STUDIORUM PROGRESSUS

Evidence of Catecholamine Mediation in the 'Aberrant' Behaviour Induced by Lysergic Acid Diethylamide (LSD) in the Rat

Since the first report on the properties of LSD[1] several hypotheses have been advanced to explain the potent psychotomimetic action of this compound. Based on its profound serotonin blocking activity on peripheral tissues[2] it was postulated that LSD could antagonize serotonin[3] in the brain. Later theories proposed a serotonin-like central action[4,5]. However the advent of D-2-bromolysergic acid diethylamide (BOL-148) showed this substance to be a more potent serotonin antagonist than LSD[6,7] and the discovery that it was without psychotomimetic activity cast doubt on the serotonin deficiency theory. From a study of 18 derivatives of lysergic acid CERLETTI[8] found a striking parallelism between the central activity in animals and the psychotic effects in man. BRODIE et al.[9] pointed out that besides being an indole, LSD possessed a phenylethylamine skeleton and suggested that its main action was through stimulation of central adrenergic receptors. This contention was reiterated by COSTA et al. in 1962[10].

It is clear that despite much discussion the mode of action of LSD on central nervous function is still unresolved. One reason is the lack of a suitable experimental model in animals by means of which the typical effects of LSD as seen in man can be reproduced. Attempts towards this problem have made use of the pyretogenic effect of LSD in rabbits[11,12] which is produced with doses similar in order to the human psychotic dose. However the influence on temperature regulation in the rabbit is difficult to compare with the behavioural action in man.

In the rat behavioural effects of LSD can be demonstrated only by the administration of relatively high doses. The pattern of arousal exhibited however is both reproducable and typical. The behaviour consists of a strong excitement with clearly recognizable symptoms including a stereotyped component with accompanying sympathetic stimulation. Using such 'aberrant' behaviour it has been possible to develop a method for the study of drug influences on the action of LSD. Although it may be questionable whether such behavioural disturbances induced in animals are representative of the psychotomimetic action in man, there seems to be no reason why, for lack of a better model, these effects cannot be subjected to closer examination in the hope of gaining some insight into the mode of action of LSD.

Method. Experiments were carried out in male Sprague-Dawley rats (170–220 g). The rats were kept in the laboratory for at least 24 h before use, at a constant room temperature of 22 °C. Food and water were available ad libitum up to 3 h before the test. Half an hour before injection of LSD the animals were removed from their home cages (housed 3 to a cage) and transferred to the experimental cage. The latter consisted of a Plexiglass container 21 × 36 × 15 cm fitted with a steel wire mesh floor raised 2 cm above the bottom of the cage. Experimental trials consisted of one control group injected with 5 or 3 mg/kg s.c. of LSD tartrate, and test groups injected with the same dose of LSD as well as the test substance. All groups consisted of 6 rats. Observation of the behaviour commenced immediately after the administration of LSD and lasted for 3 h. For the purpose of facilitating observation 3 rats were allocated to each experimental cage, 4 sets of 3 rats being observed simultaneously. For each drug tested a fresh LSD control was set up. The behaviour was assessed using a scoring system under blind conditions.

Two aspects of activity were rated. (a) Aberrant behaviour, and (b) locomotor excitation. The scoring system for (a) and (b) respectively was on a 0–8 continuum.

For LSD induced aberrant behaviour (LSD-AB): 0, normal activity as exhibited by 0.9% saline treated rats; 2, slight sniffing and side to side head movements; 4, strong sniffing and head movements, licking and jaw movements; 6, incessant sniffing, licking, biting of wire mesh floor; 8, as for 6 with the addition that the animals lie with their jaws firmly clamped on the wire mesh floor, practically immobile except for sporadic twitches of the body; occasionally interrupted by aggressive-like reactions. Locomotor excitation was scored as: 2, slight; 4, moderate; 6, strong; 8, very strong.

The score for each separate rat was assessed at 10 min intervals for the first hour and then subsequently every 20 min up to the termination of the trial. The scores obtained were totalled thus yielding 6 values for each group. These values were then compared for significance against the respective control using the Wilcoxon's Two-Sample Rank Order Test (non-parametric).

All drugs were injected by the s.c. route unless otherwise stated. Details of pretreatment times are given in the legend to the Figures. LSD tartrate was dissolved in 0.9% saline. Test drugs were dissolved in the same medium except where otherwise stated. Injection volume was 10 ml/kg body weight.

