J,Pharmacol.exp.Ther. 134, 160-166 (1961).
LSD 958/BOL/DES

EFFECTS OF LSD-25 ON BRAIN SEROTONIN[1, 2]

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Received for publication April 3, 1961

Proposals that autonomic, behavioral, and mental effects of *d*-lysergic acid diethylamide (LSD-25) could be explained by effects of the drug on brain serotonin (5-HT) have been based on observations of interactions in various peripheral organs (Gaddum and Hameed, 1954; Woolley and Shaw, 1954; Shaw and Woolley, 1956). Studies which could directly elucidate the mode of biochemical interaction between LSD-25 and 5-HT in brain have not revealed an effect of the drug on metabolism of the amine (Brodie *et al.*, 1956; Paasonen and Giarman, 1958). The present experiments were designed to determine the direction of any LSD-25 induced change in whole brain levels of 5-HT and to investigate effects on some central sites and mechanisms related to the metabolism of 5-HT.

METHODS. *Studies of brain serotonin.* Male Sprague-Dawley rats of 100 to 150 g were sacrificed in the morning by decapitation and exsanguination, and within 5 minutes the brain was removed and weighed (excluding cerebellum, pituitary, and olfactory bulbs). After addition of isotonic saline to make a total of 3 g, the brain was homogenized and the homogenate extracted in 20 volumes of 99% acetone (analytical reagent) with a final concentration of 95%, according to the method of Amin, Crawford, and Gaddum (1954). After 1 hour the extracts were filtered through Whatman no. 1 filter paper and the residue was washed twice with 15 cc 95% acetone. The acetone extract was then concentrated *in vacuo* at 37°C to the aqueous phase, which was then defatted by two treatments with 20 cc petroleum ether. Five minutes after each treatment, the petroleum ether phase was aspirated and discarded, and the extract was taken to complete dryness *in vacuo* at 38°C. Extracts were usually promptly assayed, and when this was not possible, they were stored overnight at −20°C and assayed the following day without loss due to storage.

5-HT was determined by bioassay with the isolated heart of *Mercenaria* (*Venus*) *mercenaria* (Twarog and Page, 1953; Welsh, 1953) maintained in sea water (filtered and UV irradiated) containing benzoquinonium 3 mg/l to block the action of acetylcholine, according to the method described by Gaddum and Paasonen (1955). Before assaying unknown extracts, all hearts were tested for sensitivity and discriminability. In our hands, these hearts usually respond to 1 mμg 5-HT and can discriminate differences of 1 mμg added to the 5-cc bath. Hearts failing to detect as little as 4 mμg were not used. After a stable base line was established, the extract was added to the 5-cc aerated bath and kept in contact with the heart for 30 seconds. The heart was then washed for 2 minutes to allow the base line to return to normal. Failures to return to normal (indicating either a spontaneous change in sensitivity or an effect induced by interfering substances) were investigated. In a given assay, all unknown extracts were measured between at least two treatments with the standard, so that any influence of the unknown extract upon the measures of 5-HT could be detected. No systematic changes in sensitivity attributable to extracts from drug treated animals were encountered. Dilutions of extracts and volumes assayed varied according to the amount of 5-HT in the extract. Usually 0.02 ml of a 3-fold dilution was used, and no volume greater than 0.25 ml regardless of dilution was used. These quantities of both the standard 5-HT and the unknown were delivered to the bath by means of ¼-cc tuberculin syringes. The standard solutions of serotonin creatine sulfate contained 10 μg of the free base in 100 cc of sea water; therefore, all data hereinafter are expressed in terms of free serotonin. Following completion of all the bioassay studies, experiments using spectrophotofluorimetric assay for 5-HT were carried out according to the method described by Udenfriend *et al.* (1958) on an Aminco-Bowman voltage stabilized spectrophotofluorometer. In control experiments it was determined that the presence of 1.5

[1] Aided by grants M-1204 and MY 3363 from the National Institute of Mental Health.

[2] A preliminary report of this work appeared in Fed. Proc. 19: 266, 1960.

μg LSD-25 or less in the final extract would not interfere with this assay for 5-HT.

