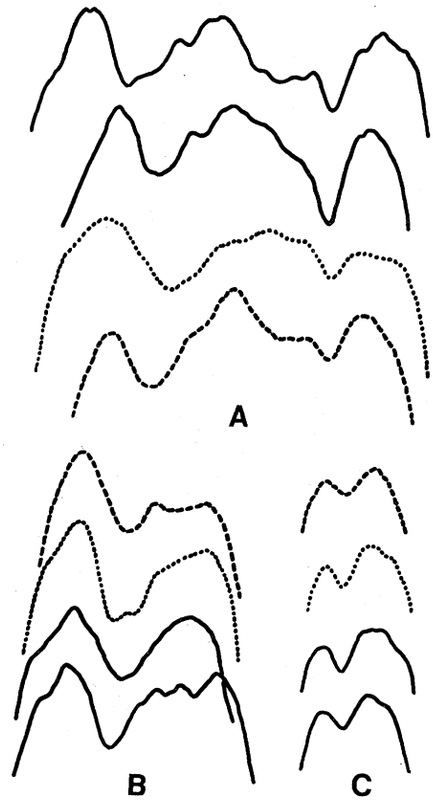


Fig. 2. Distribution profiles of fluorescent intensity for submetacentric marker chromosomes (A) and for normal No. 10's (B) and No. 19's (C). Maximums on the curves represent bright bands on the chromosomes, and minimums represent dark bands. Unbroken lines represent chromosomes from mice homozygous for the translocation (A) or from normal mice (B and C). Dotted and dashed lines represent chromosomes from two different heterozygous cells. The curves for the submetacentrics (A) have been arranged with the region corresponding to the short arms on the right. Curves for No. 10's (B) are arranged with the centromere region to the right, whereas the curves for the No. 19's (C) have the centromere regions to the left.

possible to assign each linkage group to the metaphase chromosome carrying it by applying fluorescent staining to metaphase material from stocks carrying various translocations. Other chromosomal rearrangements, such as inversions (*8*), may also be useful for this purpose. When more linkage groups have been assigned to chromosomes identified by fluorescent staining and a generally accepted standard fluorescent karyotype for the mouse has come into existence, it may prove desirable to adjust the numbering system for mouse linkage groups so that each linkage group has the same number as its chromosome, the chromosomes being numbered according to their relative length within the standard fluorescent karyotype.

MURIEL NESBITT
UTA FRANCKE

*Department of Psychiatry, University of California at Los Angeles, Los Angeles 90024*

**References and Notes**

1. T. Caspersson, L. Zech, E. J. Modest, G. E. Foley, U. Wagh, E. Simmonsson, *Exp. Cell Res.* **58**, 128 (1969).
2. U. Francke and M. Nesbitt, *Cytogenetics*, in press.
3. E. P. Evans, M. F. Lyon, M. Daglish, *ibid.* **6**, 105 (1967).
4. B. M. Slizynski, *J. Genet.* **55**, 122 (1957).
5. M. F. Lyon, *Mouse News Lett.* **40**, 26 (1969).
6. T. Caspersson, L. Zech, C. Johansson, E. J. Modest, *Chromosoma* **30**, 215 (1970).
7. T. C. Carter, M. F. Lyon, R. J. S. Phillip, *J. Genet.* **54**, 462 (1956).
8. T. H. Roderick and N. L. Hawes, *Proc. Nat. Acad. Sci. U.S.* **67**, 961 (1970).
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## Persistent Increase in Brain Serotonin Turnover after Chronic Administration of LSD in the Rat

Abstract. *Lysergic acid diethylamide at doses of 20 micrograms per kilogram per day was administered orally to rats for 1 month. Eighteen hours after the final dose a 25 to 30 percent increase in the synthesis and turnover of serotonin was noted, as well as a moderate but significant increase in the concentration of tryptophan (18 percent) and serotonin (13 percent) in the brain.*

Several kinds of psychiatric disorders have been found among the chronic users of lysergic acid diethylamide (LSD-25), ranging from mild anxiety to panic reactions and long-lasting schizophreniform psychoses (*1*). Since the half-life of an acute dose of LSD-25 in man is reported to be very short (*2*), the behavioral alterations among the chronic users of the drug might be associated with some persistent metabolic effects of the chronic use of LSD-25.

Therefore, we have studied possible persistent effects on the cerebral metabolism of serotonin in the rat, produced by the chronic administration of LSD-25 in small doses similar to those encountered in man. Serotonin metabolism was selected as an object of study because some current hypotheses suggest that altered metabolism of indoleamines may be important in the mechanism of action of LSD-25 (*3*) and may also occur in various behavioral disorders in man (*4*).

