Journal of Neurochemistry, 1961, Vol. 8. pp. 72 to 80.

THE *IN VITRO* INHIBITORY EFFECT OF LSD, ITS CONGENERS AND 5-HYDROXYTRYPTAMINE ON HUMAN CHOLINESTERASES*†

E. K. ZSIGMOND‡, F. F. FOLDES and V. M. FOLDES
Department of Anesthesia of the Mercy Hospital and the Section on Anesthesiology, Department of Surgery, The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

(*Received* 17 *February* 1961)

ALTHOUGH it has been known since 1943 that D-lysergic acid diethylamide (LSD)§ is a potent psychotropic drug (STOLL and HOFMANN, 1943; STOLL, 1947), no satisfactory explanation has been offered for its mechanism of action (ROTHLIN, 1957*a*; ROTHLIN and CERLETTI, 1956). POLONI and MAFFEZZONI (1952) observed an increase of the acetylcholine (ACh) content in the brain of guinea pigs after the administration of LSD. Subsequently, THOMPSON, TICKNER and WEBSTER (1954) studied the effect of LSD on human cholinesterases, They reported that it reversibly inhibited human plasma cholinesterase (plasma ChE) and the pseudo cholinesterase of the human brain, but in concentrations which inhibited plasma ChE by 50 per cent, it had only slight effect on red cell ChE and the true cholinesterase of the brain. It was also reported by the same authors (THOMPSON *et al.*, 1955) that human cholinesterases were inhibited by LSD to a greater extent than cholinesterases of the rat, guinea pig, rabbit, chicken and monkey brain. TONINI (1955) found that rat brain cholinesterase activity was potentiated by 5-hydroxytryptamine (serotonin; 5-HT), LSD, and lysergic acid monoethylamide (LAE) in low concentrations. He, as well as FRIED and ANTOPOL (1956, 1957), who also noticed potentiation of human plasma ChE by LSD attempted to relate this effect to the psychotropic action of these compounds.

ZEHNDER and CERLETTI (1956) reported, however that the non-hallucinogenic 2-brom-D-lysergic acid diethylamide (BOL) (ROTHLIN and CERLETTI, 1952; CERLETTI and ROTHLIN, 1955) had an inhibitory effect on human plasma ChE similar to that of LSD. Although in recent years the psychotropic activity of many, newly synthetized derivatives of LSD was studied in man (SOLMS, 1953, 1956; ISBELL *et al.*, 1959; GIBERTI and GREGORETTI, 1958; GOGERTY and DILLE, 1957; CALLIERI, 1958; MURPHREE *et al.*, 1958; HOFMANN, 1959; SCHNECKLOTH *et al.*, 1957; ABRAMSON *et al.*, 1958) relatively little work has been done on the anticholinesterase effect of these compounds (GOGERTY and DILLE, 1957).

* Preliminary reports of this work were published in *Fed. Proc.* 1959 **18**, 463; and 1960 **19**, 266.

† This study was supported by U.S. Public Health Research Grant No. M-1425 (C1, C2).

‡ Present address: Allegheny General Hospital, Pittsburgh, Pennsylvania.

§ The following abbreviations are used: LSD, D-lysergic acid diethylamide bitartrate; 5-HT, 5-hydroxytryptamine creatinine sulphate; LAE, D-lysergic acid monoethylamide bimaleinate; BOL, 2-brom-D-lysergic acid diethylamide bitartrate; L-LSD, L-lysergic acid diethylamide bimaleinate; D-*iso*-LSD, D-*iso*-lysergic acid diethylamide bimaleinate; LSM, D-lysergic acid morpholide bimaleinate; MLD, D-1-methyl-lysergic acid diethylamide bitartrate; DAM, D-lysergic acid dimethylamide chlorhydrate; ACh, acetylcholine chloride; ATCh, acetylthiocholine iodide; BuCh, butyrylcholine iodide; BuTCh, butyrylthiocholine iodide; MeCh, acetyl-β-methylcholine chloride; BeCh, benzoylcholine chloride; ChE, cholinesterase.

The purpose of this study has been the investigation of the inhibitory effect of several psychotropic and non-psychotropic LSD derivatives on human cholinesterases. Because of the antagonistic effect of LSD on some of the pharmacological actions of 5-HT (GADDUM, 1953; CERLETTI and ROTHLIN, 1955; WOOLLEY and SHAW, 1954), a more detailed investigation of the earlier reported inhibitory effect of this compound on human cholinesterase (ERDÖS, BAART, SHANOR and FOLDES, 1956) was made.