Drugs tested. Dibenamine hydrochloride, phenoxybenzamine hydrochloride, pronethalol hydrochloride, propranolol hydrochloride. Reserpine (base), chlorpromazine hydrochloride, perphenazine tartrate, BOL-148 (D-2-brom-lysergic acid hydrogen tartrate). Deseril (1-methyl-D-lysergic acid hydrogen maleinate), desmethylimipramine hydrochloride, disulfiram (tetraethylthiuram disulphide), parachloro-phenyl-alanine, α-methyl-para-tyrosine (base). The amino acids, and disulfiram were administered as an aqueous suspension. Reserpine was mixed with an equal amount of tartaric acid, 5 drops of absolute ethyl alcohol, and made up to volume with distilled water.

Results. Following the injection of 5 mg/kg LSD into rats a typical and profound excitation is produced. Within

[1] W. A. STOLL, Schweizer Arch. Neurol. Psychiat. *60*, 279 (1947).
[2] J. H. GADDUM, *Ciba Foundation Symposium on Hypertension* (Churchill, London 1953).
[3] D. W. WOOLEY and E. SHAW, Proc. natn. Acad. Sci. USA *40*, 228 (1954).
[4] D. W. WOOLEY and E. SHAW, Br. med. J. *2*, 122 (1954).
[5] F. RINALDI, L. H. RUDY and H. E. HIMWICH, Am. J. Psychiat. *112*, 678 (1956).
[6] A. CERLETTI and E. ROTHLIN, Nature *176*, 785 (1955).
[7] E. ROTHLIN, J. Pharm. Pharmac. *9*, 569 (1957).
[8] A. CERLETTI, in *Neuro-Psychopharmacology*, First Int. Meet. Neuro-Psychopharmac., Rome 1958 (Eds. P. B. BRADLEY, P. DENIKER and C. RADOUCO THOMAS; Elsevier, Amsterdam 1959), vol. 1, p. 117.
[9] B. B. BRODIE, S. SPECTOR and P. A. SHORE, Pharmac. Rev. *11*, 548 (1959).
[10] E. COSTA, G. L. GESSA, C. HIRSCH, R. KUNTZMAN and B. B. BRODIE, Ann. N.Y. Acad. Sci. *96*, 118 (1962).
[11] A. HORITA and J. M. DILLE, Science *120*, 1100 (1954).
[12] A. HORITA and J. H. GOGERTY, J. Pharmac. exp. Ther. *122*, 195 (1958).

5 min the rats exhibit piloerection, mydriasis and commence a light but persistent aimless sniffing. This is accompanied by an increase in locomotor activity in the initial phase of drug action, but which is gradually supervined by an intensification of sniffing. Incessant head movements from side to side, together with compulsive licking and chewing movements appear within 10–15 min after administration. This activity culminates in what appears to be an abnormal or 'aberrant' pattern of behavioural characterized as a strong tendency to bite or gnaw the wire mesh floor of the cage (see Figure 1). The maximum intensity of this syndrome is reached 45–60 min after receiving LSD. At this point locomotor activity may be impaired and the animals exhibit a reduced pelvic elevation with the hind limbs placed flat on the floor in a splayed position. Occasionally when rats are in contact with each other, some exhibit an aggressive-like reaction denoted as a rearing on the hind legs and facing the other animal with both fore-paws extended. This is accompanied by frequent teeth chattering and vocalization. Actual biting however is rarely seen. After about 80–90 min this activity subsides until 100–120 min few symptoms are observed. The animals then appear completely exhausted.

The effects of α- and β-adrenergic-blocking agents, namely dibenamine, phenoxybenzamine, propranolol and pronethalol on LSD-induced aberrant behaviour are given in Figure 2.

A clear dose related inhibitory action was obtained for all compounds with the exception of dibenamine. This was the weakest antagonist of this group and produced clear inhibition of LSD-AB only in the highest dose. At this level locomotor excitation was also reduced. Phenoxybenzamine was particularly effective, producing a profound inhibition of LSD-AB in all doses tested, and qualitatively it produced a concomitant depressant action on locomotor activity similar to dibenamine. The 2 β-adrenergic-blocking agents however, unlike the α-blocking drugs only produced an inhibition of LSD-AB whilst in the same doses left locomotor activity unimpaired.

