respiration is inhibited in the colorless alga *Prototheca zopfii* by irradiation with fluorescent and blue light as a result of the destruction of cytochrome a_3 of the respiratory chain. Another advantage of eliminating the near-ultraviolet light would be to sharpen vision by blocking light of shorter wavelength which the lens may not focus on the retina along with visible light of higher wavelength.

However, the lens may also suffer a major disadvantage as a result of the binding of these aromatic groups to its structural proteins. The additional hydrophobic groups would result in a lowered water solubility of the crystallins. In addition, there would exist an increased possibility for the cross-linking of lens crystallins through the added photooxidized groups [see (*3*)]. Indeed, one form of human cataract, the brunescent type, contains a dark-brown insoluble protein that has properties in common with the colored proteins produced in this study. Figure 1 shows the fluorescence spectrum for the insoluble protein of human brunescent cataract and the product of photooxidized tryptophan and dogfish γ-crystallin. These spectra closely resemble those of the insoluble proteins of human brunescent cataractous lenses, as described by Pirie (*3*). Although the intensities are different as a result of different protein concentrations, the qualitative spectrum is the same. It is possible that the formation of this type of cataract in humans is the result of the reaction between photooxidized amino acids in the lens with lens soluble proteins, so as to render these proteins insoluble. A contribution to the formation of other aging cataracts may also be made in this way. Aging of the skin may also be influenced in a similar fashion.

SEYMOUR ZIGMAN
Departments of Ophthalmology and *Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620,* and *Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

References and Notes

1. S. Duke-Elder, Ed., *System of Ophthalmology* (Mosby, St. Louis, 1958), vol. 1, p. 656; G. F. Cooper and J. G. Robson, *J. Physiol. (London)* **203**, 403 (1969); *ibid.*, p. 411.
2. A report that near-ultraviolet photooxidized PABA combines firmly with lens proteins, thereby coloring them brown, was presented by Dr. L. Fowlkes at the Ophthalmic Biochemistry Conference at Woods Hole, Massachusetts, in June 1970.
3. A. Pirie, *Invest. Ophthalmol.* **7**, 634 (1968).
4. S. Zigman, *Biol. Bull.* **139**, 444 (1970).
5. ——— and S. J. Bagley, *ibid.* **137**, 415 (1969).
6. B. Epel and W. L. Butler, *Science* **166**, 621 (1969).
7. I thank Miss T. Yulo for excellent technical assistance. Supported by research grants from the Rochester Eye and Human Parts Bank and the National Eye Institute (EY-00459) of the Public Health Service.

19 October 1970; revised 18 November 1970 ■

Receptors: Localization and Specificity of Binding of Serotonin in the Central Nervous System

Abstract. *Formation of a Schiff base between the ethylamine residue of serotonin and an appropriate carbonyl residue at the receptor site may be among the forces holding serotonin onto the receptor. Reduction of this imine may provide a means of permanently labeling receptors as a preliminary to their isolation.*

Mounting evidence indicates that reversible attachment of endogenous or exogenous biologically active substances onto specific sites of macromolecular structural elements of the living cell may result in conformational changes of the latter, which, in turn, may have important functional implications (*1*). Information about the nature of such changes could be obtained if the macromolecules under consideration could be isolated and their structure studied. This approach proved most successful in enzyme chemistry. However, receptors do not cause appreciable changes in the interacting micromolecules. Thus, attempts at their isolation must depend on efforts to tag the receptor molecules through permanent specific association with isotopically labeled agonists or antagonists (*2*). Unfortunately, the transient associations of biologically active micromolecules with receptors involve, mostly, a host of weak interactions (the free energy change, ΔG, which accompanies formation of such weak bonds, is in the range of -1 to -7 kcal/mole) like hydrophobic segregation, van der Waals forces, electrostatic attraction between integral or fractional charges, and, to a lesser extent, hydrogen bonding (*3*). Complexing by charge transfer (*4*) may also serve as a stabilizing factor, especially in cases in which the system includes components with delocalized electrons (*5*).

