Biochemical Observations on LSD-25 and Deseril

Since the discovery of the hallucinogenic action of Delysed (LSD-25, *d*-lysergic acid diethylamide)[1] and of its high potency and specificity in blocking the peripheral effects of serotonin[2], many attempts have been made to elucidate these properties and to correlate them[3–15]. A study of the serotonin-antagonistic qualities of a large number of lysergic acid derivatives revealed that Deseril (UML 491, 1-methyl-*d*-lysergic acid butanolamide) was one of the most active compounds[16,17]. As this drug was practically devoid of central effects, it could also be used clinically and has proved to be of value in the symptomatic treatment of carcinoid patients[18,19] and in the prevention of some forms of vascular headache[20].

Although there is some evidence that the psychotomimetic action of LSD is mainly structure-specific, the question arises whether the possibility of entering the brain is identical for various lysergic acid derivatives. Depending on lipid-solubility or other factors, small changes of the molecule may lead to a selection at the blood-brain barrier (BBB). The examination of LSD and Deseril in this respect was the purpose of the following experiments[21].

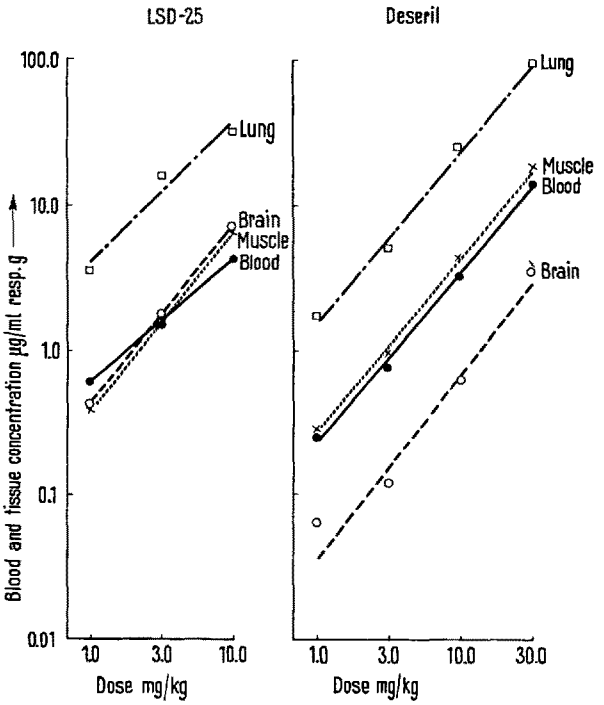
Methods and Materials. Throughout the experiments, female rats weighing 170–200 g were used. All animals were decapitated exactly 20 min after i.v. injection of the following doses of LSD: 1, 3, and 10 mg/kg respectively Deseril: 1, 3, 10, and 30 mg/kg. All doses are below the LD_{50} values (LSD: 16.5 mg/kg; Deseril: 125 mg/kg) and some are in the range of those used pharmacologically. To avoid coagulation each animal was given i.p. injections of 1.0 ml $3^0/_{00}$ heparin solution immediately prior to the drug. The blood was iced without delay and brain, lung and the muscles of both hind-legs were homogenized with chilled water. All samples were kept at −10°C until extraction (max. 72 h). For each estimation the homogenates of 2 animals were pooled. LSD and Deseril were used in the form of the tartrates and all values given are related to these salts.

As shown by AXELROD[22], LSD can be extracted from tissue in *n*-heptane and returned to 0.004 *N* HCl with good recovery. We not only confirmed this, but found, in addition, that the same procedure is suitable for Deseril. Extraction and fluorimetric estimation of LSD and Deseril can therefore be carried out as follows:

1 g tissue is homogenized with 5 ml distilled water in a Waring blender or with a Potter type homogenizer. 1 ml blood is diluted with 5 ml distilled water. 25 ml washed *n*-heptane, 0.5 ml 1 *N* NaOH and about 5 g NaCl are added to 6 ml homogenate or diluted blood in a stoppered centrifuge tube. The tube is shaken for 30 min and centrifuged. 20 ml of the heptane phase are transferred to a new centrifuge tube containing 3 ml of 0.004 *N* HCl, shaken for 10 min and centrifuged. The fluorescence of the acid phase is measured in an Aminco-Bowman spectrofluorophotometer using quartz cuvettes. The activation and fluorescence wavelengths for LSD are: 320 mμ and 435 mμ respectively and for Deseril: 330 mμ and 435 mμ respectively (uncorrected instrument values). Tissue blanks from untreated control animals as well as the corresponding internal and external standards were carried through with each extraction. All estimations were made in duplicate.