Although these 4 drugs are established adrenolytics, 2 of them, dibenamine and phenoxybenzamine are known to antagonize serotonin in peripheral tissues. Thus it seemed interesting to investigate the action of known serotonin antagonists on this phenomenon. The results of 2 such compounds, deseril and BOL-148, are given in Figure 3.

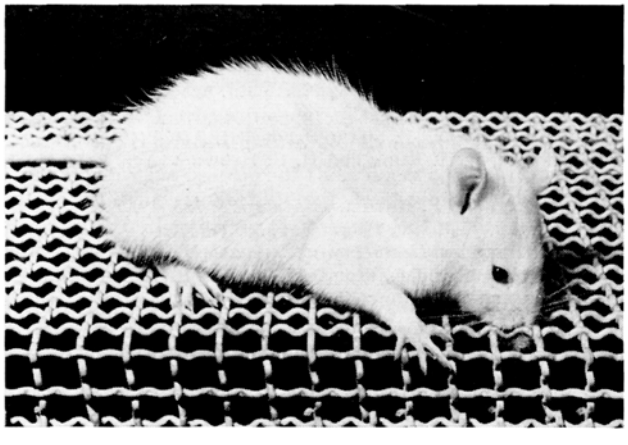

Fig. 1. LSD-induced aberrant behaviour in the rat. See text for description.

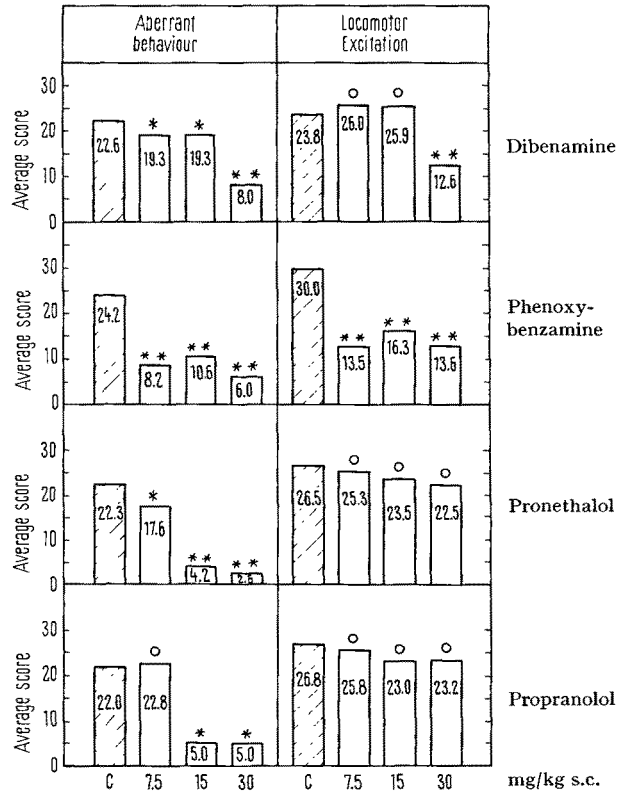

Fig. 2. Influence of adrenergic blocking drugs on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Doses of investigated compounds administered 2 h before LSD. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

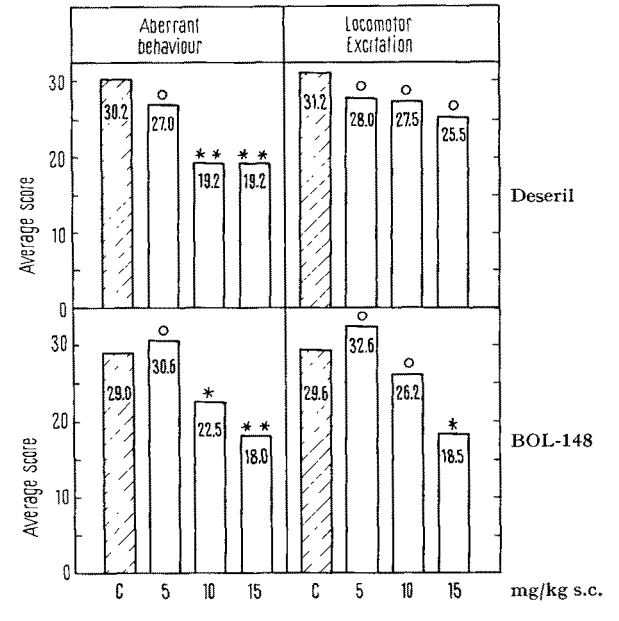

Fig. 3. Influence of serotonin antagonists on LSD-induced behaviour: C, LSD 5 mg/kg s.c. Doses of investigated compound administered 1 h before LSD. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

Both substances exerted only weak inhibitory actions on LSD-AB in the doses tested and did not antagonize the locomotor excitation, with the exception of BOL-148 which produced weak impairment in the highest dose.

