Table 1. Milligrams of chlorophyll per gram of leaf dry weight of soybean plants grown in water cultures in indicated treatments. Each value is the mean of three replications.

Treatments	Days in treatments			
	7	14	21	28
No iron				
16-hour day	4.0	2.4	2.1	2.0
9-hour day	4.3	3.4	2.1	2.0
Iron as $FeNH_4(SO_4)$*				
16-hour day	4.6	4.6	5.0	4.7
9-hour day	4.6	4.0	3.1	2.7
Iron as FeNTA*				
16-hour day	5.2	5.0	5.3	5.3
9-hour day	5.0	5.3	5.5	5.6

* Iron supplied at concentrations of 0.075×10^{-5} mole/lit.

taining a minimum amount of iron (0.1 ppm $FeSO_4$) to keep the plants a healthy green color. After 3 weeks they were transferred to water cultures containing (i) no iron, (ii) 0.075×10^{-5} mole of $FeNH_4(SO_4)_2$ per liter, and (iii) the same concentration of ferric nitrilotriacetate (FeNTA). The plants in all three iron treatments were grown in the natural daylight existing in Pasadena, California, during October, in greenhouses from 8:00 a.m. to 5:00 p.m. At 5:00 p.m. each afternoon, one-half of the plants were transferred to aerated, light-sealed cabinets in an inside room. The other half were transferred to the same room and exposed from 5:00 p.m. to midnight to radiation of 10 ft-ca (110 lu/m^2) intensity from a 100-watt incandescent bulb. In this way the plants in the various treatments were exposed to the same conditions of temperature and humidity. The nutrient solutions were changed once during the 4-week period of the study. The temperature in the greenhouse ranged from 21° to 29°C (70° to 85°F) during the normal daylight hours, and was held at 22°C. during the "night" period.

The plants were held in the treatments from 4 October to 1 November 1961. During this time the chlorophyll content of the leaves was measured at weekly intervals by a reflectance method (*2*). The results of these determinations are shown in Table 1.

All the leaves on plants grown in solutions of the chelated iron, FeNTA, maintained or increased their chlorophyll content regardless of day length. The leaves on all the plants receiving no iron lost their chlorophyll and at about the same rate, regardless of the day length. However, the leaves of the plants given $FeNH_4(SO_4)_2$ maintained their chlorophyll content in the long day, but in the short days their chlorophyll content decreased almost as rapidly as in the leaves of the plants receiving no iron. An analysis of variance showed a highly significant interaction between day length and iron compounds. In other words, exposing the plants to low light intensities during normal night-time hours delayed or prevented the development of iron-deficiency symptoms (chlorosis) where iron was not readily available.

The dry weights of these plants are shown in Table 2. The results show that increasing the availability of the iron or adding supplemental light at night increased the growth of the plants. The results also show that the more readily available the iron, the smaller the proportionate increase in growth produced by lengthening the day.

The results of this preliminary study indicate that the response of plants to iron may be influenced by day length, or the response to day length may be influenced by the iron supply. It may be that iron is absorbed only during daylight hours and that the nonchelated iron was partially precipitated out as the result of reaction with the phosphate in the nutrient solution. Thus, during the short days the plants could not acquire sufficient iron to keep the leaves green. However, if such a precipitate was formed, it was quite small, since no noticeable amount was detected when the solutions were changed or at the close of the experiment. It would be of interest to repeat these studies with a long-day variety of soybean. The iron content of the leaves was not determined, so it is not possible to conclude whether there was actually less iron in the chlorotic leaves of the plants receiving $FeNH_4(SO_4)_2$ in the short days, or whether the iron was rendered nonavailable for chlorophyll formation.

Table 2. Dry weight of soybean plants grown under short and long days in water culture containing different iron compounds. Each value is the mean of three replications of four plants each.

Plant part and iron compound	Dry weight (g)		Ratio, 16-hr day wt ÷ 9-hr day wt
	9-hr day	16-hr day	
Leaf			
None (control)	1.33	2.53	1.90
$FeNH_4(SO_4)_2$*	2.40	3.43	1.43
FeNTA*	3.77	5.00	1.33
Stem			
None (control)	0.87	1.97	2.26
$FeNH_4(SO_4)_2$	2.30	2.57	1.12
FeNTA	3.10	3.13	1.01
Root			
None (control)	0.70	0.87	1.24
$FeNH_4(SO_4)_2$	0.77	0.93	1.21
FeNTA	1.23	1.43	1.16

* Iron concentration 0.075×10^{-5} mole/lit.

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References and Notes

1. J. N. Simons, R. Swidler, H. M. Benedict, *Plant Physiol.* **37**, 460 (1962).
2. H. M. Benedict and R. Swidler, *Science* **133**, 2015 (1961).
3. Supported by the Agricultural Research Center of Stanford Research Institute.