Male Sprague-Dawley rats, weighing 150 to 160 g at the beginning of the experiment, were used. Lysergic acid diethylamide (20 $\mu$g/kg dissolved in 1.0 ml of water) was given to the rats through an oro-gastric cannula once a day at 5:00 to 7:00 p.m. for 1 month. The dose of LSD-25 was increased daily according to weight gain. Control animals received the same volume of water only. The weight gain of LSD-treated rats was 97 percent of that of the control group at the moment of death. There were no grossly evident behavioral changes in the LSD-treated animals in any period of the treatment. Sixteen to 20 hours after the last dose of LSD-25, 10 $\mu$c of uniformly labeled L-[$^3$H]tryptophan (New England Nuclear Co., 5 c/mmole) in 25 $\mu$l of a physiological saline solution were injected intracisternally (*5*) under light ether anesthesia. Fifteen minutes later the rats were decapitated. The brains were removed, weighed, and homogenized in 6.0 ml of 1.0*N* HCl in 0.5 percent (wt/vol) ascorbic acid. After centrifugation at 30,000*g* for 10 minutes, the resulting pellets were resuspended in 3.0 ml of the homogenization medium, recentrifuged, and discarded. After the addition of a drop of 2 percent (wt/vol) ethylenediaminetetraacetic acid the *p*H of the combined supernatants was adjusted to 6.9 with 1.0*N* NaOH. After centrifugation at 30,000*g* for 10 minutes, the tissue extracts were poured over Amberlite CG-50 columns at *p*H 6.1 ($H^+$ form) (*6*). Serotonin that adhered to the column was eluted with 3.5*N* HCl; its concentration in the eluate was measured fluorometrically (*7*). The *p*H of the material from the Amberlite columns' effluent was adjusted to 1.5 with 1.0*N* HCl, and passed through a Sephadex G-15 column (0.5 g of dry Sephadex in 0.1*N* HCl) (*6*). The effluent of the Sephadex column was then poured over a Dowex 50 column (200 to 400 mesh, $H^+$ form). The 5-hydroxyindoleacetic acid (5HIAA) retained by the Sephadex column was eluted with 2.5 ml of 0.02*N* $NH_4OH$ and extracted, and its concentration was then mea-

sured fluorometically (*8*). The amino acids adhering to the Dowex column were eluted with 8 ml of 0.5*N* $NH_4OH$. This eluate was neutralized and tryptophan was converted to norharman, which was extracted and measured fluorometrically (*9*). The radioactivity of various fractions was measured by liquid scintillation spectrometry (*10*). The radioactivity of [$^3$H]tryptophan was measured in an aliquot of the norharman extract (*9*).

Chronic intragastric administration of LSD-25 (20 $\mu$g/kg per day) to rats for 1 month had persistent effects on the cerebral metabolism of serotonin (Table 1). Thus, 18 hours after the final dose of LSD-25, the concentration of serotonin in brains of animals treated with LSD-25 was significantly increased by a small amount (13 percent, $P < .05$). The radioactivity of [$^3$H]serotonin derived from the intracisternally injected [$^3$H]tryptophan was found to be increased in the rats treated with LSD-25 (32 percent, $P < .02$). Furthermore, the radioactivity of [$^3$H]-5HIAA was similarly increased in the LSD-25 treated rats (27 percent, $P < .01$), while the concentration of endogenous 5HIAA was apparently unchanged. Moreover, the concentration of endogenous tryptophan, the precursor of serotonin, was increased significantly in the rats treated with LSD-25 (18 percent, $P < .02$), but the apparent increase in the radioactivity of this fraction (20 percent) failed to reach statistical significance.

Thus, it is likely that the synthesis and turnover of serotonin in the brain were persistently increased after the chronic administration of relatively small doses of LSD-25 in the rat. It is of interest that the endogenous level of 5HIAA was not found to increase, despite the clearly increased rates of formation of serotonin. This finding is not consonant with the suggestion that levels of 5HIAA alone may provide a sensitive index of neuronal serotonin turnover (*11*). The observed increase in brain serotonin synthesis, even 18 hours after the final intragastric dose of LSD-25, would not be predicted from the acute metabolic effects of LSD-25 previously reported (*12*).