The action of 4 psychotropic drugs on LSD-AB are given in Figure 4.

The 2 phenothiazines, perphenazine and chlorpromazine, exerted a dose related inhibition of the aberrant behaviour as well as reducing the locomotor excitation. The action of perphenazine was particularly strong. Animals pretreated with this substance were practically inactive.

Following a 20 h pretreatment with reserpine a striking potentiation of the behavioural effects of LSD was observed. The effect of reserpine showed a clear dose response. The rats exhibited marked sniffing, salivation, and compulsive gnawing of the wire mesh floor. In many cases the animals were aggressive showing a rage reaction, spitting, and vocalization. A slight but significant reduction in locomotor activity was also obtained.

A similar but less intense effect was produced by desmethylimipramine. In contrast to reserpine, desmethylimipramine also increased locomotor activity by a significant extent. It should be noted that in this experiment only 3 mg/kg of LSD was injected so that the potentiating effect could be better revealed.

The effect of 3 inhibitors of biogenic amine synthesis in in vivo are given in Figure 5. Para-chloro-phenylalanine in doses known to almost completely inhibit the synthesis of serotonin in the rat brain, without influencing brain levels of catecholamines was found to exert no influence on either LSD-AB or locomotor excitation. In contrast a single dose of α-methyl-para-tyrosine, a potent inhibitor of catecholamine synthesis in the brain but without effect on cerebral serotonin concentrations, produced a strong inhibition of LSD-AB and to a lesser extent LSD-induced locomotor activity. Within 20–30 min after LSD the α-methyl-para-tyrosine treated animals showed practically no aberrant behaviour.

In disulfiram treated rats only a weak reduction of LSD-AB was observed, this slight effect being evident only in the first 30 min following LSD. Disulfiram has been shown to inhibit conversion of dopamine to noradrenaline.

Discussion. The results show that α- and β-adrenergic-blocking drugs, α-methyl-para-tyrosine, and the major tranquillizers perphenazine and chlorpromazine inhibit LSD induced aberrant behaviour.

The β-adrenergic-blocking drugs, pronethalol and propranolol, exert a selective antagonism of LSD-AB since in contrast to dibenamine and phenoxybenzamine, no influence on the locomotor activity induced by LSD was demonstrated. This observation suggests that the mechanism subserving LSD-AB is particularly sensitive to compounds interacting with the β-adrenergic system.

It was previously pointed out that phenoxybenzamine and dibenamine in addition to their adrenolytic proper-