Covalent bonding usually involves large, negative values of ΔG compared to the forces mentioned above. For this reason, and in view of the expected reversibility of interactions involving receptors, covalencies are only considered in special well-documented instances (for example, fluorophosphate anticholinesterases and β-haloalkylamine compounds). In certain instances, however, covalent bonding may be associated with small values of ΔG, fully compatible with very transient complexing in the living cell. For example, changes in free energy (ΔG) associated with the fully reversible formation of Schiff bases between small molecules in solution may be estimated from spectrophotometric data (*6*). Thus, it may be calculated from data of Green and Alexander (*7*) that the ΔG associated with the formation of a Schiff base between salicylaldehyde and *n*-butylamine in aqueous solution at 25°C, at neutrality, or in slightly alkaline milieu (*p*H 8 to 9) is approximately -1 kcal/mole. Furthermore, formation of Schiff bases plays an important and well-documented role in the mechanism of action of many enzymes, like pyridoxal-linked enzymes (*8*), δ-aminolevulinic acid dehydrase (*9*), certain bacterial histidine decarboxylases linked with pyruvic acid (*10*), and monoamine oxidase (*11*); and it participates in the structure of connective tissue (*12*).

The above considerations suggest that formation of Schiff bases could participate in reversible associations of structurally suitable biologically active substances with cellular macromolecules. If substantiated, this theoretical possibility would prove most helpful in attempts to permanently tag receptors.

We have pursued this possibility in a study of the mechanism of association of indoleamines and their metabolic derivatives with brain macromolecules (*13*). Intact serotonin or tryptamine may associate with macromolecules present in the brain through formation of Schiff bases (*14*). The bond may be stabilized by reduction of the Schiff bases with sodium borohydride. The product can then be isolated by standard procedures of protein and lipid chemistry.

We now report evidence regarding the localization and specificity of these binding sites as determined from studies in vivo and in vitro. Unless otherwise indicated, young adult New Zealand white rabbits (2.5 kg) or young adult male Swiss mice (about 30 g) were routinely treated by intraperitoneal injections of pargyline (*N*-benzyl-*N*-

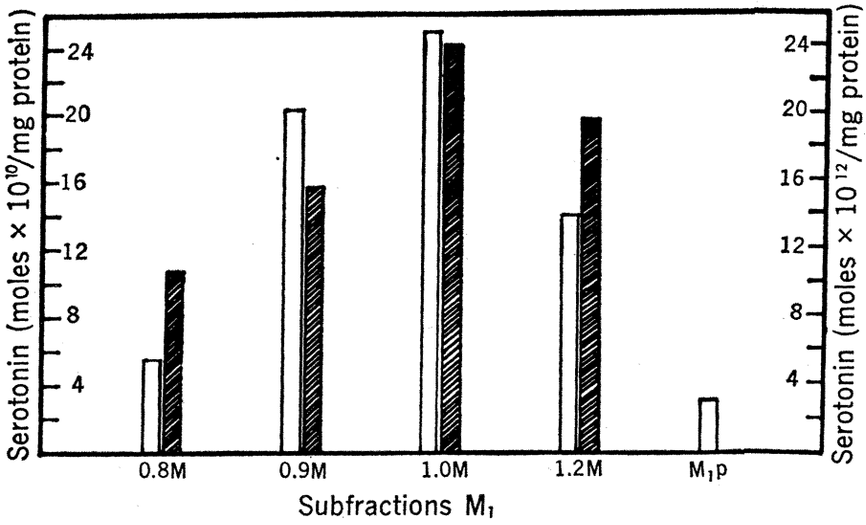

Fig. 1. Comparative localization of radioactivity from labeled serotonin in subfractions M_1 (*18*). Empty bars are from experiments in (*19*). The quantities (10^{-10} mole/mg of protein) in the cat brain were calculated from the corresponding disintegrations per minute per milligram of protein and the specific radioactivity of the original material, as given in (*19*). Shaded bars are from work in our laboratory. Quantities (10^{-12} mole/mg of protein) for the incorporation stabilized by borohydride were calculated as in (*14*). The concentrations of labeled serotonin in the experiments of Fiszer and De Robertis (*19*) and in our study were 3.3×10^{-6} and 1×10^{-6} mole/liter, respectively.

Table 1. Distribution of [^{3}H]tryptamine in the M_1 subfractions (*16*) of rabbit brain. Animals were treated with pargyline (intraperitoneally, 75 mg/kg) 18 hours before decapitation. Brains were homogenized in sucrose bicarbonate buffer containing pargyline (see text). The homogenates were fractionated according to the method described in (*18*). Incubations with [^{3}H]tryptamine (5×10^{-7} mole/liter, specific radioactivity 247 curie/mole) were as described in the text. One enzyme unit is the amount of enzyme (in milligrams of protein) which produces a change of 0.001 absorbance unit/minute at 37°C. Succinic dehydrogenase (SDH) is measured in enzyme units according to the method described earlier (*16*). Ratio indicates the ratio of Δ (radioactivity stabilized by borohydride reduction) to the control (no borohydride).