For intracellular distribution studies, after centrifugation at 100,000 × *g* with a Spinco Refrigerated Preparative Ultracentrifuge, the fractions were treated according to the method of Giarman and Schanberg (1958), and assayed as above. For matched experiments (*e.g.*, comparisons of LSD-25 with other ergot derivatives) at least four rats in each drug and control group were assayed with the same heart. In studies with LSD-25 and reserpine, all drugs were administered intraperitoneally; usually reserpine was administered to all animals, and after various intervals some of these rats received LSD-25, and 2 hours later all the animals were sacrificed. LSD-25 was available as the tartrate (mw 398.4 (2)), *d*-2-brom lysergic acid diethylamide (BOL-148) as the bitartrate (mw 552.4), *d*-1-acetyl-lysergic acid diethylamide (ALD-25) as the bitartrate (mw 515.6), 1-methyl-*d*-lysergic acid butanolamide (UML-491) as the tartrate (mw 857), and *l*-lysergic acid diethylamide as the tartrate (mw 398.4 (2)).

Biotransformation of serotonin. Monoamine oxidase activity of untreated animals and of animals treated with 520 μg/kg LSD-25 and sacrificed 20 minutes post injection was determined as follows. Brains were weighed and homogenized in 10 volumes of a modified McIlwain medium, buffered at pH 7.4. Serotonin creatine sulfate in amounts from 2 to 20 μg (of base) was added to aliquots of the homogenate representing 250 mg of tissue. The suspension was incubated and shaken at 37°C and at the end of an hour the entire mixture was extracted and assayed as described above.

5-Hydroxytryptophan decarboxylase activity of untreated and LSD-25 treated brains was determined according to the method of Gaddum and Giarman (1956).

Control studies for validity of measurements. A random order of application of extracts to the heart, bracketing of the unknown between two measures of the known, and a minimum of two applications of a single extract were routinely employed. This permitted a replication of the measure of 5-HT in the extract and allowed detection of any "sensitizing" effects on the heart by extracts from animals placed on different drug regimens. With the 2-minute cycle described above, both qualitative and quantitative indices of change are available. LSD-25 in different quantities was added to homogenates and to the bath to determine interference, if any, with measures of 5-HT. The quantity of LSD-25 in extracts was measured spectrophotofluorimetrically by the method of Axelrod *et al.* (1957). The recovery of 5-HT added to brain homogenates was determined as follows. Rats were treated with 4 mg reserpine/kg, 22 hours later they received 520 μg LSD/kg, and after 120 minutes they were sacrificed along with untreated rats. Brain homogenates from each group of rats were pooled, and 0, 125, 250, and 500 mμg 5-HT added to 3-ml aliquots from each pool; extraction and assay proceeded as described above for fluorimetric assay and bioassay.

Statistical methods. Significance of the difference between means was determined by the Student *t* test. Such *t*'s are obtained by the Cochran-Cox method of weighting the two *t* values in terms of their respective variances (Snedecor, 1946).

RESULTS. 1. *Effects of LSD-25 on brain serotonin.* With doses of 100 μg or less of LSD-25/kg no significant effect of LSD-25 on total brain 5-HT was discerned. Animals were initially depleted of 5-HT by reserpine with the hope that from the very low initial values of 5-HT any LSD-25 induced elevations would then be clearly evident. Accordingly, rats were given 1 mg reserpine/kg and 22 hours later 130 μg LSD-25/kg. Ten minutes or 2 hours later they were sacrificed along with animals treated with reserpine 24 hours prior to sacrifice and control rats treated either with saline or with 130 μg LSD-25/kg 10 minutes or 2 hours before sacrifice.

TABLE 1

Effect of LSD-25 on brain 5-HT

Treatment	Number of Rats	Mean 5-HT mμg/g	Standard Deviation	Difference between Means*	P
Saline control	17	362	80		
LSD 130 μg/kg	14	449	118	+87	<.05
1 mg Reserpine/kg					
Reserpine (R)	13	126	55		
R and LSD 130 μg/kg	28	247	72	+121	<.001
2 mg Reserpine/kg					
Reserpine (R)	14	68	34		
R and LSD 130 μg/kg	6	129	76	+61	>.10
R and LSD 520 μg/kg	14	177	73	+109	<.001
R and LSD 1300 μg/kg	6	183	11	+115	<.001
4 mg Reserpine/kg					
Reserpine (R)	25	68	29		
R and LSD 35 μg/kg	6	67	6	−1	—
R and LSD 130 μg/kg	7	89	20	+21	<.06
R and LSD 520 μg/kg	10	144	37	+76	<.001
R and LSD 1300 μg/kg	6	181	4	+113	<.001

Animals given reserpine 22 hr prior to LSD-25 and sacrificed 10 min to 2 hr after LSD-25 (see text).