MATERIAL AND METHODS

Materials

Source of enzymes. The source of plasma ChE was Cholase, a purified, concentrated human plasma cholinesterase preparation, prepared similarly to Harvard fraction IV-6-3. One ml of Cholase corresponds in activity to 160 ml of pooled, heparinized human plasma (FOLDES *et al.*, 1957).

Freshly obtained, heparinized, washed, and haemolysed human red cells, prepared according to AUGUSTINSSON (1949), were used as the source of red cell ChE.

Human grey matter ChE was obtained from the brain of adults who died suddenly (*e.g.*, rupture of aortic aneurysm, massive coronary occlusion, electrocution, etc.). Autopsies were performed within 5 hr of death. Before removing the brain, blood was washed out of it by perfusion with 0·9% NaCl. After this, the brains were sectioned, placed in plastic bags and quick-frozen by immersion in an acetone–dry ice mixture. The sections were stored below −20°. Subsequently, the grey matter of the cortex and the basal ganglia were separated from the white matter, homogenized in a glass homogenizer with 4 vol of a tissue bicarbonate buffer (NACHMANSOHN and ROTHENBERG, 1945). Homogenization was carried out at 4° and the homogenates were stored below −20°. Under these conditions, enzyme activity remained constant for at least 6 months.

Substrates. Acetylcholine chloride (ACh), acetylthiocholine iodide (ATCh), butyrylcholine iodide (BuCh), butyrylthiocholine iodide (BuTCh), benzoylcholine chloride (BeCh) and acetyl-β-methylcholine chloride (MeCh) were used.

The compounds studied were: D-Lysergic acid diethylamide bitartrate (LSD), L-lysergic acid diethylamide bimaleinate (L-LSD), D-*iso*-lysergic acid diethylamide bimaleinate (D-*iso*-LSD), 2-brom-D-lysergic acid diethylamide bitartrate (BOL), D-lysergic acid morpholide bimaleinate (LSM), D-1-methyl-lysergic acid diethylamide bitartrate (MLD), D-lysergic acid monoethylamide bimaleinate (LAE), D-lysergic acid dimethylamide chlorhydrate (DAM) and 5-hydroxytryptamine creatinine sulphate (5-HT). The structural formulae of the LSD derivatives are shown in Fig. 1.

The inhibitory effect of the breakdown products of LSD and BOL on human cholinesterases was also investigated. These were obtained by allowing LSD and BOL solutions, brought to pH 2·0 with HCl, to stand at room temperature, exposed to light for 7–10 days. The u.v. absorption curve of the breakdown product of LSD (Fig. 2), determined in a Beckman automatic recording spectrophotometer, is similar to that of lumilysergic acid A (HELLBERG, 1958).

Methods

The inhibitory effect of the compounds studied was measured with AMMON's (1934) modification of Warburg's manometric method at 37° and at pH 7·4. The total volume of the hydrolysates was 2 ml and the gas phase was 5% CO_2 : 95% N_2. All experiments were done in duplicate. Corrections were made for alkaline hydrolysis of the substrates, both in the presence and absence of the compounds tested. The compounds were preincubated with the enzymes for 15–20 min before the addition of the substrates. The inhibitory effect was observed with at least 6 different concentrations. Three of the concentrations were above and 3 below the I_{50} values, which were determined by plotting the negative logarithms of the inhibitor concentrations against activity. The data presented are the means obtained from at least three experiments. The substrate concentration of ACh with Cholase was 22·0 mM, with red cell ChE and grey matter ChE it was 3·1 mM. Preliminary studies showed that the latter concentration was optimal for the activity of human grey matter ChE. The substrate concentrations of BuCh, MeCh and BeCh were 22·0 mM with all enzymes. ATCh and BuTCh were used in the same concentrations as their oxy-analogues. The final Cholase concentration was 1:8000 (v/v) for all substrates; that of red cells was 1:165 (v/v) for ACh and 1:55 (v/v) for MeCh and that of human grey matter homogenates was 1:75, 1:50 and 1:25 (w/v) for ACh, MeCh and BuCh, respectively. With Cholase 0·025M-$NaHCO_3$ buffer was used; with red cells and brain grey matter homogenate,

the buffers described by NACHMANSOHN and ROTHENBERG (1945) were used. Experimental conditions with the breakdown products of LSD and BOL were the same as described above. The effect of changing the pH between 6·0 and 8·0 on the LSD induced inhibition of the hydrolysis of BeCh by Cholase was determined by a modification of KALOW's (1952) u.v. spectrophotometric method in a Beckman model DU spectrophotometer. The BeCh concentration in these experiments was 0·05 mM

