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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References and Notes

1. P. Buchner, *Endosymbiosis of Animals with Plant Microorganisms* (Interscience, New York, 1965).
2. R. E. Hungate, *Ann. Entomol. Soc. Amer.* **34**, 467 (1941).
3. ———, *Proc. Trans. Tex. Acad. Sci.* **27**, 91 (1944).
4. V. B. Wigglesworth, *The Principles of Insect Physiology* (Dutton, New York, 1965); B. P. Moore, in *Biology of Termites*, K. Krishna and F. M. Weesner, Eds. (Academic Press, New York, 1969), p. 407.
5. R. W. F. Hardy, R. C. Burns, R. D. Holsten, *Soil Biol. Biochem.* **5**, 47 (1973); R. H. Burris, in *The Chemistry and Biochemistry of Nitrogen Fixation*, J. R. Postgate, Ed. (Plenum, London, 1971), p. 105.
6. R. W. F. Hardy, R. D. Holsten, E. K. Jackson, R. C. Burns, *Plant Physiol.* **43**, 1185 (1968); F. J. Bergerson and E. H. Hipsely, *J. Gen. Microbiol.* **60**, 61 (1970); J. Granhall and P. Ciszuk, *ibid.* **65**, 91 (1971); J. R. Postgate, *ibid.* **63**, 137 (1970); R. F. Ellenway, J. R. Sabine, D. J. D. Nicholas, *Arch. Mikrobiol.* **76**, 277 (1971).
7. J. R. Benemann and R. C. Valentine, *Advan. Microbial Physiol.* **8**, 59 (1972).
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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.

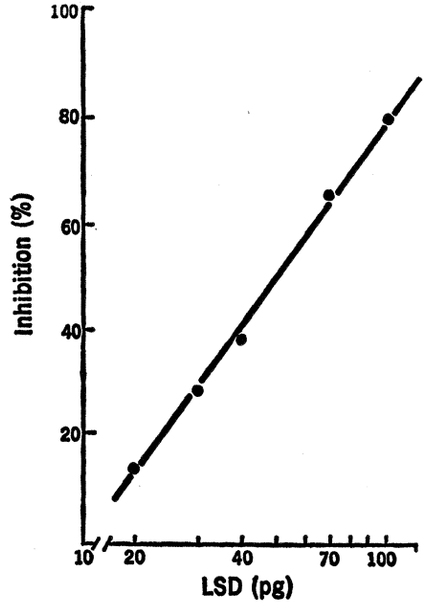

Fig. 1. Inhibition by LSD of the binding of ^{125}I-labeled LSD-copolymer by antibody to LSD. Reaction conditions are given in the text.

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

tenth bleeding. All serums showed similar limited cross-reactivity (see below).

The radioimmunoassay was carried out as follows. A solution of either unknown or standard LSD (0.1 ml) was mixed with the antibody to LSD (0.1 ml of the antiserum at the highest dilution which gives 40 to 50 percent binding of the labeled conjugate) and the ^{125}I-labeled LSD-copolymer conjugate (20,000 count/min) and diluted to a final volume of 0.5 ml with buffer (0.01*M* tris·HCl, *p*H 7.4, 0.15*M* NaCl, 0.01 percent gelatin). The mixture was incubated at 2° to 4°C for 4 hours. Antibody to rabbit gamma globulin (0.01 ml) and normal rabbit serum carrier (0.1 ml) were added to precipitate the complexes of LSD and antibody to LSD, and the mixture was incubated further for 20 hours at 2° to 4°C. After centrifugation the precipitate was counted in a gamma counter. The amount of LSD in an unknown sample was determined by the degree to which it displaced the binding of the ^{125}I-labeled LSD-copolymer complex. A standard curve is shown in Fig. 1. At 50 pg of LSD, 50 percent inhibition of the binding occurred. The standard curve is unaltered on carrying out the assay at *p*H 6.2 to 8.0.

The antibody is highly specific for LSD. The specificity was demonstrated by competitive binding studies with 50 percent inhibition of the binding of the marker as the end point (rabbit serum 19-1B). Only one compound was found to cross-react significantly. This is *l*-LSD which requires 50 ng to give 50 percent inhibition as compared to 50 pg for *d*-LSD. Other lysergic acid derivatives tested were isolysergic acid amide (1 μg), ergonovine (2 μg), ergocristine (> 10 μg), lysergic acid amide (> 10 μg), *d*-lysergic acid (LSA, 20 μg), and ergotamine (50 μg). The *N*-substituted tryptamine derivatives required more than 10 μg to give 50 percent inhibition. For example, *N*-acetylserotonin required 10 μg, psilocin (> 40 μg), psilocybin (50 μg), *N,N*-dimethyltryptamine (> 10 μg), and bufotenine (> 100 μg). Phenylisopropylamine derivatives and *N*-substituted phenylethylamine derivatives tested included *dl*-amphetamine (250 μg), 2,5-dimethoxy-4-methylphenylisopropylamine (> 200 μg), *N*-methylmescaline (> 200 μg), *N,N*-dimethylmescaline (> 200 μg), and mescaline (> 1000 μg). More than 20 miscellaneous compounds have been checked for interference in the assay. A few typical compounds are *N*-acetylmescaline (100 μg), gramine (> 100 μg), *dl*-normetanephrine (> 100 μg), niacin (> 100 μg), nicotinamide (> 100 μg) and *l*-epinephrine (> 200 μg). In all, more than 50 compounds have been tested for cross-reactivity, and the results demonstrate the high specificity of the antibody to LSD.