Subfractions M_1	Incorporation (dpm/mg protein)				
	Borohydride treatment		Δ	Ratio	SDH
	(−)	(+)			
0.8*M*	1398	4615	3217	2.3	0.0
0.9*M*	1102	14221	13119	11.9	0.0
1.0*M*	1046	29715	28669	27.4	3.6
1.2*M*	1680	37638	35958	21.4	12.2
M_1P	1802	13717	11915	6.6	21.7

Table 2. Effect of indole, 5-hydroxyindole, tryptamine, and LSD on the incorporation of serotonin in vitro. Homogenates of mouse brain prepared from animals first treated with pargyline (intraperitoneally, 100 mg/kg) 48 hours and 20 hours before being killed were incubated at 30°C for 30 minutes with uniformly tritiated serotonin in the presence or absence of displacing agents. The *K*eq are the thermodynamic equilibrium constants for the association of substances mentioned in the table with receptors.

Concentration (mole/liter)		Displacement (%)	*K*eq	Approximate Δ*G* (kcal/mole)
Displacing agent	Serotonin			
	5×10^{-7} to 7×10^{-6}		> 100,000	<(− 6.9)
		Indole		
2.50×10^{-4}	5×10^{-7}	67.8		
5.00×10^{-4}	5×10^{-7}	87.1	~ 3,000	− 4.8
1.00×10^{-3}	5×10^{-7}	96.1		
2.00×10^{-3}	5×10^{-6}	31.9		
		5-Hydroxyindole		
1.00×10^{-5}	5×10^{-6}	42.2		
2.50×10^{-5}	5×10^{-6}	56.3	~ 50,000	− 6.5
5.00×10^{-5}	5×10^{-6}	58.8		
		Tryptamine		
5.00×10^{-6}	5×10^{-6}	20.4	> 70,000	<(− 6.7)
		LSD		
1.00×10^{-5}	5×10^{-6}	5.34	≫ 6,500	≪(− 5.2)
1.75×10^{-5}	5×10^{-6}	8.14		
		Mescaline		
1.00×10^{-6}	5×10^{-6}	0.0		

methyl-3-propynylamine hydrochloride), a competitive, irreversible (*15*) inhibitor of monoamine oxidase, which attaches at the active site of the enzyme (*11*). Rabbits received one dose (75 mg/kg) 20 hours before their brains were removed; mice received two doses (100 mg/kg each), one 48 hours and one 20 hours before their brains were removed. The brains were homogenized in a mixture containing in final concentration 0.1*M* sucrose, 0.1*M* sodium bicarbonate buffer (*p*H 8.9), and 0.4 m*M* pargyline (calculated osmolarity, 0.3051). Samples of this homogenate were then incubated at 30°C for 30 minutes with shaking in the presence of labeled serotonin or tryptamine and other compounds as indicated in the tables and figures. At the end of the incubation, samples were treated with sodium borohydride and then acidified. The resultant precipitate was repeatedly washed with 10 percent trichloroacetic acid, and the radioactivity and the amount of proteins in the precipitate and the combined washings were measured as described previously (*14*). Succinoxidase was determined by a modification (*16*) of the method of Slater and Bonner (*17*). Fractionation of the homogenate, after osmotic disruption of the separated crude synaptosome-containing fraction, and subfractionation of the latter in discontinuous sucrose gradients were performed by the methods described by Azcurra and De Robertis (*18*). The individual fractions were then used instead of the homogenate for incubation.