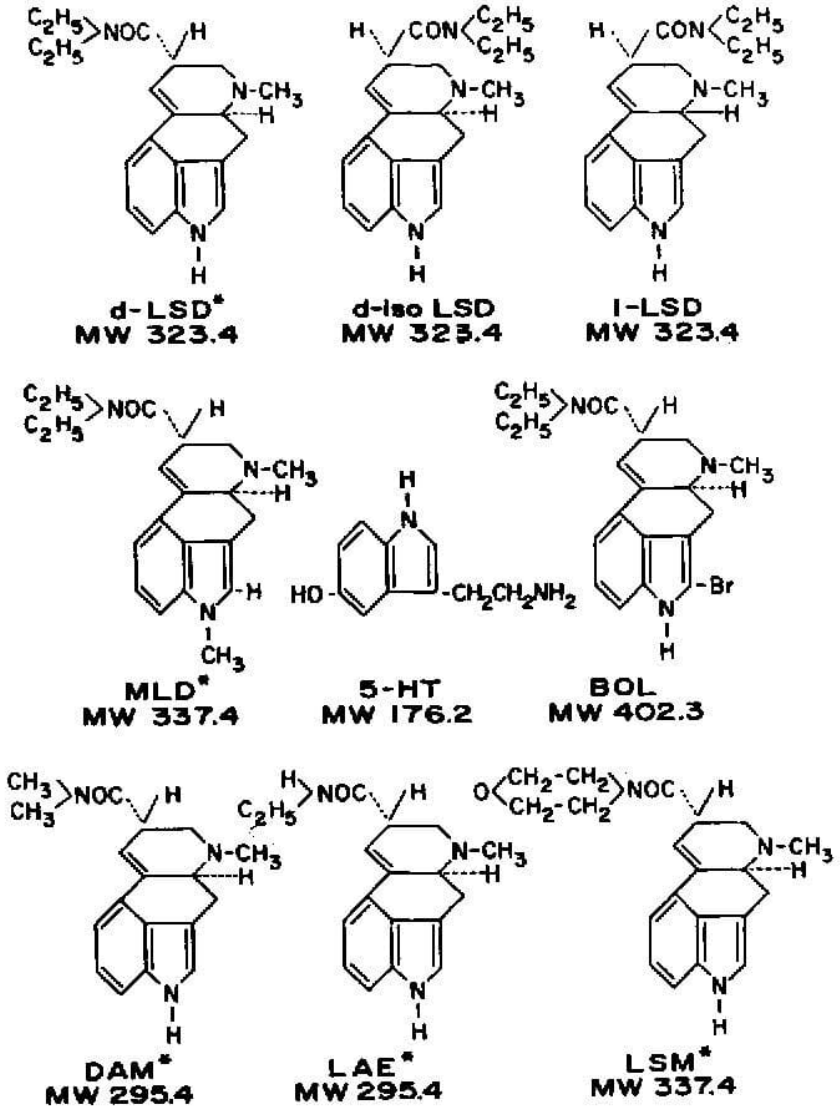

FIG. 1.—The structural formulae of the compounds tested. The compounds marked with an asterisk have hallucinogenic activity.

and that of Cholase was 1:30,000 (v/v). Readings were taken at 30 sec intervals until 50 per cent of the substrate was hydrolysed. SÖRENSEN's (1909) phosphate buffers were used in these experiments. The temperature was kept constant at 37°. The total volume of the system was 4 ml. The light path was 10 mm. In a few experiments, pooled, heparinized human plasma was used instead of Cholase.

RESULTS

The inhibitory effect of the compounds tested on the hydrolysis of ACh, BuCh and BeCh by plasma ChE is summarized in Table 1. There is no relationship between the inhibitory effect of the compounds tested on the activity of plasma ChE and their psychotropic activity. Although the inhibitory effect of D-LSD, the most potent