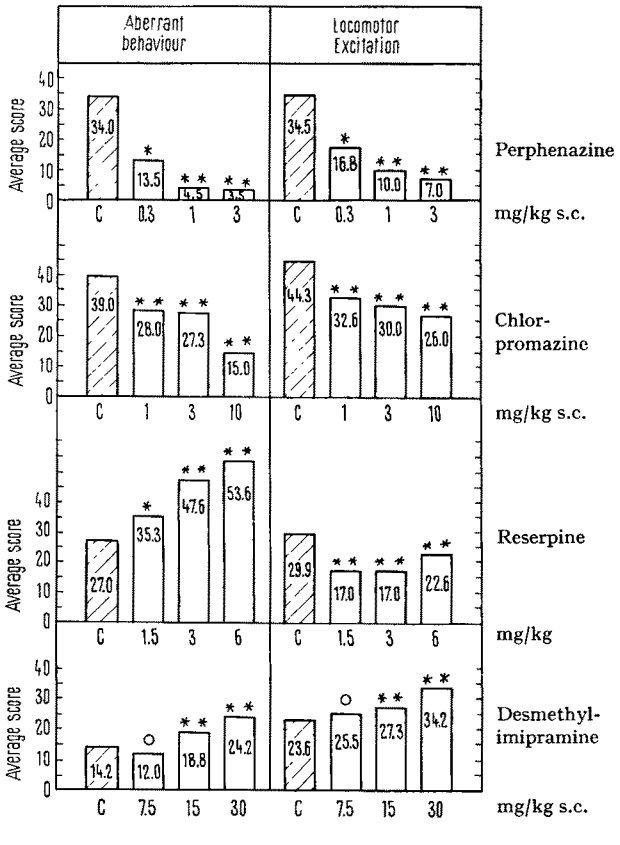

Fig. 4. Influence of psychotropic drugs on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Reserpine given i.p. 20 h before LSD. All other compounds administered 2 h before LSD. LSD 3 mg/kg s.c. was used in the experiment with desmethylimipramine. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as the average total score obtained for each group in 3 h.

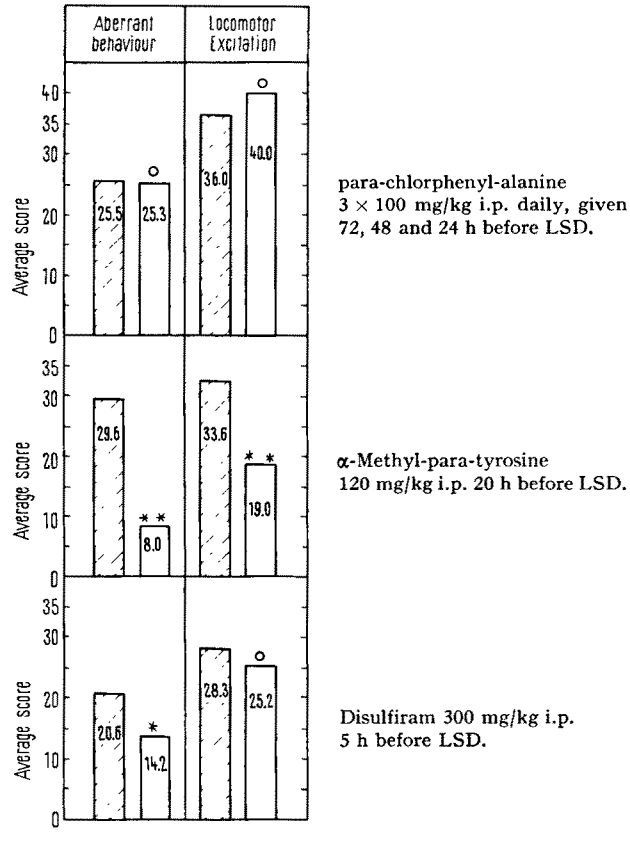

Fig. 5. Influence of biogenic amine synthesis inhibitors on LSD-induced behaviour. C, LSD 5 mg/kg s.c. Statistical significance (p): 0, > 0.05, *, < 0.05, **, < 0.01. Results expressed as average total score obtained for each group in 3 h.

ties, possess serotonin antagonistic characteristics[13-15]. That this latter property was not responsible for their profound inhibitory action on LSD-AB is indicated by the weak antagonism obtained with BOL-148 and deseril. DOEPFNER[16] has pointed out that deseril does not enter the brain as readily as LSD. However in the present experiments relatively high doses were adopted. Furthermore BOL-148 has been shown to exert clear antagonism of the potentiating action of serotonin on barbiturate anaesthesia[17] which is of central origin. The fact that BOL-148 has also been detected in the brain in the same way as has LSD indicates that it is unlikely that these drugs were not present in the brain in sufficient amounts to exert an antagonism to serotonin.

Confirmatory evidence that serotonin does not play a major role in the central action of LSD was obtained with para-chloro-phenylalanine. This compound has been shown to be a potent and selective inhibitor of serotonin biosynthesis[18]. The dose regimen adopted in the present investigations is known to cause almost complete reduction in brain concentrations of serotonin without affecting catecholamine levels. Despite this fact para-chlorophenylalanine failed to exert any influence on LSD-AB or the accompanying locomotor excitation.

It would appear therefore that the antagonism against LSD-AB produced by the adrenergic blocking agents reflects a true central adrenolytic action, and furthermore suggests that the action of LSD is subserved by an adrenergic component.

Similarly the clear and significant inhibition of LSD-AB by α-methyl-para-tyrosine, a selective inhibitor of catecholamine biosynthesis[19], gives strong support to the contention that catecholamine mediation is responsible for the excitement observed with LSD in the present experimental situation. It is of interest that besides causing an increase in brain levels of serotonin, LSD produces a significant concomitant lowering of noradrenalin and dopamine concentrations in the rat brain[20-22]. Thus it could be that LSD release catecholamines centrally.