* Differences between means of LSD-25 treated animals and of the appropriate saline or reserpine treated rats.

TABLE 2

Duration of LSD-25 effect on brain 5-HT

	2 hr after LSD (22–24 hr after Reserpine)		96 hr after LSD (118–120 hr after Reserpine)		% Difference	P
	N	Mean 5-HT mμg/g	N	Mean 5-HT mμg/g		
2 mg Reserpine						
Reserpine	14	68	4	141		
Reserpine plus 520 μg LSD/kg	14	177	8	226	+61	<.05
4 mg Reserpine						
Reserpine	25	68	7	153		
Reserpine plus 1300 μg LSD/kg	4	182	8	207	+35	<.05

Rats were injected with 2 or 4 mg reserpine/kg, 22–24 hr later with 520 or 1300 μg LSD-25, and sacrificed 2 or 96 hr after the single injection of LSD-25.

Table 1 shows that brain 5-HT levels were approximately doubled in the reserpine-LSD-25 treated group. There was no significant difference between animals sacrificed at 10 minutes or 120 minutes after LSD-25. The same order of effect on levels of brain 5-HT can be seen with animals given 2 or 4 mg reserpine/kg and 22 hours later 520 μg LSD-25/kg; these rats were sacrificed 2 hours following LSD-25. All treatments with LSD-25 in doses of 130 μg/kg or more, with or without reserpine, showed higher brain 5-HT levels than the matched control groups (table 1).

2. *Effects of order of administration and dose.* Twelve rats pretreated with doses of 130 to 520 μg LSD-25/kg were injected 2 hours later with 2 mg reserpine/kg and sacrificed 6 to 18 hours following reserpine. No effect on reserpine induced depletion of 5-HT was observed, a finding previously reported by Brodie *et al.* (1956).

When the effects of 130 or 520 μg LSD-25/kg are compared in rats receiving 1, 2, or 4 mg reserpine/kg, the dose of reserpine significantly influenced the magnitude of the LSD-25 effect, indicating some competitive interaction.

Statistically significant ($P < .05$) dose effects of 35, 130, and 520 μg LSD-25/kg were apparent in studies with 4 mg reserpine/kg; differences in effect of dosage in studies with 2 mg reserpine/kg did not prove to be significant. There appears to be an upper limit to the LSD-25 effect, since experiments with doses of 1300 μg/kg did not induce markedly higher levels of 5-HT than did doses of 520 μg/kg. Nor was a cumulative effect of LSD-25 seen, since 130 μg LSD-25 given at 24-hour intervals for 8 days to 10 animals produced no greater increases than a single dose of 130 μg/kg when animals were sacrificed 2 hours following the last dose. Three daily doses of 130 μg LSD-25/kg given at 24, 48, and 70 hours following 1 mg reserpine/kg to 14 animals produced no greater elevation than a single dose when animals were sacrificed within 2 hours following LSD.

In summary, the onset of the LSD-25 effect was found within 10 minutes following injection and the effect persisted unchanged over the following 2 hours. Within limits, the amount of LSD-25 did not appreciably influence the magnitude of effects on brain 5-HT. A single dose was as effective as several daily doses. In reserpine treated rats, the immediate effect of LSD-25

TABLE 3

Distribution of brain 5-HT (homogenates centrifuged at 100,000 × g for 25 min)

Treatment	Number of Rats	5-HT Content, mμg/Whole Brain				Mean % of Total 5-HT in Supernatant
		Particulate		Supernatant		
		Mean	S.D.	Mean	S.D.	
Bioassay						
Saline control	12	329	44	138	12	30
LSD, 520 μg/kg	20	653*	68	181	13	22
Reserpine, 2 mg/kg	8	60	5	69	7	53
R and LSD, 520 μg/kg	8	321*	23	64	9	17
Fluorimetric assay						
Reserpine, 2 mg/kg	8	168	7	—	—	—
R and LSD, 520 μg/kg	8	220*	21	—	—	—

* Difference between mean of LSD-25 treated group and matched control or reserpine treated group significant beyond .001 level.

on brain 5-HT depended both on the dose of reserpine and the dose of LSD-25.