The attachment of serotonin which can be stabilized with borohydride has essentially the same distribution among subsynaptosomal fractions as the "binding" reported by Fiszer and De Robertis (*19*), despite widely different experimental conditions (Fig. 1). In both instances, membranous material stemming from synaptosomal membranes (*18*) is most heavily labeled. Quantitative differences are, most probably, due to differences in experimental conditions. Fiszer and De Robertis (*19*) used the hypothalamic region, basal ganglia, and gray areas of the midbrain of cats—areas rich in serotonergic neurons (*20*). We used the whole brains of rabbits. Furthermore, the concentration of labeled serotonin in our incubation mixtures (Fig. 1) was 3.3 times smaller than that used by Fiszer and De Robertis (*15*). Both factors would tend to decrease the amount of radioactivity bound per milligram of protein in our studies. In addition, Fiszer and De Robertis incubated their samples at

0°C. They assumed that at this temperature monoamine oxidase would not be very active. Since they did not attempt to stabilize the association of serotonin with brain macromolecules, their washings of the complex were by necessity fewer and milder (*15*). However, in our study, monoamine oxidase was irreversibly blocked by initial treatment of the animals with pargyline and by the presence of pargyline in the incubation mixtures (30°C). The stabilized complex was washed exhaustively with acid, a treatment which would be expected to remove most nonspecific binding (*21*). When these differences are considered, qualitative agreement as shown in Fig. 1 is remarkable.

Data in Table 1 are from an experiment with tritiated tryptamine. Increased labeling, due to treatment with borohydride, as expressed by the ratio of the Δ (radioactivity stabilized by borohydride reduction) to the control (no borohydride) is highest in the M_1 1*M* fraction. Distribution of succinic dehydrogenase activity, taken as an indication of the presence of mitochondrial fragments, is entirely different from that of binding stabilized by borohydride. This finding confirms the conclusion that incorporation stabilized by borohydride occurs at synaptic membranes. Distributions of the latter similar to those shown in Fig. 1 were also obtained with serotonin or tryptamine labeled with ^{14}C.

The reversibility and specificity of this binding were tested with the aid of indole, substituted indole derivatives, and other compounds (Fig. 2). Labeled serotonin was administered intracranially. The animals were decapitated 30 minutes later and the displacing agent (nonlabeled serotonin or indole) was added to the homogenate of the brain. Treatment with borohydride followed. Only radioactivity which remained at the acceptor sites was fixed. Evidently serotonin is approximately 40 times more effective as a displacing agent than indole. A relatively more quantitative evaluation of the affinity of various agents for the acceptors was obtained by studies in vitro of homogenates (Table 2). Values of the equilibrium constants, *K*eq, and Δ*G* were calculated from data that had been obtained from several experiments by means of the expressions:

$$\frac{[Rt]}{[RS]} - 1 = \frac{1}{Ks[S]} \quad (1)$$

$$\frac{[RS]}{[Rt]} = \frac{Ks[S]}{1 + Ks[S] + Kd[D]} \quad (2)$$

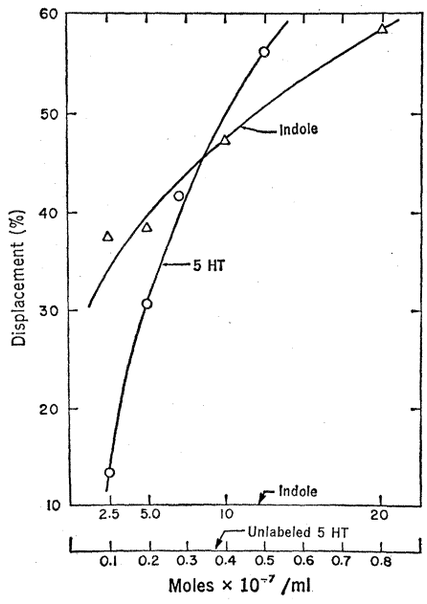

Fig. 2. Effect of unlabeled serotonin or indole on the radioactivity from serotonin administered in vivo, attached to brain macromolecules. Uniformly tritiated serotonin (5 nmole, 5 μc) was injected endocranially and intraventricularly into mice previously treated with pargyline. Thirty minutes later the animals were killed, their brains were homogenized, and samples of the homogenates were incubated for 30 minutes at 30°C with either unlabeled serotonin or indole, at concentrations indicated in the appropriate abscissas. Treatment with borohydride was as described in the text. Maximum incorporation of radioactivity in the acid-washed precipitate (zero displacement) was 34,000 disintegrations per minute (dpm) per milligram of protein (~1.5×10^{-8} mole/kg brain). *5HT*: 5-hydroxytryptamine.

where [*Rt*] is the quantity of acceptors per gram of brain tissue; [*S*] is the molar concentration of labeled serotonin (or tryptamine); [*RS*] is the quantity of the complex per gram of brain; *K*s is the equilibrium constant for the formation of the complex; [*D*] is the molar concentration of the displacing agent; and *K*d is the equilibrium constant for the formation of a complex with the displacing agent.