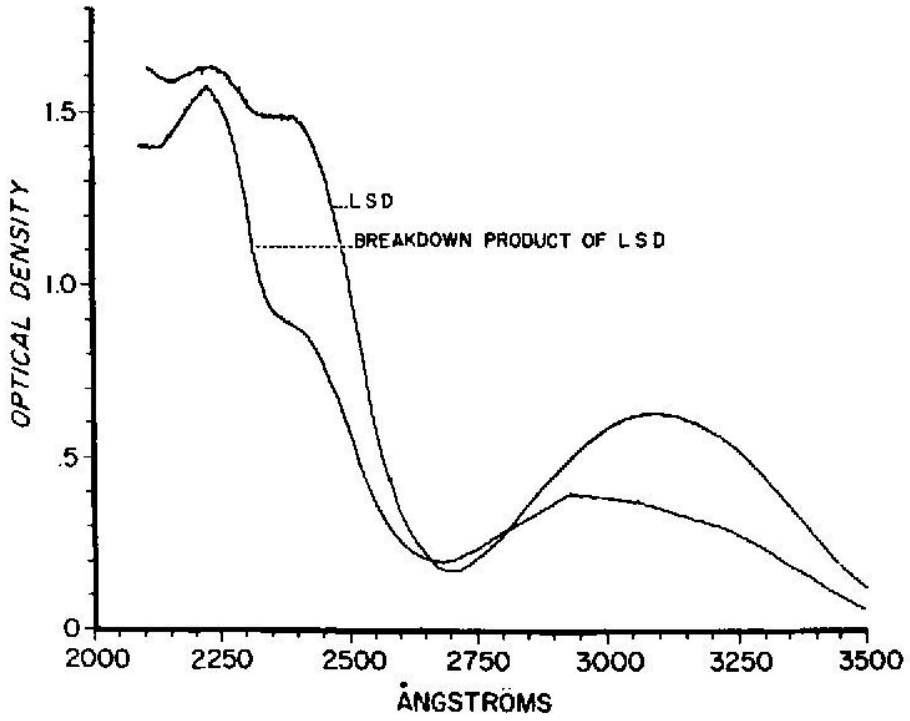

FIG. 2.—The u.v. absorption curves of LSD and its breakdown product. The concentration of the compounds was 0·033 mM.

TABLE 1.—THE INHIBITORY EFFECT OF LYSERGIC ACID DERIVATIVES AND 5-HT ON HUMAN PLASMA CHOLINESTERASE

Compounds	I_{50} values (M)		
	ACh	BuCh	BeCh
	115·5* ± 5·1	232·3 ± 21·1	36·2 ± 3·6
Hallucinogenic			
D-LSD	$1{\cdot}4 \times 10^{-6}$	$3{\cdot}3 \times 10^{-6}$	$5{\cdot}3 \times 10^{-6}$
MLD	$6{\cdot}7 \times 10^{-5}$	—	—
LAE	$1{\cdot}5 \times 10^{-4}$	—	—
DAM	$2{\cdot}8 \times 10^{-5}$	—	—
LSM	$1{\cdot}0 \times 10^{-4}$	$6{\cdot}0 \times 10^{-3}$	Insignificant
Non-hallucinogenic			
L-LSD	$1{\cdot}0 \times 10^{-5}$	—	—
D-*iso*-LSD	$7{\cdot}6 \times 10^{-6}$	—	—
BOL	$4{\cdot}9 \times 10^{-5}$	$1{\cdot}6 \times 10^{-5}$	$9{\cdot}7 \times 10^{-5}$
5-HT	$9{\cdot}4 \times 10^{-4}$	$2{\cdot}6 \times 10^{-3}$	Insignificant

* Uninhibited rate: μmoles hydrolysed by 1 ml of plasma in 30 min ± S.E.M. Values are means of at least 3 experiments.

hallucinogenic compound, is the greatest, L-LSD, D-*iso*-LSD (ROTHLIN, 1957*a*, *b*) and BOL (CERLETTI and ROTHLIN, 1955), which are psychotropically inactive, inhibit this enzyme to a greater extent than the psychotropically active MLD (ISBELL *et al*, 1959), LAE (ROTHLIN, 1957*a*), DAM (ISBELL *et al.*, 1959), and LSM (GOGERTY and DILLE, 1957). Comparison of the structural formulae of the compounds (Fig. 1) with their I_{50} values (Table 1) indicates that substitution of both ethyl radicals on the amino

nitrogen with methyl groups as in DAM, or one with a hydrogen, as in LAE, or the enclosure of the two ethyl groups into a morpholine ring, as in LSM, or the substitution of a methyl group on the indole nitrogen decreased the inhibitory effect of the compounds on plasma ChE. Stereo-isomeric changes, as in L-LSD and D-*iso*-LSD, also decreased the inhibitory effect on plasma ChE.

