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Studies on deoxyribonucleic acid metabolism in human cells treated with lysergic acid diethylamide*

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CHROMOSOMAL abnormalities have been reported in cultures *in vitro* of human leukocytes,[1] as well as in leukocytes of a patient previously treated with lysergic acid diethylamide (LSD).[2] Abnormalities have been observed in meiotic chromosomes of mice [3,4] and in barley seeds.[5]

However, Loughman *et al.*,[6] Sparkes *et al.*[7] and Bender and Siva Sankar[8] did not find any chromosomal abnormalities in leukocytes from persons exposed to LSD. No chromosome aberrations were observed in *Drosophila melanogaster*[9] or in *Vicia faba*, Chinese hamster and human leukocytes,[10] or in *Allium* meristematic cells.[11]

LSD has been reported to be mutagenic in *Drosophila*.[12–14] However, Grace *et al.*[9] were not able to demonstrate an increase in chromosomal damage or mutation due to LSD treatment. Zetterberg[15] has demonstrated that LSD had no significant influence on back-mutations in *Ophisotoma*.

As a teratogenic agent, LSD has been reported to increase the number of stillborn and stunted fetuses[16] and to induce brain malformations in certain strains of mice.[17] It has also been reported that LSD induces lens anomalies in mice,[18] and congenital malformations in hamsters.[19] However, no teratogenic effect has been demonstrated in rats,[20] in mice or hamsters,[21,22] in rabbits,[23] or in man.[24,25]

On the molecular level, Yielding and Sterglanz,[26] Wagner,[27] and Smythies and Antun[28] have reported that LSD binds to native DNA. This investigation was undertaken to determine if the reported LSD–DNA complex might interfere with normal DNA metabolism in human cells grown *in vitro*.

LSD (Sandoz Batch LSD-25 No. 88601) was supplied by the National Institute of Mental Health. It was applied to the tissue culture medium and filter-sterilized just prior to use.

Tissue culture. Human amnion (AV_3) cells from the American Type Culture Collection (Rockville, Md.) were used for these experiments. The AV_3 cells were grown in Eagles' minimal essential medium, supplemented with 10% calf serum and 60 μg/ml of anti-PPLO agent (Grand Island Biological Company). Cells were plated on 60-mm Falcon plastic petri dishes for experiments.

DNA synthesis assay. To determine if LSD treatment affected DNA synthesis, the method described by Bollum,[29] as modified by Regan and Chu,[30] was utilized. Cells (8×10^5 cells/60 mm petri dish) were inoculated and incubated for 1 day before assay. The old medium was decanted and new medium containing ^{3}H-thymidine (2·5 μc/ml; 2·0 c/m-mole), with or without various concentrations of LSD, was used. At various times after exposure to ^{3}H-thymidine or ^{3}H-thymidine plus LSD, duplicate plates for each treatment were sonicated with a Sonifier (Heat Systems Company, Melville, N.Y.) for 10 sec.[31]

Ultra-violet induced pyrimidine dimer assay. AV_3 cells were labeled for 15 hr in ^{3}H-thymidine (1 μc/ml; 2·0 c/m-mole). The ultra-violet light irradiation was performed with one 15 W germicidal lamp, mounted in a Microvoid transfer hood. The ultra-violet light was predominantly 2537 Å and the incident dose-rate to the cells was 25 ergs/mm^2/sec. Prior to irradiation, the medium was decanted from plates, the edge of the monolayer of cells was scraped with a rubber "policeman", and the cells were washed twice with Hanks' buffered saline. The cells were irradiated in 0·5 ml of Hanks' saline.

To determine if LSD interferes with the production of ultra-violet-induced pyrimidine dimers, 3 ml medium, with or without LSD (20 μg/ml), was put on the cells 0·5 hr prior to the irradiation. Media were decanted, and 0·5 ml of Eagle's medium, with or without LSD, was added and the cells were irradiated. Immediately after the irradiation, cells were harvested and fixed in cold 5% trichloroacetic acid (TCA) and the insoluble residue was analyzed for pyrimidine dimers by two-dimensional chromatography after hydrolysis in formic acid.[32,33]

To determine if LSD interfered with normal excision of ultra-violet-induced pyrimidine dimers, AV_3 cells, irradiated in normal medium, were either collected immediately for pyrimidine dimer analysis or reincubated in medium, with or without LSD (20 μg/ml or 0·002 μg/ml). After 24 hr, these irradiated cells were collected and the pyrimidine dimers remaining in the TCA-insoluble residue were analyzed as above.