6 March 1964

Serotonin Binding to Nerve-Ending Particles of the Rat Brain and Its Inhibition by Lysergic Acid Diethylamide

Abstract. *The binding of serotonin to nerve-ending particles and other preparations from rat brain has been examined. By investigating the amount bound as a function of serotonin concentration from 10^{-7}M to 10^{-2}M, it was possible to identify three major components having K_{assoc} (association constant) values of 2×10^6, 5×10^4, and 5×10^2. The component having the highest binding constant was not present in liver and appeared to be confined to the cortex and midbrain regions. This component is inhibited by* d*-lysergic acid diethylamide at low concentrations. Solubilization of this binding component has been achieved.*

As part of a general investigation into the biochemistry of events at the synapse, the binding of serotonin to preparations of nerve-ending particles from whole rat brain has been examined. Previous studies have implicated serotonin in neural events (*1*). Many studies have been made on its binding to tissues; in this report we distinguish between a low-affinity nonspecific binding and a binding of very high affinity which is specific to certain brain components. Any tissue preparation may contain one or more binding compo-

nents, distinguishable by their association constants; $K_{assoc} = [MS]/[M]\,[S]$, where $[M]$ is the concentration of a binding macromolecule expressed in binding equivalents, $[S]$ is the concentration of serotonin, and $[MS]$ that of the complex.

Preparations of nerve-ending particles were made from whole rat brain by a combination of differential and isopycknic centrifugation slightly modified from that described by Whittaker (*2*). Other centrifugal fractions from brain were examined, as well as preparations of liver mitochondria, erythrocyte ghosts, and bovine serum albumin. All the preparations were dialyzed into and washed with 500 volumes of 0.1*M* sodium phosphate buffer, *p*H 7.2. The experiments were done in this buffer throughout at 2°C. The binding was measured by equilibrium dialysis and by a method in which the macromolecular exclusion properties of Sephadex were used. The results of both methods agree. Particular attention was paid to estimating and accounting for the serotonin which was adsorbed on the dialysis membranes and the Sephadex grains. We had been concerned that the action of monoamine oxidase (MAO) in removing serotonin would falsify the results, but uniform attainment of equilibrium, and determination of the temperature coefficient of MAO action on serotonin convinced us that its removal by MAO was negligible at 2°C and at concentrations of serotonin below $10^{-4}M$, even in preparation rich in MAO. Figure 1 shows the way in which the amount of serotonin bound varies with serotonin concentration. In the case of nerve-ending particles the plot has the form of the titration of a tribasic acid with a base. From the

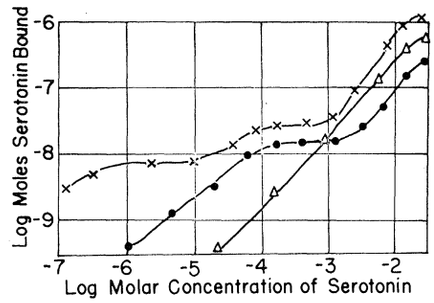

Fig. 1. Moles of serotonin bound as a function of serotonin concentration, determined by equilibrium dialysis. Crosses, nerve-ending particle fraction; circles, liver mitochondrial preparation; triangles, bovine serum albumin. All preparations contained 1 mg of protein per milliliter.

graph, the quantity and K_{assoc} of the three binding components can be evaluated, but graphical procedure according to Scatchard (*3*) and a least squares curve fitting procedure (*4*) have been used in practice. Clearly, the component with highest affinity was present only in nerve-ending particles, and the component with lowest affinity was found even in bovine serum albumin. It is recognized that the components identified on the basis of their K_{assoc} values may, in fact, contain more than one substance.

The component with the lowest K_{assoc} value was found in all brain fractions, liver mitochondria, erythrocyte ghosts, and bovine serum albumin. Because of the ubiquity of this binding it is not considered to be of very great neurobiological interest. Bovine serum albumin binds approximately 120 moles of serotonin per mole of protein and the individual K values computed according to the procedure of Klotz (*5*) lie between 10^4 and 10^{-2}. It is thought that the widespread binding of cations to proteins must be avoided or allowed for in the conduct and interpretation of binding experiments.

The compound with intermediate-strength binding was present in liver mitochondria and most brain fractions, but was absent in erythrocyte ghosts and bovine serum albumin. The correlation between MAO concentrations in the brain fractions and amounts of the intermediate-strength binding (see Table 1) suggested that it was associated with MAO activity. Harmine was found to inhibit competitively the component with intermediate-strength binding with a K_i of 1.2×10^{-7} and to be a competitive inhibitor of brain MAO with a K_i of 1.8×10^{-7}. We have reservations about firmly ascribing the component with the intermediate-strength binding to the activity of MAO because harmine is a peripheral antagonist of serotonin (*6*). Nevertheless, the similarity of the inhibitory constants is suggestive.