Similarly, no correlation could be detected between the antiserotonin activity of the compounds (CERLETTI and ROTHLIN, 1955; CERLETTI and DOEPFNER, 1958; and

TABLE 2.—THE INHIBITORY EFFECT OF LYSERGIC ACID DERIVATIVES AND 5-HT ON HUMAN RED CELL CHOLINESTERASE

Compounds	I_{50} values (M)	
	ACh 302·4* ± 3·5	MeCh 59·4 ± 4·9
Hallucinogenic		
D-LSD	$4·9 \times 10^{-5}$	$9·0 \times 10^{-5}$
MLD	$7·1 \times 10^{-6}$	—
LAE	$4·3 \times 10^{-3}$	—
DAM	$4·3 \times 10^{-4}$	—
LSM	$1·5 \times 10^{-3}$	$2·4 \times 10^{-3}$
Non-hallucinogenic		
L-LSD	$2·0 \times 10^{-3}$	—
D-*iso*-LSD	$4·2 \times 10^{-4}$	—
BOL	$6·8 \times 10^{-5}$	$1·0 \times 10^{-4}$
5-HT	$4·3 \times 10^{-3}$	$5·4 \times 10^{-3}$

* Uninhibited rate: μmoles hydrolysed by 1 ml of red cells in 30 min ± S.E.M. Values are means of at least 3 experiments.

ISBELL *et al.*, 1959) and their anti-plasma ChE effect. Furthermore, there was little difference between the inhibitory effect of 5-HT and that of some of its antagonists, *e.g.* MLD and DAM (ISBELL *et al.*, 1959), on the hydrolysis of plasma ChE.

The inhibitory effect of the compounds on the hydrolysis of ACh and MeCH by red cell ChE is summarized in Table 2. With the exception of MLD, the inhibitory effect of all compounds tested was lower for red cell ChE than for plasma ChE, and the relative potency of the compounds for the two enzymes was about the same. MLD, however, had a greater inhibitory effect on red cell ChE than on plasma ChE.

The inhibitory effect of the compounds on the hydrolysis of ACh and MeCH by human grey matter ChE was about the same as that on the hydrolysis of these substrates by human red cell ChE. Similarly, the inhibitory effect of the compounds on the hydrolysis of BuCh by human grey ChE and by human plasma ChE were of the same order of magnitude. This is in line with the finding that most of the human grey matter ChE is true cholinesterase (NACHMANSOHN and ROTHENBERG, 1945).

The results with ATCh and BuTCh substrates were similar to those obtained with their oxy-analogues.

The breakdown products of LSD and BOL inhibited the hydrolysis of various substrates by human plasma ChE and red cell ChE to about the same extent as their parent compounds.

LSD, in concentrations ranging from 10^{-11} to 10^{-8}M, neither accelerated nor inhibited the activity of the enzymes tested.

Changing the pH of the reaction medium from 6·0 to 7·0 increased three-fold the inhibitory effect of LSD on the hydrolysis of BeCh by plasma ChE. Further increase of the pH from 7·0 to 8·0 caused no change in inhibitory potency. Since the p*K*b value of LSD is 7·12 (FISHER, 1956), it seems that there is no close relationship between the degree of ionization and the anticholinesterase activity of LSD.

TABLE 3.—THE INHIBITORY EFFECT OF LYSERGIC ACID DERIVATIVES AND 5-HT ON HUMAN GREY MATTER CHOLINESTERASE

Compounds	I_{50} values (M)		
	ACh 164·9* ± 10·1	MeCh 83·0 ± 5·2	BuCh 21·6 ± 0·6
Hallucinogenic			
D-LSD	$6·3 \times 10^{-5}$	$1·1 \times 10^{-4}$	$2·5 \times 10^{-5}$
MLD	$2·8 \times 10^{-5}$	—	—
LAE	—	$1·0 \times 10^{-2}$	$3·3 \times 10^{-3}$
DAM	$3·8 \times 10^{-4}$	—	—
LSM	$3·1 \times 10^{-3}$	$6·5 \times 10^{-3}$	$1·4 \times 10^{-3}$
Non-hallucinogenic			
L-LSD	—	$4·1 \times 10^{-3}$	$1·0 \times 10^{-4}$
D-*iso*-LSD	—	$3·0 \times 10^{-4}$	$9·0 \times 10^{-5}$
BOL	$3·0 \times 10^{-5}$	$1·8 \times 10^{-4}$	$1·6 \times 10^{-5}$
5-HT	$7·6 \times 10^{-3}$	$9·8 \times 10^{-3}$	$8·4 \times 10^{-3}$

* Uninhibited rate: μmoles hydrolysed by 1 g of wet tissue in 30 min ± S.E.M. Values are means of at least 3 experiments.

5-HT was a weak inhibitor of the hydrolysis of all substrates by the three enzymes tested. Its inhibitory effect was additive with that of LSD.

The results of the experiments with freshly obtained, pooled, human plasma were the same as those obtained with Cholase.