The question arises, which of the 2 amines, noradrenaline or dopamine contributes to the effects of LSD? Disulfiram, a compound which inhibits the conversion of dopamine to noradrenaline[23,24], produced only a small although significant attenuation of LSD-AB. Since after disulfiram brain levels of noradrenaline but not those of dopamine are reduced, it could be argued that a persistance of aberrant behaviour in this experiment suggests a dopamine mediated effect. However GOLDSTEIN[25] has pointed out that disulfiram does not completely inhibit brain noradrenaline synthesis, and thus the possibility exists that in the present experiment a sufficient reduction of this catecholamine was not achieved. The definite proof concerning the responsible catecholamines implicated in the observed action of LSD must await further data from studies on the biochemical mechanisms involved.

The action of perphenazine and chlorpromazine in antagonizing LSD-AB may reflect an action on central adrenergic mechanisms. Chlorpromazine possesses adrenolytic properties but is also a potent dopamine antagonist[26,27]. Recent studies with thioridazine carried out on the present experimental situation show that it exerts similar inhibitory effects as does chlorpromazine and perphenazine. Chlorpromazine and thioridazine are known to produce an elevation of homovanillic acid and dopamine in the corpus striatum[28-31]. It should be of interest therefore to examine catecholamine concentrations in the basal ganglia in animals treated with LSD, before and after pretreatment with neuroleptics in order to ascertain if this could be the site of action.

The profound stimulation induced by LSD in reserpinized rats confirms earlier findings[17,32]. Why reserpine should potentiate the behavioural action of LSD is not clear. In reserpinized animals there is a constant overflow of newly synthesized catecholamine in adrenergic neurones[33-35] as a result of impaired intraneuronal storage. Uptake of catecholamine into the axoplasm however still occurs. Accordingly, it may be possible that LSD either sensitizes the central adrenergic receptors to the action of the available catecholamine, inhibits the re-uptake mechanism of catecholamine into the neurone, and/or possesses monoamine-oxidase inhibitory properties. This could possibly explain the stronger effect of LSD in reserpinized rats as compared to those animals receiving LSD alone. However until more detailed investigations have been carried out on this phenomenon, the reserpine potentiation of LSD can only be speculated upon.

Desmethylimipramine is a tricyclic antidepressant which is believed to act by inhibition of the re-uptake of noradrenaline into the neurone after release by nervous impulse[36-39]. Since LSD appears to act through catecholamine mediation in producing aberrant behaviour, the potentiation of this effect by desmethylimipramine should be expected and supports the assumption of the LSD mechanism.

As stated in the introduction it is possible that the aberrant behaviour produced by LSD in the rat does not reflect the psychotomimetic action in man. However the