As shown in Table 1, the components with the highest-strength binding are mainly to be found in preparations of nerve-ending particles from the brain. The nerve-ending particle appears to contain synaptic vesicles, with a portion of the postsynaptic membrane adhering to it (*7*). Because such a preparation contains these elements of the nerve cell it is suggested that the binding with the highest affinity is involved with some aspects of the neurohumoral function of serotonin; it is also significant that this type of binding is found mainly in the cortex, thalamus, and midbrain regions, and to lesser extents in the medulla, cerebellum, or cervical segments of the spinal cord.

The effects of a number of drugs and psychoactive agents on this high-affinity binding were investigated. The most interesting was the effect of *d*-lysergic acid diethylamide (LSD) which was found to inhibit the binding at extremely low concentrations, $K_i = 10^{-7}$. To rule out the possibility that LSD was interacting with serotonin regardless of the macromolecular phase, the binding of LSD to this fraction was investigated and found to have a K_{assoc} of 10^7 and to be inhibited by serotonin. The effect of the nonhallucinogenic congener of LSD, 2-bromo-*d*-lysergic acid diethylamide (BOL 148) (*8*) was at first found to be erratic. Very little inhibition of binding could be demonstrated if the BOL was solubilized in glass-distilled water at 2°C, but if the BOL was treated with phosphate buffer,

Table 1. Distribution of components in centrifugal fractions from rat brain. Each result shown is the mean of three determinations.

Fraction		Monoamine oxidase (%)	Component (%)	
			$K_{assoc} = 5 \times 10^{+4}$	$K_{assoc} = 2 \times 10^{+6}$
	Sedimented at 1000g for 10 minutes			
Nuclear		41	44	19
	Sedimented at 18,000g for 30 minutes			
Mitochondrial	Denser than 1.4*M* sucrose	29	24	12
Nerve ending particles	Density less than 1.4*M* sucrose; greater than 0.7*M* sucrose	11	12	54
Myelin fragments	Lighter than 0.7*M* sucrose	5	4	4
	Sedimented at 30,000g for 90 minutes			
Cell membrane (*15*)		8	11	10
	Not sedimented below 30,000g for 90 minutes			
Supernatant		7	5	<1

*p*H 7.2, at 50°C for 1 hour it became extremely inhibitory with a strength comparable to that of LSD.

Gaddum (*9*) and Woolley and Shaw (*6*) originally proposed that the hallucinogenic action of LSD was caused by its antagonism to serotonin, as could be demonstrated on a smooth muscle preparation. However, there is an anomally in that the nonhallucinogenic BOL 148 (*10*) is also an antagonist of serotonin in such preparations (*11*). By contrast, the fact that LSD is antagonistic toward the binding of serotonin to nerve-binding particles, while mildly solubilized BOL 148 is not, is compatible with the original proposal. Nevertheless, if BOL 148 is converted to a serotonin antagonist in vivo, as it is under relatively mild laboratory conditions, then the anomaly remains. Also, it remains possible that LSD interferes with other neurohumoral agents besides serotonin.

Reserpine inhibits some components of the high-affinity binding, but the effect is much weaker than that of LSD. Calcium ions at high concentration were found to have little effect and chlorpromazine (*12*) could not be shown to be an inhibitor. Attempts to solubilize the LSD-sensitive substance with surface active agents such as Tween and lysolecithin were unsuccessful because the binding constant was changed and the inhibitory effect of LSD was lost. Such changes also occurred slowly on allowing it to stand in buffer at 2°C. Solubilization and precipitation from *n*-butanol with retention of activity and tenfold purification has been achieved. The LSD-sensitive binding is destroyed by cholera vibrio neuraminidase, suggesting that a ganglioside is involved.

Woolley has reported the existence in a variety of tissues of a serotonin lipid receptor, which he has proposed is a participant in calcium transport in vivo (*13*). The property of transporting calcium ions from an aqueous phase to a lipid phase in the presence of serotonin appears to be a fairly general characteristic of many types of lipids (*14*). Although we have not examined the calcium transporting activity of the components with the high-affinity binding, it does not seem likely that it is identical with the serotonin receptor described by Woolley, because the liver is a fairly rich source of the receptor lipid (*13*) whereas we were unable to detect the component with the high-affinity binding in liver preparations.

The existence of serotonin in nerve-ending particles has been shown (*2*), and it is hoped that investigation of its binding to macromolecules in the nerve-ending particles will throw further light on its role in neurotransmission.