DISCUSSION

The findings presented indicate that LSD and some of its congeners have a significant inhibitory effect on both true and pseudo-cholinesterases. This finding is in contradistinction to those of THOMPSON *et al.* (1955), who reported that LSD only inhibited plasma ChE and had no significant inhibitory effect on human red cell and brain cholinesterases. The discrepancy between the findings of THOMPSON *et al.* (1955) and those of ours may be explained by the fact that the substrate concentration used by them was considerably higher (15 mM) than the concentration (3·1 mM) found to be optimal in our study. The 0·049 mM-LSD concentration which gave 50 per cent inhibition with the substrate concentration used in this work only gave a 15 per cent inhibition with the concentration used by THOMPSON (ZSIGMOND *et al.*, 1959).

Except for MLD, all compounds investigated inhibited human plasma ChE to a greater extent than human red cell ChE or grey matter ChE. In agreement with

THOMPSON *et al.* (1955), rabbit cholinesterases were inhibited less than human cholinesterases by LSD derivatives in equivalent concentrations (ZSIGMOND *et al.* Unpublished data).

Plotting the reciprocal of the inhibited and uninhibited rate ($1/v$) against the reciprocal of the substrate concentration ($1/S$) according to LINEWEAVER and BURK (1934) resulted in straight lines intersecting the ordinate at the same point both with plasma ChE (Fig. 3) and red cell ChE (Fig. 4). This indicates that LSD is a true

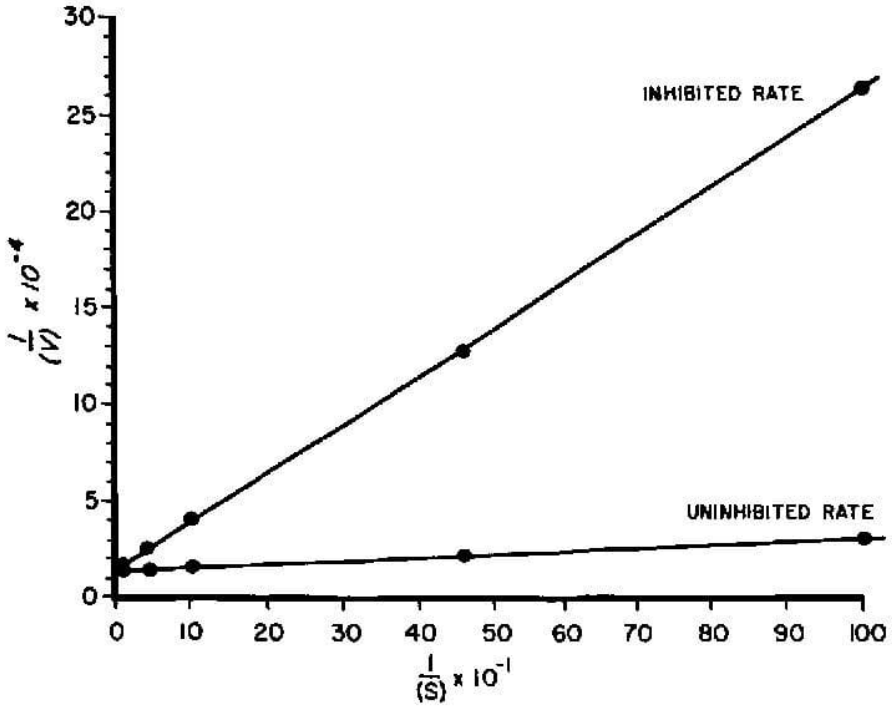

FIG. 3.—The influence of changing substrate concentration on the inhibitory effect of LSD on the hydrolysis of ACh by plasma cholinesterase. The figure indicates competitive inhibition.

competitive inhibitor of the hydrolysis of ACh by both enzymes. Probably the other structurally closely-related compounds studied are also competitive inhibitors.

Although ATCh and BuTCh are hydrolysed about 30 per cent faster than the corresponding oxy-esters, LSD inhibited their hydrolysis by cholinesterases to the same extent as that of their oxy-analogues. In contrast to the findings of TONINI (1955) and FRIED and ANTOPOL (1957), it could not be demonstrated that LSD in low concentrations accelerated the hydrolysis of ACh by human plasma ChE.

No correlation was found between the *in vitro* inhibitory effect and the hallucinogenic activity of the compounds tested. BOL, L-LSD and D-*iso*-LSD, compounds without any significant hallucinogenic effect (CERLETTI and ROTHLIN, 1955; ISBELL *et al.*, 1959; MURPHREE *et al.*, 1958; ROTHLIN, 1957*a*), inhibited cholinesterases to about the same extent as the highly hallucinogenic LSD. On the other hand, MLD, LAE, DAM and LSM, which are hallucinogenic, had less inhibitory effect on plasma ChE than the non-hallucinogenic compounds. Although the inhibitory effect of the hallucinogenic MLD on red cell ChE and grey matter ChE was the greatest, the more strongly hallucinogenic LSD inhibited these enzymes to the same extent as the non-hallucinogenic BOL. The breakdown products of LSD (see Fig. 2) and BOL which

have no psychogenic effects (ROTHLIN, 1957*b*) also inhibited cholinesterases to the same extent as the mother compounds.