The acute effects of a single high dose of LSD-25 on serotonin metabolism in the brain include a decrease in the conversion of tryptophan to serotonin and a transient increase in the level of endogenous serotonin. Comparison of these findings with the present observations is made difficult since very high doses of LSD-25 (130 to 5000 $\mu$g/kg) were used in the former experiments, and since the route of administration of LSD-25 and the labeled precursor, as well as the time elapsed between the last dose and killing, were different. Single doses of LSD-25 below 130 $\mu$g/kg have been reported not to alter endogenous serotonin levels in the brain (*12*). Furthermore, in acute experiments using the same methods employed in the present study no alterations of serotonin metabolism were found 1 hour after the small dose of LSD-25 (20 $\mu$g/g) (*13*). By using the same method, we have previously found a moderate decrease of serotonin turnover and an increase of serotonin concentration concomitant with a fall of 5HIAA 45 minutes after a single injection of a large dose of LSD-25 (600 $\mu$g/kg) (*14*). The latter results are quite different from the present data with small amounts of chronically administered LSD-25, but similar to previous results with large doses of the drug (*12*).

The relatively small doses of LSD-25 used in the present experiments have also been found previously to lead to a reversible cessation of the firing of units in the midbrain raphe nuclei (*15*), which are known to contain serotonin (*16*). However, this fact cannot be correlated readily with the present metabolic findings, as the reported cessation of the firing lasted only a few minutes after an acute dose of LSD-25, and chronic effects were not studied. Since the electrical stimulation of the raphe cells can increase the turnover of serotonin in the brain (*17*), it would be well to study firing rates of these cells after the chronic administration of LSD-25.

The increase in the concentration of tryptophan in the brain which is noted might at least partially account for the observed stimulation of serotonin synthesis, as the activity of the first step of the synthesis, tryptophan hydroxylation, is believed not to be saturated with the endogenous levels of the substrate (*18*). Recently it has been shown that the acute administration of LSD-25 or other psychotomimetic substances is also followed by increased tryptophan concentrations in the brain (*19*).

In conclusion, the oral administration of relatively small doses of LSD-25 to rats, similar to those used by man, for 1 month, resulted in increased rates of formation and turnover of serotonin in the brain. These changes persisted for at least 18 hours after the final dose of the drug. Unlike the acute effects of very large doses of LSD-25, it is possible that the chronic use of LSD-25 by man may cause similar persistent metabolic changes. While there is yet no evidence that changes of serotonin metabolism occur in psychoses, it would be of interest to study this possibility in chronic users of LSD-25, who sometimes develop profound psychiatric abnormalities (*1*).

Table 1. Effects of chronic administration of LSD-25 (20 $\mu$g/kg per day, or its vehicle intragastrically) on the tryptophan–serotonin–5-hydroxy-3-indoleacetic acid pathway in the rat brain. Rats were treated once a day for a month; 18 hours after the last dose of LSD-25 the animals were given 10 $\mu$c of [$^3$H]tryptophan intracisternally and decapitated 15 minutes later. Data are accumulated from two to four separate experiments and are expressed as means ± S.E.M., with the number of animals in parentheses. The probability was calculated by Student's *t*-test. Abbreviation: dpm, disintegrations per minute.

| Measurement | Control | LSD-25 treated | Percent of control |
|---|---|---|---|
| | *Tryptophan* | | |
| Content (nmole/g) | 25.10 ± 0.69 (25) | 29.51 ± 1.47 (24) | 118* |
| Disintegrations per minute per whole brain | 765,929 ± 58,611 (13) | 920,819 ± 83,547 (12) | 120 |
| Specific activity (dpm/nmole) | 15,985 ± 1,197 (13) | 16,749 ± 1,123 (12) | 105 |
| | *Serotonin* | | |
| Content (nmole/g) | 2.84 ± 0.06 (25) | 3.20 ± 0.15 (26) | 113† |
| Disintegrations per minute per whole brain | 16,454 ± 1,139 (25) | 21,703 ± 1,700 (24) | 132* |
| Specific activity (dpm/nmole) | 3,035 ± 212 (25) | 3,640 ± 266 (24) | 120† |
| | *5-Hydroxy-3-indoleacetic acid* | | |
| Content (nmole/g) | 1.71 ± 0.07 (20) | 1.68 ± 0.08 (18) | 98 |
| Disintegrations per minute per whole brain | 4,461 ± 278 (20) | 5,661 ± 322 (18) | 127‡ |
| Specific activity (dpm/nmole) | 1,367 ± 101 (20) | 1,763 ± 107 (18) | 129‡ |

* $P < .02$. † $P < .05$. ‡ $P < .01$.

