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Nitrogen Fixation in Termites

Abstract. *Nitrogen fixation, measured by the reduction of acetylene to ethylene, was found in workers of the dry-wood termite* Kalotermes minor. *The soldiers and reproductive castes fixed little or no nitrogen. The fixation rates ranged between 24 and 566 micrograms of nitrogen fixed per month per gram (wet weight) of termite. Nitrogen fixation can be a significant source of nitrogen for these termites.*

Microorganisms living in symbiosis with insects are known to furnish essential vitamins and amino acids required for insect growth and development (*1*). Although their diet can be low in protein, the evidence for nitrogen fixation by herbivorous insects such as termites is not conclusive and has been discounted (*2–4*). The acetylene assay for nitrogen fixation has provided a method for reinvestigating this problem. This assay depends on the ability of nitrogenase to reduce acetylene to ethylene through a reaction which gives a reliable and sensitive measure of nitrogen fixation [see (*5*) for reviews]. Acetylene reduction has been used to detect nitrogen fixation in the guts of ruminants and mammals, including man (*6*). The results indicated that nitrogen fixation is nutritionally insignificant, at least under the conditions of the tests.

Table 1 shows results obtained with *Kalotermes minor*. Since all known nitrogen-fixing organisms are prokaryotic [see (*7*) for a review], it is likely that the nitrogen-fixing agent is the intestinal bacterial flora of the termites. Although the inhibitory effect of anaerobic conditions observed in one experiment (Table 1, assay 4) might suggest aerobic nitrogen-fixing bacteria, the death of the termites in this experiment could also account for the low activities if the bacteria stopped functioning as soon as the termites died. Also notable is the low rate of nitrogen fixation in the soldier and reproductive castes. The different feeding habits of these types might account for the low nitrogen-fixing activity of their intestinal flora. Variations in nitrogenase activity were observed between different batches (Table 1, assays 1 and 2). The cause of these variations remains to be established; the age of the workers might be a contributing factor.

Kalotermes minor, collected from May to October 1972 in San Diego County, California, exhibited a wide range of nitrogen fixation rates under air—from 24 to 566 μg of N fixed per month per gram (wet weight) of termite. Other termites collected during this time, *Cryptotermes brevis* and *Zootermopsis angusticollis*, were able to fix nitrogen; however, only low rates [about 10 μg of N fixed per month per gram (wet weight)] were recorded. If these organisms exhibit variations in nitrogenase activity similar to those observed with *K. minor*, then further collecting might turn up cases of nutritionally significant amounts of nitrogen fixed even in these species. Since fungi play an important role in the nutrition and nitrogen economy of termites (*2*), a symbiotic relation between nitrogen-fixing bacteria and fungi might exist in some termite nests. The cockroach *Periplaneta americana* did not reduce acetylene even following 3 months on a starch or sucrose diet.

It is difficult to ascertain the nitrogen requirements of termites and quantitate the importance of nitrogen fixation. This is due to cannibalism and the fact that the nitrogen content of wood is not directly related to its nutritional value to termites (*2*). From Hungate's data (*2*) on the nitrogen content of termites [an

Table 1. Acetylene reduction by *Kalotermes minor*. The termites were removed on 29 September 1972 from a pine post collected in southeast San Diego the previous day. Live termites were incubated at room temperature (24°C) in stoppered 7-ml serum flasks under the atmospheres indicated below, and the reaction was started by introducing 16.5 percent acetylene. After 150 minutes ethylene was determined by gas chromatography. (The termites in assay 4 died during the experiment.) No ethylene was detected in the absence of acetylene. A factor of 0.25 was used to convert acetylene reduction to nitrogen fixation (*6*). Ethylene determinations were accurate within 3 percent. Acetylene reduction was reproducible for at least three successive days as long as the termites were fed wood; *N*, number of termites per flask.

Assay	Type	Weight (g)	*N*	Atmosphere	Ethylene produced (nmole)	Est. N fixed per month per gram termite (wet weight) (μg)
1	Workers	0.692	66	Air	46	139
2	Workers	1.027	101	Air	108	212
3	Workers	1.155	120	Oxygen (20%)-argon (80%)	141	246
4	Workers	0.819	79	Argon	12	30
5	Reproductives	0.050	5	Air	<0.5	<20
6	Soldiers	0.525	30	Air	2.5	10

average of 17.5 mg per gram (wet weight) for *Kalotermes*] it would take 7 to 8 years to double the nitrogen content of the termites reported in Table 1; even at the highest observed rate (566 μg of N per month per gram) 30 months would be required. Therefore, although nitrogen fixation must now be considered a major source of nitrogen for at least some termites under some circumstances, only further studies will reveal whether nitrogen fixation can be the only source of nitrogen for these wood-eating insects. Considering the variation and magnitudes of the fixation rates, one can see why even careful and comprehensive nitrogen balance studies have failed to detect nitrogen fixation by termites (*2*, *3*).