3. *Duration of LSD-25 effect in reserpine treated rats.* An effect of LSD-25 on the post-reserpine recovery curve of 5-HT in brain can be detected up to 96 hours following a single injection of 520 μg LSD-25/kg in animals pretreated with 2 or 4 mg of reserpine/kg. Animals were injected with LSD-25 24 hours after their reserpine injection, and sacrificed at 72 or 96 hours after their LSD-25 injection. No significant differences were detected beyond 96 hours; the time course between 2 and 72 hours after LSD-25 was not studied. Thus, effects of a *single* dose of LSD-25 are apparent during the height of reserpine effects on brain 5-HT and during the period of recovery from reserpine effects (table 2).

4. *Effect of LSD-25 on the distribution of brain serotonin.* Since the finding that brain 5-HT increased following a single dose of LSD-25 was most clearly apparent in animals treated with reserpine, a common receptor site for such effects was inferred. Giarman and Schanberg (1958) have demonstrated that the depletion of 5-HT induced by reserpine occurred largely in intracellular granules of brain. Accordingly, animals were treated with 520 μg LSD-25/kg and sacrificed 20 to 120 minutes later, and the particulate and nonparticulate fractions of brains were assayed following highspeed centrifugation. The results clearly indicated high levels of 5-HT in the particulate fraction and little or no change in the supernatant. The increase in the particulate appears to account for the increases observed in the previous studies with whole brain (table 3). Pretreatment with 2 mg reserpine/kg followed in 24 hours by 520 μg LSD-25/kg similarly showed the increase to be in the particulate. Spectrophotofluorimetric assay of the particulate fraction also showed this increase.

5. *The effect of LSD-25 on the biotransformation of serotonin.* The above results may be ascribed to increased synthesis or decreased destruction of 5-HT. In three experiments with animals treated with 520 μg LSD-25/kg and sacrificed 20 minutes later, the destruction of 5-HT by the LSD-25 treated brains was not different from that observed with the untreated brains. The net synthesis of 5-HT from 2×10^{-4} M 5-hydroxytryptophan following either the administration of 520 μg LSD-25/kg to rats 20 minutes prior to sacrifice or the addition of 1 μg of LSD-25 per ml of brain homogenate did not differ from that observed in untreated rat brain. 520 μg LSD-25/kg did not influence the rise and fall in brain 5-HT observed following intraperitoneal injection of 100 mg 5-hydroxytryptophan/kg.

TABLE 4

Comparison of effect on brain 5-HT of LSD derivatives

	Reserpine Alone	520 μg LSD-25	671 μg ALD-52	718 μg BOL-148	1300 μg BOL-148
2 mg Reserpine/kg					
Mean 5-HT (mμg/g)	71	168*	113†	76	—
Standard deviation	39	50	69	47	—
Number of rats	18	14	16	18	—
4 mg Reserpine/kg					
Mean 5-HT (mμg/g)	68	144*	—	64	114
Standard deviation	29	37	—	43	60
Number of rats	25	10	—	12	6

Animals were sacrificed 20 to 120 min after the ergot derivative was administered and 18 to 24 hr after the administration of reserpine.

* Difference from reserpine alone, significant $P < .05$.

† Difference from reserpine alone, significant $P < .10$.

6. *Effects of related compounds and procedures.* Eighteen to 24 hours after treatment with 2 mg reserpine/kg rats received 520 μg *l*-lysergic acid diethylamide/kg or 520 μg UML/kg or 5 mg metamphetamine/kg, and were sacrificed 20 to 120 minutes later. No rise in brain 5-HT was observed in 21 animals. No rise was observed in 7 animals treated with aversive doses of electroshock to the feet. Following reserpine pretreatment, BOL-148 did show an effect in elevating brain 5-HT, but one less potent than that induced by LSD-25 or ALD-52 (table 4). Animals pretreated with 4 mg reserpine/kg required a dose of BOL-148 which was over twice the equivalent dose of LSD-25 to show an effect. No effect of 1300 μg BOL/kg was seen without pretreatment with reserpine either by bioassay or fluorimetric assay.

7. *Validity and specificity of LSD-25 effect.* A series of observations indicated that pretreat-

ment with LSD-25 does not lead to artifactitious, high measures of 5-HT. 120 minutes after 520 μg LSD-25/kg (with or without 4 mg reserpine/kg 24 hours previously) the quantity of LSD-25 in extracts prepared for bioassay was less than 1 mμg/g. In agreement with Welsh (1953), we found that the heart shows a prompt and sustained response (over 120 minutes) to 3 to 4 mμg/cc of LSD-25 in the 5-cc bath; in concentrations below 2 mμg/cc, LSD-25 must remain in contact with the heart for many minutes beyond the 2-minute cycle employed in these studies for maximal effects to be observed.