The radioimmunoassay has been used to detect the presence of LSD in urines from users of the drug. Control urines from 50 normal subjects (including ten pregnant women) were assayed without dilution or modification. Two samples showed some nonspecific binding. On dilution of the urine to 1 : 5 or 1 : 10 this nonspecific interference disappeared. As a result all urine samples were diluted 1 : 10 before assay although, in general, urine samples can be assayed directly without dilution. LSD added to a control urine can be assayed with adequate precision (98 ± 3 percent).

Table 1. Analyses of urine samples for LSD.

Tube No.	LSD found (ng/ml)	Dose (μg)	Samples collected
		Patient J.L.	
1–4	0.00	0	Controls
5	1.65	300	First
6	3.15		Second
7	54.65		Third
8	49.65		Fourth
9	34.65		Rest of 24 hr
		Patient J.L., second series	
10	19.65	0	First
11	24.65	300	Second
12	49.95		Rest of 24 hr
		Patient J.R.	
13–15	0.00	0	Controls
16	19.84	300	First
17	7.34		Second
18	19.84		Third
19	22.34		Rest of 24 hr
		Patient R.L.	
20	0.00	0	Controls
21	24.70	300	First
22	14.70		Second
23	14.70		Rest of 24 hr
		Patient L.W.	
24	0.00	0	Controls
25	2.40	400	First
26	12.40		Second
27	2.90		Third
28	11.90		Rest of 24 hr
		Patient B	
29, 30	0.00	0	Controls
31	0.12	200	First
32	Tube not sent		Second
33	1.40		Third
34	1.40		Fourth
35	4.90		Rest of 24 hr
		Patient G	
36, 37	0.00	0	Controls
38	1.55	250	First
39	9.80		Second
40	5.30		Third
41	5.30		Rest of 24 hr
		Patient S	
42	0.00	0	Controls
43	7.25	400	First
44	2.25		Second
45	7.25		Third
46	13.25		Rest of 24 hr
		Patient M	
47	0.00	0	Controls
48	5.80	300	First
49	5.30		Second
50	5.80		Rest of 24 hr
		Patient R.L., second series	
51	9.70	0	First
52	6.20	250	Rest of 24 hr

Urine samples were taken from a group of behavioral scientists given LSD under medical supervision. Eight different subjects were used and urine samples were collected both before and at intervals of up to 24 hours after administration of the drug; 50 urine samples were supplied coded and were assayed. Fifteen samples gave negative results and the remainder showed amounts of LSD ranging from 1.5 to 55 ng/ml. The results were then correlated with the data for the urine samples (Table 1). All control samples were negative, and also one of the other samples taken immediately after administration of LSD. All the remaining samples, collected after LSD was given, were positive. The slopes of the inhibition curves with standard LSD and urine from LSD users were identical. This offers further proof that the assay measures LSD or a closely related metabolite.

These data indicate that the radioimmunoassay can detect LSD or possibly a closely related metabolite in urine. The assay can also be used to detect LSD in serum, bile, gastric fluids, and other biological material (*3*). It can detect as little as 20 pg of LSD, which should make it a useful tool in research.

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References and Notes

1. J. Axelrod, R. O. Brady, B. Witkop, E. V. Evarts, *Ann. N.Y. Acad. Sci.* **11**, 435 (1957); G. J. Aghajanian and O. H. L. Bing, *Clin. Pharmacol. Ther.* **5**, 1203 (1964); H. Van Vunakis, J. T. Farrow, M. B. Gjika, L. Levine, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1128 (1971); A. R. Sperling, *Microgram* **2**(2), 51 (1969); S. Agurell, *Acta Pharm. Suecica* **2**, 357 1965).
2. W. M. Hunter and F. C. Greenwood, *Nature* **194**, 495 (1961).
3. A. Taunton-Rigby, S. E. Sher, A. P. Chase, P. R. Kelley, paper presented at 25th annual meeting of American Academy of Forensic Sciences, Las Vegas, 1973.
4. We thank Dr. A. A. Kurland of the Maryland Psychiatric Research Center, Baltimore, for supplying the urine samples, and J. Woiszwillo for technical assistance. Supported by the Office of Scientific Support, Bureau of Narcotics and Dangerous Drugs, under contract J-70-24.

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