[13] J. H. GADDUM, in *Symposium on 5-Hydroxytryptamine* (Ed. G. P. LEWIS; Pergamon Press, London 1958), p. 195.
[14] V. ERSPAMER, Archs int. Pharmacodyn. Thér. *93*, 293 (1953).
[15] J. H. GADDUM, A. KHAN-HAMEED, D. E. HATHWAY and F. F. STEPHENS, Q. Jl exp. Physiol. *40*, 49 (1955).
[16] W. DOEPFNER, Experientia *18*, 256 (1962).
[17] M. TAESCHLER and A. CERLETTI, Pharmac. exp. Ther. *120*, 179 (1957).
[18] K. KOE and A. WEISSMAN, J. Pharmac. exp. Ther. *154*, 499 (1966).
[19] S. SPECTOR, A. SJOERDSMA and S. UDENFRIEND, J. Pharmac. exp. Ther. *147*, 86 (1965).
[20] D. X. FREEDMAN, J. Pharmac. exp. Ther. *134*, 160 (1961).
[21] D. X. FREEDMAN, Am. J. Psychiat. *119*, 843 (1963).
[22] S. A. ROSECRANS, R. A. LOVELL and D. V. FREEDMAN, Biochem. Pharmac. *16*, 2011 (1967).
[23] J. MUSSACHIO, I. J. KOPIN and S. SNYDER, Life Sci. *3*, 769 (1964).
[24] M. GOLDSTEIN, B. ANAGNOSTE and E. LAUBER, Life Sci. *3*, 763 (1964).
[25] M. GOLDSTEIN, Pharmac. Rev. *18*, 77 (1966).
[26] A. PLETSCHER, M. DA PRADA and G. FOGLAR, 5th Int. Congr. Neuro-psychopharmac. (Excerpta Medica, Amsterdam 1967), p. 101.
[27] J. M. VAN ROSSUM, 5th Int. Congr. Neuro-psychopharmac. (Excerpta Medica, Amsterdam 1967), p. 304.
[28] A. PLETSCHER and M. DA PRADA, 5th Int. Congr. Neuro-psychopharmac. (Excerpta Medica, Amsterdam 1967), p. 304.
[29] H. NYBAECK, G. SEDVALL and I. J. KOPIN, Life Sci. *6*, 2307 (1967).
[30] D. F. SHARMAN, Br. J. Pharmac. Chemother. *28*, 153 (1966).
[31] D. F. SHARMAN, Br. J. Pharmac. Chemother. *30*, 620 (1967).
[32] Z. VOTAVA, S. N. GLISSON and H. E. HIMWICH, Int. J. Neuropharmac. *6*, 543 (1967).
[33] J. GLOWINSKI and J. AXELROD, Pharmac. Rev. *18*, 775 (1966).
[34] L. STJAERNE, Acta physiol. scand. *62*, Suppl. 228 (1964).
[35] L. L. IVERSEN, *Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge Univ. Press. 1967), p. 147.
[36] J. AXELROD, G. HERTTING and L. POTTER, Nature *194*, 297 (1962).
[37] F. SULSER and J. V. DINGELL, in *Antidepressant Drugs of Non-MAO-Inhibitor type* (Proceedings of a Workshop; U.S. Dept. of Health, Education and Welfare 1966).
[38] L. L. IVERSEN, J. AXELROD and J. GLOWINSKI, 5th Int. Congr. Neuro-psycho-pharmac. (Excerpta Medica, Amsterdam 1967), p. 326.
[39] J. GLOWINSKI and J. AXELROD, Nature *204*, 1318 (1964).

behavioural syndrome produced parallels the strong central effects previously reported by CERLETTI.

The work presented here supports the concept that LSD acts, at least partly, through catecholamine mediation.

Zusammenfassung. Der Einfluss verschiedener Pharmaka auf das durch LSD hervorgerufene bizarre Verhalten der Ratte wurde untersucht. Die Resultate zeigen, dass α- und β-Blocker sowie α-Methyltyrosine eine starke Hemmung dieses Verhaltens erzeugen, im Gegensatz zu den Serotonin-Antagonisten Deseril, BOL-148 und Parachlorphenylalaninen, die nur schwache oder keine Hemmung hervorrufen. Es wird gefolgert, dass das bizarre Verhalten durch Wirkung von LSD an Katecholaminstrukturen hervorgerufen wird. Die Wirkung von 4 psychotropen Pharmaka am bizarren Verhalten wird im Rahmen dieser Hypothese diskutiert.

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Institute of Medical and Biological Research, Sandoz Ltd., Basel (Switzerland),
2 May 1968.

PRO EXPERIMENTIS

Serendipitous Precise and Unique Staining of Cell Nuclei

We have recently been interested in developing new techniques and substrates for demonstration of enzymes in tissues, peripheral blood, bone marrow cells and bacteria. Previously we have described the application of the indigogenic principle to the histochemical demonstration of leucine aminopeptidase[1], B-glucosidase[2], B-galactosidase[3], *N*-acetyl-B-glucosaminidase[4], alkaline and acid phosphatase[5], sulfatase[6], B-xylosidase[7], endo and exo nucleases (phosphodiesterases[8]) serum alkaline and acid phosphatase by disc electrophoresis[9,10] and bacterial DNase[11]. The indolyl substrates were synthesized according to the methods described recently by HORWITZ and co-authors[12,13]. The indolyl substrates offer the advantage of precise enzyme localization with no or very little diffusion. Moreover, the substrates offer a simple and direct method for demonstration of the enzymes without the need for a coupling reaction. The principal of the indigogenic reaction is that a hydrolysis of the specific indolyl substrate occurs in the presence of the enzyme yielding a chromogenic highly insoluble indigo at the enzyme site. The addition of the redox system potassium ferro-ferricyanide is frequently included in the incubation medium to effect and accelerate the oxidation of the intermediate indoxyl to indigo[14].