200 mμg LSD-25 added directly to the homogenate did not change the measured values for brain 5-HT in either LSD-25 treated or the untreated portions of the homogenate, nor was the sensitivity for the standard solution of 5-HT changed. Addition of 300 mμg LSD-25 did not change measures for 5-HT although a slight and persistent rise in the base line occurred following application of the first extract of the LSD-25 treated homogenate. Since such extract-contingent effects were not observed with extracts from *in vivo* treated animals, and since the measured amounts of LSD-25 in the extracts were below the threshold for effects in this assay, it seems probable that the drug did not interfere with measures for 5-HT. Nor was the drug detectable fluorimetrically 96 hours after a single dose.

TABLE 5

Effect of LSD-25 on brain 5-HT (fluorimetric assay)

Treatment	Number of Rats	Mean 5-HT mμg/g	Standard Deviation	Difference between Means*	P
LSD 1300 μg/kg					
Saline control	4	538	38		
LSD, sacrificed 30 min	4	644	46	+106	<.06
Saline control	5	488	23		
LSD, sacrificed 60 min	7	587	17	+99	<.01
Saline control	8	530	60		
LSD, sacrificed 4 hr	8	536	57	+6	—
LSD 520 μg/kg					
Saline control	4	594	30		
LSD, sacrificed 60 min	4	672	21	+78	<.05
LSD 260 μg/kg					
Saline control	4	533	18		
LSD, sacrificed 30 min	4	630	51	+97	<.06
Total LSD 260, 520, and 1300 μg/kg					
Saline controls	17	535	28		
LSD, sacrificed 30 or 60 min	19	626	34	+91	<.001
BOL 1300 μg/kg					
Saline control	4	581	31		
BOL, sacrificed 30 min	4	592	32	+11	—
Saline control	4	594	24		
BOL, sacrificed 60 min	4	578	38	−16	—

* Brain 5-HT of each experimental group was assayed along with that of a saline control group; differences are between means of the LSD or BOL treated animals and of the corresponding control group. 5-HT in the brain of each individual rat was individually determined; *i.e.*, results are not based upon pooled brains.

Pretreatment with reserpine and LSD-25 did not increase the efficiency of extraction of 5-HT either by the acetone or the butanol procedures, since recovery of added 5-HT ranged from 86 to 98% with the acetone extraction and from 92 to 105% with the butanol extraction with no differences attributable to pretreatment. Since liver concentrates both metabolized and unchanged LSD-25 (Axelrod *et al.*, 1957), higher false values for 5-HT contingent upon such substances should be found in that organ. The liver of 12 animals, each showing elevation in brain 5-HT (2 hours following 130 μg LSD-25/kg and 24 hours after 1 mg reserpine/kg), showed no difference in measures of 5-HT compared with the liver of 6 control animals given 1 mg reserpine/kg. Finally, the effects of LSD-25 on brain 5-HT as defined by the bioassay can be essentially replicated by the spectrophotofluorimetric assay (table 5).

Discussion. These results indicate that LSD-25 leads to increases in whole brain levels of 5-HT. The effect is contingent upon the psychoactive *d*-LSD-25, since the *levo* preparation was without effect. While no systematic attempt was undertaken to compare the two assay procedures, the effect was evident with both bioassay and fluorimetric assay. Tests with both procedures indicated that the noted elevations could not be ascribed to the presence of metabolized or unchanged quantities of LSD-25 and that premedication did not alter the precision with which 5-HT was measured. While it seems unlikely in view of recovery experiments that substances other than 5-HT were falsely read as 5-HT by both procedures, chromatographic methods would definitely settle the issue.

There are previous reports of no effect of LSD-25 on 5-HT in rabbit brain measured

spectrophotofluorimetrically (Brodie *et al.*, 1956) and no effect in rat brain measured with bioassay (Paasonen and Giarman, 1958). The lack of effect observed in both studies may be explained by differences in dose regimen, time of sacrifice (4 hours following medication), and, in view of the small differences to be measured, the number of animals employed (two in each study).