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References and Notes

1. V. Erspamer, review in *Progr. Drug Res.* **3**, 151 (1961).
2. V. P. Whittaker, *Biochem. J.* **72**, 694 (1959).
3. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
4. R. M. Marchbanks and F. Rosenblatt, in *Collected Technical Papers*, F. Rosenblatt, Ed. (Cognitive Systems Research Program, Cornell Univ., Ithaca, N.Y., 1963), vol. 2, pp. 69–72.
5. I. M. Klotz, *Arch. Biochem.* **109**, 9 (1946).
6. D. W. Woolley and E. Shaw, *Proc. Natl. Acad. Sci. U.S.* **40**, 228 (1954).
7. E. G. Gray and V. P. Whittaker, *J. Anat.* **96**, 79 (1962).
8. Courtesy of Sandoz Pharmaceutical Inc.
9. J. H. Gaddum, in *Ciba Founda. Symp. Hypertension-Humoral Neurogenic Factors, 1955*, G. E. W. Wolstenholme and M. P. Cameron, Eds. (Little, Brown, Boston, 1954), p. 75.
10. A. Cerletti and E. Rothlin, *Nature* **176**, 785 (1955).
11. H. Isbell, E. J. Miner, C. R. Logan, *Psychopharmacologia* **1**, 20 (1959).
12. Courtesy of Smith, Kline and French Laboratories.
13. D. W. Woolley, *Proc. Natl. Acad. Sci. U.S.* **44**, 1202, (1958).
14. R. S. Dombro, L. S. Bradham, N. K. Campbell, D. W. Woolley, *Biochim. Biophys. Acta* **54**, 516 (1961).
15. V. Hanzon and G. Toschi, *Sci. Rept. Ist. Super. Sanita* **1**, 19 (1961).
16. This research was supported by ONR contract NONR 401 (40) and by NSF grant GP-971.

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Reduction of Dimethylsulfoxide to Dimethylsulfide in the Cat

Abstract. *A peculiar sweetish odor was noted in the exhaled breath of cats treated with dimethylsulfoxide. By means of gas chromatographic and mass spectrographic techniques, the responsible compound was identified as dimethylsulfide.*

Dimethylsulfoxide has been advocated as a medium for the frozen storage of biological material such as bone marrow (*1*) and whole organs (*2*) intended for transplantation. Recently (*3*) dimethylsulfoxide has been advocated for a wide variety of possible medical uses ranging from the experimental prevention of peritoneal adhesions to tranquilization (*3*).

While investigating the pharmacology of this compound in cats, we noticed a peculiar sweetish odor in the expired air within a minute of the intravenous injection of the compound. A comparison of this odor with those of related chemical compounds led to the suspicion that the gaseous metabolite was dimethylsulfide.

The transformation of the sulfoxide to the sulfide represents an unusual metabolic pathway. The reduction of sulfoxides has hitherto been observed only in microorganisms (*4*). Bennett (*5*), however, showed that rats grew normally when DL-methionine sulfoxide was substituted for dietary DL-methionine. This observation suggests the possibility that DL-methionine sulfoxide may be reduced to DL-methionine.

To verify the occurrence of dimethylsulfide after intravenously administering dimethylsulfoxide to cats, we trapped expired air in an empty absorption bottle immersed in ice. The condensate was analyzed by gas chromatography. The retention time of the unknown sample was identical with

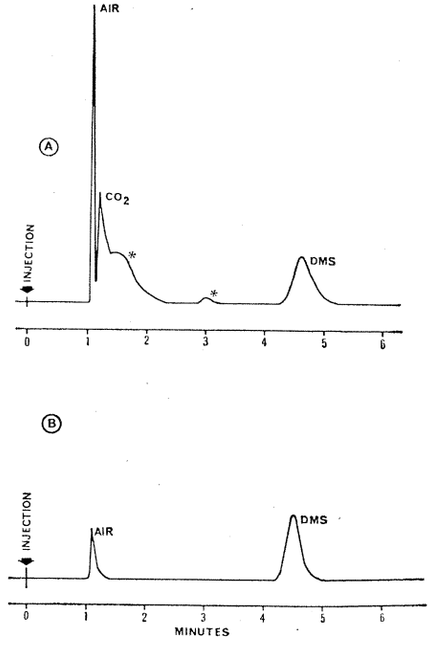

Fig. 1. Gas chromatograms of cat's exhaled breath condensate (*A*) and dimethylsulfide (*B*) (*7*). *A*, 20 μl of condensate, attenuation × 1. Ordinate, recorder excursion; abscissa, time in minutes. *B*, 3 μl of 1 percent of dimethylsulfide in water, attenuation × 16. (*) Unidentified peaks.