No correlation could be demonstrated between the anti-serotonin and the anticholinesterase activity of the compounds tested. There was no major difference in the anticholinesterase effect of LSD and BOL which are potent antagonists of 5-HT (GADDUM, 1953; CERLETTI and ROTHLIN, 1955) and that of L-LSD and D-*iso*-LSD which did not inhibit 5-HT (ROTHLIN 1957*a*; CERLETTI and DOEPFNER, 1958). Furthermore, the anticholinesterase effect of 5-HT and that of its pharmacological antagonists D-LSD and BOL, were found to be additive.

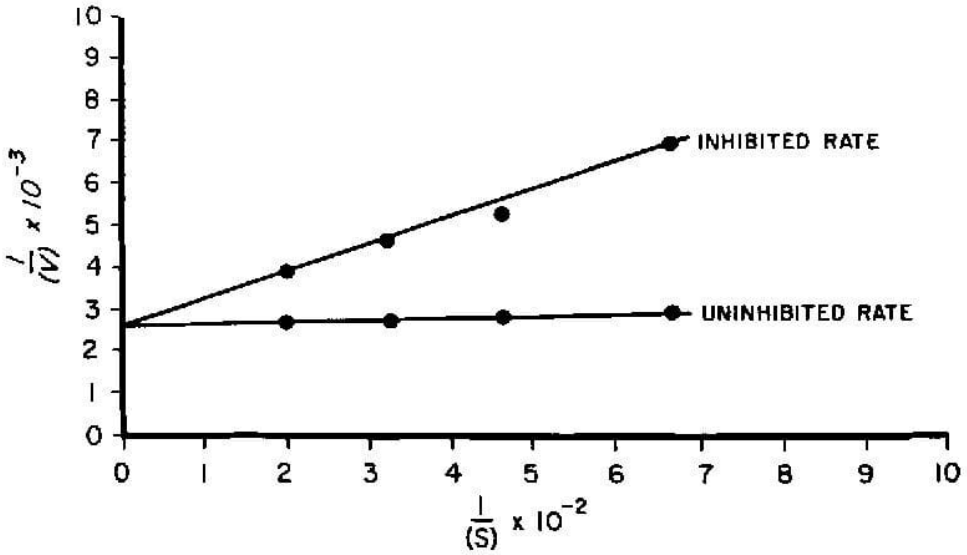

FIG. 4.—The influence of changing substrate concentration on the inhibitory effect of LSD on the hydrolysis of ACh by red cell cholinesterase. The figure indicates competitive inhibition.

Comparison of the structural formulae and the anticholinesterase activity of the compounds tested suggests the possible significance of ethyl substitution on the amino nitrogen. Those compounds in which two ethyl groups are attached to the amino nitrogen, D-LSD, MLD, L-LSD, D-*iso*-LSD and BOL, were more potent inhibitors of both true and psuedo-cholesterases than LAE where one of the ethyl groups is substituted with hydrogen, or DAM in which both ethyl groups are substituted by methyl radicals, or LSM in which both ethyl groups were incorporated in a morpholine ring. Substitution of a methyl group on the indole nitrogen of LSD significantly decreased the inhibitory effect of the resulting compound, MLD, on plasma ChE and slightly increased its inhibitory effect on red cell ChE and grey matter ChE.

SUMMARY

The inhibitory effect of eight LSD derivatives and of 5-hydroxytryptamine on human plasma, red cell and brain grey matter cholinesterases was investigated. With the exception of MLD, plasma cholinesterase was inhibited more by all compounds tested than red cell or grey matter cholinesterases. No correlation was found between the *in vitro* anticholinesterase activity and the hallucinogenic or anti-serotonin activity of the compounds tested.

Acknowledgements—We would like to express our gratitude to Dr. J. H. HINK, Jr. of Cutter Laboratories, Berkeley, California, for generous supplies of Cholase; to Dr. R. BIRCHER of Sandoz Pharmaceuticals Division of Sandoz, Inc., Hanover, New Jersey, for supplies of LSD derivatives; to Mr. M. E. SPEETER of the Upjohn Company, Kalamazoo, Michigan, for supplies of 5-hydroxytryptamine; and to Dr. LEO A. PIRK of Hoffmann-LaRoche Inc., Nutley, New Jersey for supplies of acetylthiocholine iodide and butyrylthiocholine iodide.