These equations are modified versions of those used by Klotz *et al.* (*22*) for the binding of organic ions by proteins, and they are based on the simplifying assumption that each interacting site is independent of others, either on the same or in neighboring macromolecules. This assumption is partly true, as evidenced from results plotted in Fig. 3 according to Eq. 1. However, in linear plots of incorporation stabilized by borohydride as a function of concentration of the agonist, sigmoidal deviations from the expected hyperbolic pattern are often observed, as those observed in a recent study by Paton and Rang (*23*). They indicate the possibility of allosteric changes of affinity with increasing concentration of the agonist. For such reasons, values of *K*eq and Δ*G* in Table 2 are given as approximations (determined from the initial parts of the curves). A more de-

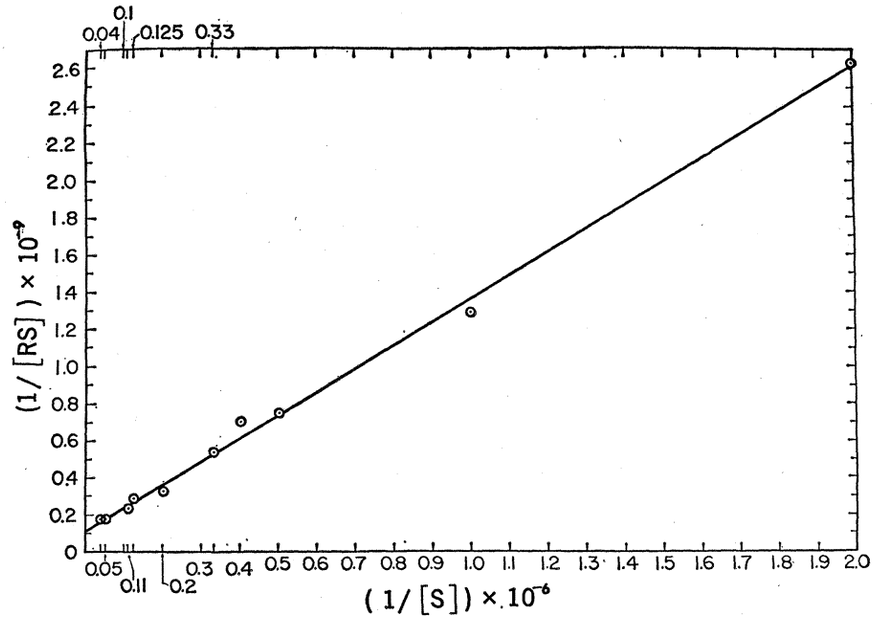

Fig. 3. Reciprocals of moles of borohydride stabilizable incorporation per gram of brain (1/[*RS*]) plotted as a function of the reciprocals of the molar concentration of serotonin (1/[*S*]). Data from incubations in vitro (30 minutes, 30°C) of homogenates of mouse brain prepared from animals first treated with pargyline.

Table 3. Incorporation of radioactivity from labeled [^{3}H]serotonin in egg albumin. Uniformly labeled [^{3}H]serotonin (0.05 μc/ml) was incubated with crystalline egg albumin (Fisher, 3 mg/ml for 30 minutes at 30°C). Treatment with borohydride and processing were as described in the text.

Serotonin (mole/liter)	Incorporation (dpm/mg protein)	
	Without $NaBH_4$	With $NaBH_4$
1×10^{-6}	620	578
4×10^{-6}	316	248

tailed mathematical treatment must await purification of the acceptors. Preliminary results in Table 2 indicate that the 5-hydroxyl group and the 3-ethylamine residue are essential for a firm attachment of the agonist, serotonin, on serotonergic receptors (Fig. 4). Despite important structural differences, LSD-25 is also a relatively efficient displacing agent of serotonin. The equilibrium constant cited in Table 2 represents the lowest conservative limit. Values up to 60,000 were obtained in a number of recent studies. In this instance, inconsistencies are highly suggestive of an allosteric role played by the bulk of the LSD molecule which must overflow in some way beyond the limits of the indole acceptor (*25*). When egg albumin is used instead of brain homogenates there is no incorporation stabilized by borohydride (Table 3). The possibility that monoamine oxidase is the acceptor may be excluded because, in our studies, monoamine oxidase was blocked by pargyline (*11*).