Of more direct relevance to these findings is the mechanism by which LSD-25 induced an increase in the particulate fraction. The fact that neither decreased destruction nor increased synthesis could be demonstrated does not rigorously exclude these mechanisms. On the other hand, an effect of LSD-25 on the active transport of 5-hydroxytryptophan by brain slices could not be demonstrated by Schanberg and Giarman (1960). Thus it would appear that availability of the substrate to the synthesizing enzyme is not an explanation for the noted effects. Udenfriend and Weissbach (1958) have presented data suggesting a rapid synthesis and release of 5-HT in brain, the estimated half-life being in the order of minutes. It is thus possible that within 10 minutes after injection of LSD-25 an increment of 5-HT in the particulate could occur without "expense" to the supernatant by an increase in binding of the newly formed amine. From presently available evidence, it would be descriptively convenient to characterize the observed effects of LSD-25 as inducing a binding of 5-HT by the affected storage site and as stimulating the recovery of binding capacity after pretreatment with reserpine. The mechanism of such effects remains to be elucidated (Green, 1961).

The nature of the receptor substances in the particulate fraction is similarly obscure. A complex of receptor sites could be expected; *i.e.*, sites for storage and release, and sites for activity with respect to function. The view that the functionally significant receptors for 5-HT and LSD-25 are lodged in the postsynaptic neural elements may be valid (Marrazzi and Hart, 1955), although it seems equally likely that these receptors are lodged in intracellular particles; their alteration, in turn, could conceivably change the requirements for the response of a neuron to an input and thus influence neural function.

While the problem of the locus of receptors relevant for neural activity cannot presently be resolved, the particulate receptors for binding and release of 5-HT are presumably sites at which LSD-25 is active in terms of effects on 5-HT. The postulated receptor sites in brain probably differ from peripheral receptors since peripheral antiserotonin potency (Cerletti and Doepfner, 1958) does not correlate with the potency of the tested ergot derivatives in inducing binding. These central receptors appear to have a specific affinity for specific molecular configurations *e.g.*, *d*-LSD-25 and not *l*-LSD-25. A closely related set of receptors for drugs which induce release of 5-HT and drugs which induce binding may be inferred. The effect of increasing the dose of reserpine from 1 to 2 or 4 mg/kg was to reduce the quantity of 5-HT which was bound following a dose of 130 to 520 μg LSD-25/kg. This dose interaction effect could indicate a competitive interaction at related receptor sites; perhaps it reflects the difference between the rates of two separate processes—binding and release. While LSD-25 was more effective than BOL-148 in inducing binding of 5-HT, the quantities of both drugs in whole brain are not different (Filbert *et al.*, personal communication). Therefore, accessibility to receptor sites rather than the concentration of drug in whole brain may account for the noted differences in binding of 5-HT. An effect of BOL-148 on binding was not apparent until rats were pretreated with reserpine. Whether reserpine pretreatment alters the accessibility of the receptor sites to BOL-148 or facilitates detection of some binding action of the drug can in part be determined by differential centrifugation studies.

The long duration of reserpine induced impairment of binding (Brodie, 1957; Freedman and Benton, 1961) and of LSD-25 induced enhancement of binding following reserpine cannot readily be explained. While a number of drug interaction studies may further elucidate the relationships of the involved receptors, the mechanism of binding and the chemical identification of the receptor substances remain the critical unsolved problems in the pharmacology of brain 5-HT and LSD-25.

SUMMARY

LSD-25 in doses of 130 to 1300 μg/kg induced a small and significant rise in levels of rat brain serotonin within 10 to 120 minutes following administration. A single dose was as effective as

several daily doses. In reserpine treated animals, LSD-25 induced a 2-fold elevation in brain serotonin, and effects were found 96 hours following a single dose of LSD-25. A dose-effect interaction of LSD-25 and reserpine was evident. Peripheral antiserotonin potency does not correlate with the effect on brain serotonin since the order of potency in elevating brain serotonin following reserpine induced depletion was: LSD-25, ALF-52, and BOL-148.

With high-speed centrifugation the effects of LSD-25 on brain serotonin are largely in the particulate fraction. LSD-25 *in vivo* or *in vitro* did not increase the destruction of serotonin or change the net synthesis of serotonin from 2×10^{-4} M 5-hydroxytryptophan. These findings are interpreted as an effect of LSD-25 on inducing binding of brain serotonin and stimulating the binding of serotonin following reserpine induced depletion.

ACKNOWLEDGMENTS. The author gratefully acknowledges the technical assistance of Miss Hedi Suter and Mr. Stephen Pachl; the aid of Mr. Saul Schanberg with centrifugation studies; the counsel of Dr. N. J. Giarman; and the aid of Dr. K. A. Chandler with statistics.

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