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Lysergic Acid Diethylamide: Radioimmunoassay

Abstract. *A radioimmunoassay for* d-*lysergic acid diethylamide (LSD) is described. Antibodies to LSD were obtained by immunizing rabbits with a conjugate of LSD and human serum albumin. The specificity of the antibody was shown by competitive binding studies. The method has been used to detect the presence of LSD in human urines. Picogram amounts can be measured by this assay.*

The conventional assay of *d*-lysergic acid diethylamide (*d*-LSD) in biological fluids has depended on time-consuming extraction procedures, coupled with insensitive detection methods, such as fluorometric techniques or thin-layer chromatography (*1*). We describe here methods for producing antibodies to LSD and the development of a sensitive and specific radioimmunoassay, capable of detecting picogram amounts of LSD.

Antibodies were produced in rabbits by immunization with a conjugate of LSD and human serum albumin (HSA). The LSD-HSA complex was prepared as follows: HSA (200 mg) was dissolved in water (1 ml) and 3*M* sodium acetate (2 ml) at room temperature. A 37 percent formaldehyde solution (4 ml) was added dropwise, and the mixture was stirred for 5 minutes. *d*-LSD (25 mg) in water (1 ml) was added slowly, and the resulting solution was stirred at room temperature for 2 hours. Uncoupled LSD and other small molecules were removed by dialysis at 4°C against distilled water for 4 days. The solution was lyophilized, and the product was stored at −20°C.

That the LSD-HSA conjugate was free of uncoupled LSD was shown by thin-layer chromatography on silica F plates (E. Merck) with a solvent system consisting of chloroform and methanol (1 : 4, by volume). With this system the R_F of LSD is 0.66 and that of the conjugate is 0.00. The presence of LSD in the conjugate could be shown by the fluorescence of the LSD under ultraviolet light. Attempts were made to estimate the number of LSD residues per molecule of protein by hydrolysis. However, the conditions necessary to remove the LSD residue (boiling 5 percent sodium hydroxide solution) resulted in decomposition.

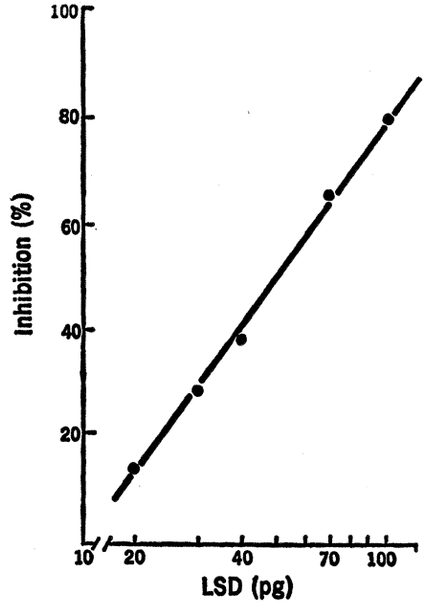

Fig. 1. Inhibition by LSD of the binding of ^{125}I-labeled LSD-copolymer by antibody to LSD. Reaction conditions are given in the text.

The extent of conjugation was estimated from the ultraviolet spectrum of the LSD-HSA at *p*H 7.0 (which shows a maximum at 310 nm). With the use of an extinction coefficient for LSD of 8800 at 310 nm, the number of LSD residues per molecule of protein was about ten. A second conjugate of LSD with bovine serum albumin (LSD-BSA) was prepared by the same procedure. The number of LSD residues attached to the BSA was about ten, estimated from the ultraviolet spectrum of the conjugate at *p*H 7.0.

Eight New Zealand female rabbits were immunized with the conjugates, four with LSD-BSA and four with LSD-HSA. Each rabbit was injected intramuscularly in the thigh with 0.5 mg of conjugate in complete Freund's adjuvant. The rabbits were injected once every 3 weeks and were bled 10 to 14 days after each injection. The antigen-binding capacity of the rabbit serum was determined with the use of a conjugate of LSD which was labeled with ^{125}I. This conjugate was prepared by forming a complex of LSD with a synthetic copolymer consisting of L-glutamic acid, L-alanine, L-lysine, and L-tyrosine in the proportion of 36 : 24 : 35 : 5 (Pilot Chemical, Watertown, Mass.) by the same methods as those used to prepare the LSD-HSA. The LSD-copolymer conjugate was labeled with ^{125}I (*2*) and had a specific activity of 150 to 300 c/mmole. Separation of unreacted ^{125}I was by chromatography on Sephadex G-10 in a buffer consisting of 0.5*M* sodium chloride and 0.01*M* sodium phosphate, *p*H 7.4.

All eight rabbits responded to the immunogens. Titers increased from 1 to 500 at the first bleeding to between 1 to 250,000 and 1 to 500,000 at the