Results. Almost instantaneously after the injection of even a dose as small as 1 mg/kg LSD, the animals showed hyperactivity with convulsive movements and, in the higher dose range, generalized body tremors. After Deseril no behavioural changes could be observed, except a slight hypersensitivity to noise and handling.

The drug concentrations found in the various tissues 20 min after the injection of increasing doses of LSD and Deseril are plotted in the Figure and show for both com-



Blood and tissue concentration curves of LSD and Deseril 20 min after i.v. injection of various doses of the drugs. Each point represents the mean of 2 experiments.

[1] W. A. STOLL, Schweiz. Arch. Neurol. Psychiat. *60*, 279 (1947).
[2] J. H. GADDUM, J. Physiol. (London) *121*, 15 P (1953).
[3] B. BERDE and A. CERLETTI, Helv. physiol. Acta *14*, 325 (1956).
[4] B. BERDE and A. CERLETTI, Z. Inn. Med. *129*, 149 (1957).
[5] B. BERDE, W. DOEPFNER, and A. CERLETTI, Helv. physiol. Acta *18*, 537 (1960).
[6] A. CERLETTI and E. ROTHLIN, Nature *176*, 785 (1955).
[7] A. CERLETTI, Transactions of the Second Conference on Neuropharmacology, Princeton, May 1955 (Josiah Macy Jr. Foundation, New York 1956).
[8] A. CERLETTI, in Proceedings of the 1st International Congress of Neuro-Psychopharmacology, Rome, September 1958 (Elsevier, Amsterdam, London, New York, Princeton 1959), p. 117.
[9] H. ISBELL, E. J. MINER, and C. R. LOGAN, Psychopharmacologia *1*, 20 (1959).
[10] H. KONZETT, XXe Congr. Internat. de Physiologie, Bruxelles (July 30 to August 4, 1956), p. 518.
[11] K. NEUHOLD, M. TAESCHLER, and A. CERLETTI, Helv. physiol. Acta *15*, 1 (1957).
[12] E. ROTHLIN, A. CERLETTI, H. KONZETT, W. R. SCHALCH, and M. TAESCHLER, Exper. *12*, 154 (1956).
[13] M. TAESCHLER and A. CERLETTI, J. Pharmacol. exp. Ther. *120*, 179 (1957).
[14] H. WEIDMANN and A. CERLETTI, Helv. physiol. Acta *15*, 376 (1957).
[15] H. WEIDMANN and A. CERLETTI, Helv. physiol. Acta *16*, C38 (1958).
[16] A. CERLETTI and W. DOEPFNER, J. Pharmacol. exp. Ther. *122*, 124 (1958).
[17] A. CERLETTI and W. DOEPFNER, Helv. physiol. Acta *16*, C55 (1958).
[18] A. FANCHAMPS, W. DOEPFNER, H. WEIDMANN, and A. CERLETTI, Schweiz. med. Wschr. *90*, 1040 (1960).
[19] R. LANZ, Schweiz. med. Wschr. *90*, 1046 (1960).
[20] F. SICUTERI, Int. Arch. Allergy *15*, 300 (1959).
[21] A detailed communication concerning tissue distribution, half-life etc., covering further derivatives of lysergic acid is in preparation.
[22] J. AXELROD, R. O. BRADY, B. WITKOP, and E. V. EVARTS, Ann. N. Y. Acad. Sci. *66*, 435 (1957).

pounds linearity. The highest concentrations were found in the lungs. This finding, as far as LSD is concerned, accords with earlier observations in mice (experiments with C^{14}-labelled LSD[23]) and cats (fluorimetric estimation[22]). The levels of LSD and Deseril in blood and muscle are about the same but definitely lower than in the lungs. In the brain, however, the two compounds behave quite differently. Whereas in the case of LSD the brain values closely resemble those found in muscle and blood, much smaller amounts of Deseril could be detected in this tissue. This difference in the brain-concentration is probably due to different degrees of penetration across the BBB.

More significant than absolute values in such distribution-studies are the ratios: tissue/blood, particularly if different drugs are to be compared at different dose-levels. In Table I the values for these ratios are summarized. LSD and Deseril accumulate 6 to 10 times in the lung and the muscle/blood ratio figures around 1.

