products of bacterial metabolism may serve this function. A greater knowledge of the metabolism of the hindgut bacteria, especially that of the spirochaetes, is needed to resolve this question.

A challenging area for study in termite metabolism is to investigate how termites obtain their nitrogen requirements. No single aspect of the problem, whether it be dinitrogen-fixation, amino acid metabolism or uric acid accumulation, is completely understood, nor is it clear what is the exact role of the gut flora or fauna or both in any of these processes.

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