JOSÉ-LUIS DÍAZ*
MATTI O. HUTTUNEN†
*Psychiatric Research Laboratories, Massachusetts General Hospital, Boston 02114*

**References and Notes**

1. J. Cohen and K. J. Ditman, *J. Am. Med. Ass.* **181**, 161 (1962); J. T. Underlieder, D. D. Fisher, M. Fuller, *ibid.* **197**, 389 (1966).
2. G. K. Aghajanian and O. H. L. Bing, *Clin. Pharmacol. Ther.* **5**, 611 (1964).
3. G. K. Aghajanian and D. X. Freedman, in *Psychopharmacology: A Review of Progress*, D. H. Efron, Ed. (Government Printing Office, Washington, D.C., 1968), p. 1185; J. H. Gaddum, *J. Physiol.* **121**, 15P (1953); N. J. Giarman and D. X. Freedman, *Pharmacol. Rev.* **17**, 1 (1965).
4. M. M. Airaksinen and W. M. McIsaac, *Ann. Med. Exp. Fenn.* **46**, 367 (1968); G. Curzon, *Br. J. Psychiat.* **115**, 1367 (1969); D. W. Wooley and E. N. Shaw, *Proc. Nat. Acad. Sci. U.S.* **40**, 228 (1954).
5. S. M. Shanberg, J. J. Schildkraut, I. J. Kopin, *J. Pharmacol. Exp. Ther.* **157**, 311 (1967).
6. J. Jonsson and T. Lewander, *Acta Physiol. Scand.* **78**, 43 (1970).
7. N.-E. Andén and T. Magnusson, *ibid.* **69**, 87 (1967); D. F. Bogdanski, A. Pletscher, B. B. Brodie, S. Udenfriend, *J. Pharmacol. Exp. Ther.* **117**, 82 (1956).
8. E. Giacalone and L. Valzelli, *J. Neurochem.* **13**, 1236 (1966).
9. W. D. Denckla and H. K. Dewey, *J. Lab. Clin. Med.* **69**, 160 (1967); R. R. Lin, E. Costa, N. H. Neff, C. T. Wang, S. H. Ngai, *J. Pharmacol. Exp. Ther.* **170**, 232 (1969). The method for separation of tryptophan and metabolites was developed by J. Macon.
10. I. J. Kopin, J. Axelrod, E. Gordon, *J. Biol. Chem.* **236**, 2109 (1961).
11. N.-E. Andén, H. Corrodi, K. Fuxe, T. Hökfelt, *J. Pharmacol. Chemother.* **34**, 1 (1968); P. M. Díaz, S. H. Ngai, E. Costa, *Advan. Pharmacol.* **6B**, 75 (1968); W. D. Reid, L. Volicer, H. Smookler, M. A. Beaven, B. B. Brodie, *Pharmacology* **1**, 329 (1968).
12. D. X. Freedman, *J. Pharmacol. Exp. Ther.* **134**, 160 (1961); ———, R. Gottlieb, R. A. Lovell, *Biochem. Pharmacol.* **19**, 1181 (1970); R. C. Lin, S. H. Ngai, E. Costa, *Science* **166**, 237 (1969); J. Shubert, H. Nyback, H. Sedvall, *Eur. J. Pharmacol.* **10**, 215 (1970).
13. J. Macon, personal communication.
14. J.-L. Díaz and M. O. Huttunen, in *Homenaje al Dr. Dionisio Nieto*, C. Pérez de Francisco and H. Pérez-Rincón, Eds. (National Univ. of Mexico, in press).
15. G. K. Aghajanian, W. E. Foote, M. H. Sheard, *Science* **161**, 706 (1968).
16. A. Dahlström and K. Fuxe, *Acta Physiol. Scand.* **232** (Suppl.), 1 (1965).
17. G. K. Aghajanian, J. A. Rosecrans, M. H. Sheard, *Science* **156**, 402 (1967).
18. M. M. Airaksinen, E. Giacalone, L. Valzelli, *J. Neurochem.* **15**, 55 (1968).
19. J. R. Tonge and B. E. Leonard, *Life Sci.* **9**, 1327 (1970).


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* Permanent address: Instituto de Investigaciones Biomédicas, UNAM, and Instituto Nacional de Neurología, SSA; Mexico City, Mexico.

† Present address: Departments of Medical Chemistry and Psychiatry, University of Helsinki, Helsinki, Finland.