The determination of the u.v. absorbtion curve of LSD and its breakdown product was carried out by Dr. E. G. ERDÖS, Division of Fundamental Research, Mellon Institute, Pittsburgh, Pa.

REFERENCES

ABRAMSON H. A., SKLAROFSKY B., BARON M. O. and FREMONT-SMITH N. (1958) *Arch. Neurol. Psychiat., Chicago* **79,** 201.

AMMON R. (1934) *Pflüger's Arch. ges. Physiol.* **233,** 486.

AUGUSTINSSON K. -B. (1949) *Arch. Biochem.* **23,** 11.

CALLIERI B. (1958) *Rass. Neuropsichiat.* **11,** 198.

CERLETTI A. and DOEPFNER W. (1958) *J. Pharmacol.* **122,** 124.

CERLETTI A. and ROTHLIN E. (1955) *Nature, Lond.* **176,** 785.

ERDÖS E. G., BAART N., SHANOR S. P. and FOLDES F. F. (1956) *Abstr. XX int. physiol. Congr.* 274.

FISHER R. (1956) *Molecular Structure and Functional Activity of Nerve Cells.* p. 68. American Institute of Biological Sciences, Waverly Press, Baltimore, Md.

FOLDES F. F., ERDÖS E. G., BAART N. and SHANOR S. P. (1957) *Proc. Soc. exp. Biol., N.Y.* **94,** 500.

FRIED G. H. and ANTOPOL W. (1956) *Anat. Rec.* **125,** 610.

FRIED G. H. and ANTOPOL W. (1957) *Fed. Proc.* **16,** 357.

GADDUM J. H. (1953) *J. Physiol.* **121,** 15P.

GIBERTI F. and GREGORETTI I. (1958) *Sist. nerv.* **10,** 97.

GOGERTY J. H. and DILLE J. M. (1957) *J. Pharmacol.* **120,** 340.

HELLBERG H. (1958) *Pharm. Weekbl.* **93,** 1.

HOFMANN A. (1959) *Acta physiol. Pharm. neerl.* **8,** 240.

ISBELL H., MINER E. J. and LOGAN C. R. (1959) *Psychopharmacologia,* **1,** 20.

KALOW W. (1952) *J. Pharmacol.* **104,** 122.

LINEWEAVER H. and BURK D. (1934) *J. Amer. chem. Soc.* **56,** 658.

MURPHREE H. B., DEMAAR E. W. J., WILLIAMS H. L. and BRYAN L. L. (1958) *J. Pharmacol.* **122,** 55*a*.

NACHMANSOHN D. and ROTHENBERG M. A. (1945) *J. biol. Chem.* **158,** 653.

POLONI A. and MAFFEZZONI G. (1952) *Sist. nerv.* **4,** 578.

ROTHLIN E. (1957*a*) *J. Pharm., Lond.* **9,** 569.

ROTHLIN E. (1957*b*) *Ann. N.Y. Acad. Sci.* **66,** 668.

ROTHLIN E. and CERLETTI A. (1952) *Helvet. physiol. Acta* **10,** 319.

ROTHLIN E. and CERLETTI A. (1956) *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry.* Grune and Stratton, New York, N.Y.

SCHNECKLOTH R., PAGE I. H., DEL GRECO F. and CORCORAN A. C. (1957) *Circulation* **16,** 523.

SOLMS H. (1953) *Schweiz. med. Wschr.* **83,** 356.

SOLMS H. (1956) *Praxis* **45,** 746.

SÖRENSEN S. P. L. (1909) *Biochem. Z.* **22,** 352.

STOLL W. A. (1947) *Schweiz. Arch. Neurol. Psychiat.* **60,** 279.

STOLL A. and HOFMANN A. (1943) *Helvet. chim. Acta,* **26,** 944.

THOMPSON R. H. S., TICKNER A. and WEBSTER G. R. (1954) *Biochem. J.* **58,** 19.

THOMPSON R. H. S., TICKNER A. and WEBSTER G. R. (1955) *Brit. J. Pharmacol.* **10,** 61.

TONINI G. (1955) *Boll. Soc. ital. Biol. sper.* **31,** 768.

WOOLEY D. W. and SHAW E. (1954) *Science,* **119,** 587.

ZEHNDER K. and CERLETTI A. (1956) *Helvet. physiol. Acta,* **14,** 264.

ZSIGMOND E. K., FOLDES F. F., FOLDES V. and ERDÖS E. G. (1959) *Fed. Proc.* **18,** 463.