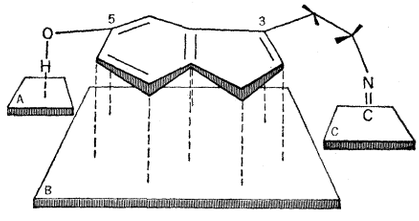

Fig. 4. Working schematic representation of the attachment of serotonin at acceptor sites in the brain. The indole system of the molecule becomes rather weakly attached (at *B*), probably through hydrophobic segregation and complexing by charge transfer. Hydrogen bonding of the hydroxyl moiety (at *A*), increases the firmness of the association by approximately 20-fold. Similarly, the ethylamine side chain enhances the affinity of the molecule over 20-fold. This is partly due to Schiff base formation (at *C*). The role of the (hydrophobic) aliphatic carbon atoms in the side chain has not yet been studied.

Because the term "receptor" was historically derived as a pharmacological concept (*26*) and presupposes higher organization than is biochemically feasible, the general term "acceptor" should be used in in vitro studies to refer to those macromolecules which bind serotonin. At present only circumstantial evidence can link acceptors to receptors. Such evidence (*27*) includes localization of the material prior to its isolation, specificity of binding, and quantities commensurate with present concepts (*28*). In our study, the distribution of the attachment of serotonin (nerve-ending membranes) is identical to that obtained by De Robertis and his co-workers and is highly suggestive of the expected distribution of such receptors. Our methodology permits stabilization of the labeled agonist on the acceptors. This not only reveals a novel mechanism of attachment, but it also permits convenient and detailed study of the specificity of the attachment of serotonin. Our studies indicate that this attachment is highly specific. The equilibrium constant indicates high affinity ($Keq \geq 10^{+5}$; $\Delta G \sim -7$ kcal/mole), and it may be predicted that this attachment will occur easily at concentrations at which serotonin occurs in vivo. Furthermore, the quantities detected and those extrapolated from our studies (1×10^{-8} to 5×10^{-8} mole per kilogram of brain; Fig. 1 and Fig. 2) are in harmony with those expected for receptors.

SPYRIDON G. A. ALIVISATOS
FRIEDA UNGAR
PRAHLAD K. SETH
LEROY P. LEVITT
ANTHONY J. GEROULIS
THOMAS S. MEYER

Department of Biochemistry,
Chicago Medical School,
2020 West Ogden Avenue,
Chicago, Illinois 60612