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## Nonconversion of o,p'-DDT to p,p'-DDT in Rats, Sheep, Chickens, and Quail


Abstract. *The finding of appreciable quantities of* p,p'*-DDT after feeding* o,p'*-DDT to rats led to the proposal of a theory that an isomeric metabolic conversion occurs. The presence of* p,p'*-DDT as an impurity in supposedly pure samples of* o,p'*-DDT is the correct explanation for the appearance of* p,p'*-DDT. Purified* o,p'*-DDT and* $^{14}C$*-labeled* o,p'*-DDT yielded no data to support the idea that* o,p'*-DDT is converted to the* p,p'*-DDT isomer.*


Klein *et al.* (*1*, *2*) reported the supposed isomeric conversion of *o,p'*-DDT to *p,p'*-DDT in the rat (*3*). This conversion was based on the finding of appreciable quantities of *p,p'*-DDT after feeding *o,p'*-DDT to rats. Such a conversion would involve either (*1*) splitting off the *o*-chlorophenyl group from the ethane chain with subsequent recombination to form a *p,p'* molecule or (*2*) replacement of the *o*-Cl by H, and chlorination of the para position. Both of these mechanisms appear to be unlikely biological metabolic reactions (*4*). The purpose of our study was to demonstrate that the conversion of *o,p'*-DDT to *p,p'*-DDT does not occur biologically and to provide a more logical explanation for the appearance of *p,p'*-DDT after feeding pure *o,p'*-DDT.

An explanation for the supposed conversion can be given by the presence of *p,p'*-DDT as an impurity in the *o,p'*-DDT which was fed. When a dilute (1 μg/ml) solution of *o,p'*-DDT (over 99 percent pure, Aldrich) was analyzed by gas-liquid chromatography (GLC) (*5*), a single peak with a retention time characteristic of *o,p'*-DDT is observed. When, however, a concentrated (100 μg/ml) solution was analyzed, the presence of *p,p'*-DDT was also noted. Thin-layer chromatography (TLC) of an equivalent sample of this solution and subsequent GLC analysis resulted in essentially quantitative recovery of *p,p'*-DDT from this supposedly pure *o,p'*-DDT solution. Thus, the simple step of analyzing a concentrated solution of *o,p'*-DDT, rather than an extremely dilute one, demonstrated the presence of *p,p'*-DDT.

During the past 2 years, we have conducted experiments in which *o,p'*-DDT was fed to rats (*Rattus norvegicus*), sheep (*Ovis aries* L.), Japanese quail (*Coturnix coturnix japonica*), and chickens (*Gallus domesticus*). Analysis of body lipid revealed that *o,p'*-DDT was the major pesticidal residue but that significant quantities of *p,p'*-DDT were found (Table 1). In all cases, enough *p,p'*-DDT was ingested as an impurity to account for the *p,p'*-DDT found in the animals at the end of the experiment.

Analysis of three batches of commercial *o,p'*-DDT are shown in Table 2. These batches (Aldrich) contained much more *p,p'*-DDT than did earlier samples from the same source, and they contained significantly more *p,p'*-DDT than a sample obtained from the Pesticide Chemicals Branch of the U.S. Department of Agriculture (USDA-ENT 3983). The USDA *o,p'*-DDT has been prepared by isolation from technical DDT 25 years ago in a study of the composition of technical DDT (*6*).

As additional proof that *p,p'*-DDT is not formed from *o,p'*-DDT, pure *o,p'*-DDT was prepared for subsequent feeding trials. Samples (60 mg) of commercial *o,p'*-DDT (Aldrich) were chromatographed on 150 g of aluminum oxide (Merck) in a glass column [27 mm (inside diameter) by 300 mm]. The chromatogram was developed with 300 ml of *n*-hexane, and each 10-ml fraction was analyzed by GLC for the presence of *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE. Those fractions containing only *o,p'*-DDT, usually fractions 13 to 15, were combined to provide pure *o,p'*-DDT for the feeding trials. By this technique, a product was obtained which was 99.974 percent pure *o,p'*-

Table 1. *p,p'*-DDT in rats, sheep, Japanese quail, and chickens after feeding impure *o,p'*-DDT.

| Species | *o,p'*-DDT in diet (ppm) | Time (days) | *p,p*-DDT impurity (%) | *p,p'*-DDT intake (mg) | *p,p'*-DDT retained (mg) | Retention (%) |
|---|---|---|---|---|---|---|
| Rat | 20 | 98 | 0.4 | 0.118 | 0.065 | 55 |
| | 40 | 98 | 0.4 | 0.235 | 0.116 | 49 |
| | 100 | 22 | 1.3 | 0.453 | 0.322 | 71 |
| Sheep-ewe | 10 | 120 | 0.5 | 8.400 | 0.625 | 7 |
| Lamb | 10 | 87 | 0.5 | 2.958 | 1.012 | 34 |
| Japanese quail | 100 | 45 | 0.4 | 0.144 | 0.104 | 72 |
| Chicken | 150 | 98 | 0.6 | 9.700 | 5.004 | 52 |