In our recent studies on the intracellular localization of exonuclear and endonuclear 'phosphodiesterase' activity[8] one of the controls which were employed was an incubation of fresh frozen sections of various tissues in potassium ferro-ferricyanide at pH 5.2. We were surprised to observe selective and unique staining of only the cell nuclei of various tissues. We thus made this observation by serendipity.

Methods. Tissues from mouse and rat were used for this study. Representative pieces of tissue from each organ were removed and cut into blocks 2–4 mm in thickness, and quick-frozen by placing the tissue in a glass tube and immersing it in a Dewar flask containing acetone and dry ice at − 70 °C. The tissues were embedded in optimal cutting temperature compound, purchased from Lab-Tek, composed of water-soluble glycols and resins matched to a specific cutting zone temperature of − 20 °C to − 35 °C. The embedded tissue was then placed on the quick-freeze bar of a Lab-Tek cryostat for 1 min until the embedding medium was frozen, and became the proper consistency for cutting 6 μ sections at − 20 °C. After cutting, the sections were attached to warm slides. All solutions were maintained at 4 °C for preservation of enzymatic activity. The slides were then air dried to prevent the formation of ice crystals and stored at − 25 °C until incubated in the specific solution. Fresh frozen sections were incubated for 6 h in solutions consisting of 1 ml 0.05 *M* potassium ferrocyanide, 1 ml 0.05 *M* potassium ferricyanide, 10 ml 0.2 *M* acetate buffer pH 5.2. The reaction was observed hourly until 20 h. After incubation the slides were washed briefly in tap water and mounted in glycerol gel for microscopic examination.

Results. Selective and unique blue staining of only the cell nuclei of mouse and rat kidney, liver, skeletal muscle, spleen, gastrointestinal mucosa, gastrointestinal smooth muscle, pancreas and epididymis. The chromatin of the

[1] B. PEARSON, P. WOLF and M. ANDREWS, Lab. Invest. *12*, 712 (1963).
[2] B. PEARSON, M. ANDREWS and F. GROSE, Proc. Soc. exp. Biol. Med. *108*, 619 (1961).
[3] B. PEARSON, P. L. WOLF and J. VAZQUEZ, Lab. Invest. *12*, 1249 (1963).
[4] P. L. WOLF, J. P. HORWITZ, J. VAZQUEZ, J. CHUA and M. DAROOGE, Am. J. clin. Path. *44*, 307 (1965).
[5] P. WOLF, J. P. HORWITZ, J. VAZQUEZ, J. CHUA, M. S. Y. PAK and E. VON DER MUEHLL, Experientia *23*, 182 (1967).
[6] P. WOLF, J. P. HORWITZ, J. VAZQUEZ, J. CHUA, M. S. Y. PAK and E. VON DER MUEHLL, Proc. Soc. exp. Biol. Med. *124*, 1207 (1967).
[7] P. WOLF, J. P. HORWITZ, J. FREISLER, J. VAZQUEZ and E. VON DER MUEHLL, Enzymologia *34*, 20 (1968).
[8] P. WOLF, J. P. HORWITZ, J. V. FREISLER, J. VAZQUEZ and E. VON DER MUEHLL, Biochim. biophys. Acta, J. Enzymol. *159*, 212 (1968).
[9] E. EPSTEIN, P. L. WOLF, J. P. HORWITZ and B. ZAK, Clin. Chem. *37*, 530 (1967).
[10] E. EPSTEIN, P. L. WOLF, J. P. HORWITZ and B. ZAK, Clin. Chem. *13*, 694 (1967).
[11] P. L. WOLF, J. HORWITZ, R. MANDEVILLE, J. VAZQUEZ and E. VON DER MUEHLL, J. Am. med. Ass. *204*, 23 (1968).
[12] J. P. HORWITZ, J. CHUA, R. J. CURBY, A. J. TOMSON, M. A. DAROOGE, B. E. FISHER, J. MAURICIO and I. KLUNDT, J. med. Chem. *7*, 574 (1964).
[13] J. P. HORWITZ, J. CHUA, M. NOEL, J. T. DONATTI and J. FREISLER, J. med. Chem. *9*, 447 (1966).
[14] A. G. E. PEARSE, in *Histochemistry* (Little, Brown and Co., Boston 1960), p. 888.