References and Notes

1. J.-P. Changeux, J. Thiéry, Y. Tung, C. Kittel, *Proc. Nat. Acad. Sci. U.S.* **57**, 335 (1967); A. Karlin, *J. Theor. Biol.* **16**, 306 (1967).
2. *Enzymes and Drug Action*, J. M. Mongar and A. V. S. de Rueck, Eds. (Little Brown, Boston, 1962), p. 435.
3. E. W. Gill, in *Progress in Medicinal Chemistry*, G. P. Ellis and G. B. West, Eds. (Butterworths, Washington, D.C., 1965), p. 39.
4. J. Weiss, *J. Chem. Soc.* **245**, 18 (1942); R. S. Mulliken, *J. Phys. Chem.* **56**, 801 (1952); R. S. Mulliken, *J. Amer. Chem. Soc.* **74**, 811 (1952); B. Pullman and A. Pullman, *Quantum Biochemistry* (Interscience, New York, 1963).
5. S. G. A. Alivisatos and P. K. Seth, in *Methods and Techniques of Neuroscience*, R. Fried, Ed. (Dekker, New York, in press).
6. D. E. Metzler, *J. Chem. Soc.* **79**, 485 (1957); L. Davis, F. Roddy, D. E. Metzler, *J. Amer. Chem. Soc.* **83**, 127 (1961); R. J. Johnson and D. E. Metzler, *Methods Enzymol.* **18A**, 433 (1970).
7. R. W. Green and P. W. Alexander, *Aust. J. Chem.* **18**, 329 (1965).
8. E. E. Snell, P. M. Fasella, A. Braunstein, R. Fanelli, in *Chemical and Biological Aspects of Pyridoxal Catalysis* (Pergamon, New York, 1963).
9. D. L. Nandi and D. Shemin, *J. Biol. Chem.* **243**, 1236 (1968).
10. W. D. Riley and E. E. Snell, *Biochemistry* **7**, 3520 (1968); D. Hodgins and R. H. Abeles, *J. Biol. Chem.* **242**, 5158 (1967).
11. L. Hellerman and V. G. Erwin, *J. Biol. Chem.* **243**, 5234 (1968).
12. A. J. Bailey, *Biochim. Biophys. Acta* **160**, 447 (1968).
13. S. G. A. Alivisatos, F. Ungar, S. S. Parmar, *Biochem. Biophys. Res. Commun.* **25**, 495 (1966); S. G. A. Alivisatos and F. Ungar, *Biochemistry* **7**, 285 (1968); ———, S. S. Parmar, P. K. Seth, *Biochem. Pharmacol.* **17**, 1993 (1968); S. G. A. Alivisatos, F. Ungar, P. K. Seth, L. P. Levitt, *ibid.* **19**, 401 (1970); S. G. A. Alivisatos, *Neurosci. Res. Program Bull.* **8**, 34 (1970).
14. S. G. A. Alivisatos, A. D. Papaphilis, F. Ungar, P. K. Seth, *Nature* **226**, 455 (1970).
15. For definitions of inhibitory activity see J. F. Moran, C. R. Triggle, D. J. Triggle, *J. Pharm. Pharmacol.* **21**, 38 (1969).
16. E. De Robertis, A. Pellegrino de Iraldi, G. Rodriquez de Lores Arnaiz, L. Salganicoff, *J. Neurochem.* **9**, 23 (1962).
17. E. C. Slater and W. D. Bonner, Jr., *Biochem. J.* **52**, 185 (1952).
18. E. De Robertis, *Science* **156**, 907 (1967); J. M. Azcurra and E. De Robertis, *Int. J. Neuropharmacol.* **6**, 15 (1967).
19. S. Fiszer and E. De Robertis, *J. Neurochem.* **16**, 1201 (1969).
20. J. H. Gaddum and N. J. Giarmann, *Brit. J. Pharmacol.* **11**, 88 (1956); A. Carlsson, B. Falck, N-A. Hillarp, *Acta Physiol. Scand.* **56** Suppl., 196 (1962).
21. R. H. McMenamy, *J. Biol. Chem.* **240**, 4235 (1965).
22. I. M. Klotz, H. Triwush, F. M. Walker, *J. Amer. Chem. Soc.* **70**, 2935 (1948); I. M. Klotz, in *The Proteins*, H. Neurath and K. Bailey, Eds. (Academic Press, New York, ed. 1, 1953), vol. 1, p. 727. The term "displacing agent" is used somewhat loosely in this context. It is understood that for the lapse of time that a receptor site is empty, either serotonin or the displacing agent will have the chance to occupy this site. Thus, statistically, the number of available sites for serotonin, for any interval of time, will be limited in the presence of the displacing agent.
23. W. D. M. Paton and H. P. Rang, *Proc. Roy. Soc. London, Ser. B.* **163**, 1 (1965); H. P. Rang, *Ann. N.Y. Acad. Sci.* **144**, 756 (1967).
24. A similar displacing effect of LSD was also reported by R. M. Marchbanks [*Biochem. Pharmacol.* **16**, 1971 (1967)] for the "high affinity binding" of serotonin to synaptosomes [R. M. Marchbanks, F. Rosenblatt, R. D. O'Brien, *Science* **144**, 1135 (1964)], which is, most probably, identical to the "borohydride stabilizable incorporation" reported here.
25. M. J. Greenberg, *Brit. J. Pharmacol.* **15**, 375 (1960).
26. Reviewed by H. O. Schild, in *Importance of Fundamental Principles in Drug Evaluation*, D. H. Tedeschi and R. E. Tedeschi, Eds. (Raven, New York, 1968), p. 257.
27. Efforts to isolate receptors until 1968 have been reviewed by S. Ehrenpreis, J. H. Fleisch, T. W. Mittag [*Pharmacol. Rev.* **21**, 131 (1969)]. Efforts from 1969 through 1970 are reviewed in (*5*).
28. See, for example, P. G. Wasser, *Ann. N.Y. Acad. Sci.* **144**, 737 (1967).

29. Supported in part by grants from the State of Illinois Department of Mental Health (101-13-RD), PHS (5 R01 MH15410), American Cancer Society, Inc. (P-424A), and W. and M. Deree Foundation, M. and C. Caditz, and J. Daros. P.K.S. was a postdoctoral trainee of the Schweppe Foundation. A.J.G. and T.S.M. were predoctoral trainees.

10 December 1970