With regard to the brain, the ratios vary considerably: those of LSD range from 0.68 to 1.63, those of Deseril only around 0.2. Assuming a relatively free passage of LSD across the BBB into the brain, values for Deseril expressed as a percentage of those for LSD are of interest (Table II). Only 3 and 10 mg/kg are considered in this Table, because after the injection of 1 mg/kg Deseril, the brain-concentration is in a range where the accuracy of the fluorimetric method is doubtful. On the other hand, 30 mg/kg LSD is not included because of its toxicity. As the figures show, the distribution of Deseril in lung and muscle is similar to that of LSD, whereas only 12.0% and 14.6% respectively are found in the brain.

Tab. I. Tissue/blood ratio of LSD and Deseril at various dose levels 20 min after i.v. injection

	Dose mg/kg i.v. 1	3	10	30
Tissue/Blood	Tissue/Blood ratio of LSD			
Lung/Blood	5.88	10.00	7.47	
Muscle/Blood	0.66	1.06	1.58	
Brain/Blood	0.68	1.08	1.63	
	Tissue/Blood ratio of Deseril			
Lung/Blood	7.0	8.05	7.45	6.61
Muscle/Blood	1.16	1.26	1.31	1.30
Brain/Blood	0.25	0.16	0.20	0.25

Tab. II. % tissue concentration of Deseril expressed as a percentage of that of LSD calculated from the tissue/blood ratios of Table I

Tissue	Dose mg/kg 3	10
Lung	80	100
Muscle	119	83
Brain	14.6	12

Discussion. The distribution and fate of LSD after parenteral administration in mice have been investigated in our laboratories with various methods[23,24]. Using a bioassay (serotonin-antagonistic effects on the isolated rat uterus), as well as C^{14}-labelled LSD, it was shown that, as early as 10–15 min after intravenous injection, a tissue-equilibrium, i.e. a maximum of tissue concentration, is obtained. In preliminary experiments, using the fluorimetric method, it was observed that the distribution of Deseril in the rat is as rapid as that of LSD. An analysis of the distribution of each substance at various doselevels and in various organs, therefore seemed of interest. The tissue-concentrations plotted in the Figure confirm, by and large, the hypotheses: they are dose-dependent and, as far as LSD is concerned, accord with earlier results.

The concentration ratio tissue/blood given in Table I and the distribution ratio of Deseril in relation to LSD in Table II, show almost identical values in lung and muscle. From our findings it becomes obvious that Deseril penetrates into peripheral tissues. In contrast to this, only about 13% of the amount of Deseril to be expected reaches the brain. Since Deseril does not elicit LSD-like symptoms, even when given in doses 8 times greater than those of LSD, the difference in the brain levels obtained does not explain why Deseril lacks central effects. Our results contribute to the analysis of the hallucinogenic activity of LSD itself, only in so far as they add further support to the view that it is structure-specific. On the other hand, however, they show that with regard to structure/activity relationships such factors have to be taken into account in the comparison of peripheral and central effects.

Zusammenfassung. Bei Ratten wurde die Verteilung von LSD und Deseril 20 min nach intravenöser Verabreichung fluorimetrisch bestimmt. Auf Grund der ermittelten Quotienten: Gewebe/Blut ergab sich, dass beide Lysergsäurederivate ein gleiches Verteilungsschema in Lunge und Muskel, d. h. in peripheren Organen aufweisen. Im Gegensatz hierzu konnten von Deseril im Gehirn nur ca. 13% des zu erwartenden Wertes nachgewiesen werden. Die Bedeutung dieses Befundes wird diskutiert.

W. Doepfner

Medizinisch-Biologische Forschungsabteilung der Sandoz A.G., Basel (Switzerland), April 6, 1962.

[23] A. Stoll, E. Rothlin, J. Rutschmann, and W. R. Schalch, Exper. *11*, 396 (1955).

[24] U. Lanz, A. Cerletti, and E. Rothlin, Helv. physiol. Acta *13*, 207 (1955).

Action répressive de l'oxygène sur la biosynthèse de la fumarique-réductase d'*Aerobacter aerogenes*

L'une des principales fonctions physiologiques des réductases bactériennes est de permettre à de nombreuses espèces anaérobies facultatives d'utiliser différents accepteurs d'hydrogène minéraux ou organiques. Ces enzymes d'oxydoréduction n'interviennent pas dans le métabolisme oxydatif aérobie mais jouent par contre un rôle essentiel dans le métabolisme oxydatif anaérobie. Nous avons précédemment établi que l'oxygène atmosphérique réprime la biosynthèse de la nitrate-réductase chez *Aero-*