Alkaline sucrose gradients. To determine if LSD might interfere with the synthesis of new DNA (i.e. preventing small fragments from being linked together), the relative molecular weights of DNA molecules, synthesized in the presence or absence of LSD, were determined by a modified alkaline

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sucrose gradient technique[34] described by McGrath and Williams.[35] AV_3 cells were incubated in ^{3}H-thymidine (2 μc/ml; 15·9 c/m-mole), with or without LSD (20 or 0·2 μg/ml). The 3·6-ml gradients of 5–20% sucrose contained 0·3 M NaOH, 2 M NaCl and 0·01 M EDTA. At the bottom was a cushion of 0·2 ml of 60% alkaline sucrose. At the top was 0·2 ml of 1 M NaOH into which approximately 5000 cells were gently layered. After remaining at 22° for 1 hr, the samples were spun for 90 min at 30,000 rev/min in a Beckman SW-56 rotor. The bottom of each centrifuge tube was punctured and 30 $\pm$ 1 fractions were collected on filter paper disks. The disks were washed with cold 5% TCA, then ethyl alcohol, then dried and counted in a scintillation spectrometer.

To determine if LSD broke pre-existing DNA, cells were labeled with ^{3}H-thymidine (2 μc/ml; 15·9 c/m-mole) for 24 hr. The medium was decanted and nonradioactive medium, with or without LSD, was added to each plate for 12 hr. Cells were collected and placed on a gradient as described above.

Although the molecular basis of chromosome aberrations has not been worked out (see references 36, 37), there is some evidence that a lesion in DNA leads to chromosome breaks.[38,39] With the reported observations that LSD does interact with DNA *in vitro*[26–28] and that LSD can induce chromosome aberrations[1,3–5] and with the assumption that a molecular lesion in DNA can lead to a chromosome break, the following experiments were undertaken to determine if any abnormal DNA metabolism could be detected in LSD-treated human cells grown *in vitro*.

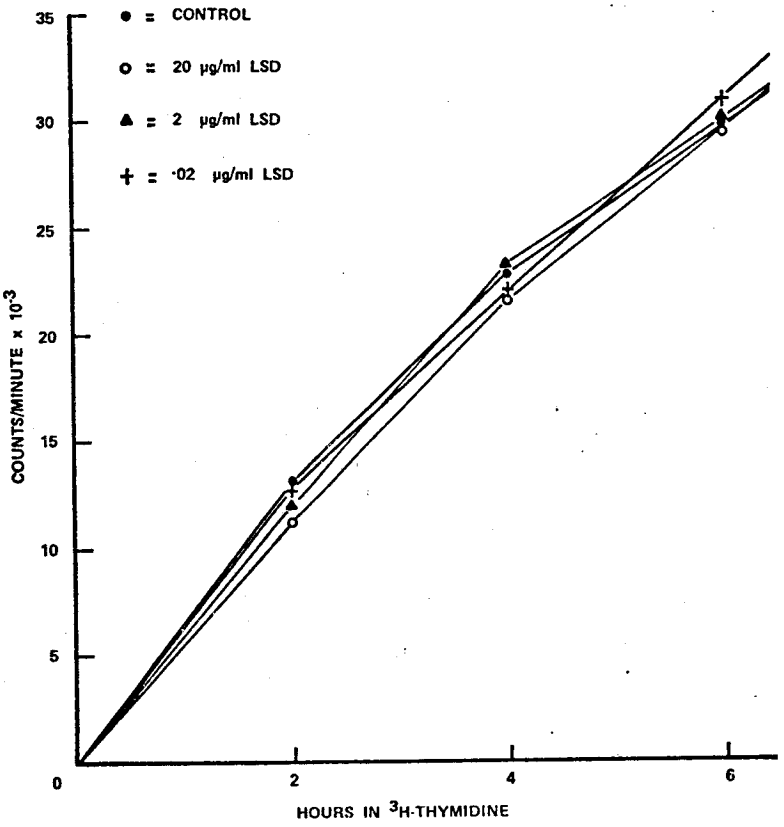

FIG. 1. Incorporation of ^{3}H-thymidine into LSD-treated human amnion cells.

From the data in Fig. 1, it is apparent that LSD (up to 20 μg/ml) had no significant effect on the rate of ^{3}H-thymidine incorporation into DNA. The rationale of this experiment stemmed from the observation that many drugs that bind to DNA induce, directly or indirectly, chromosome aberrations.[40] Many of these drugs form lesions which interfere with DNA synthesis.[41,*] The absence of any detectable effect might be due to the insensitivity of the assay method.

* J. E. Trosko, unpublished results.

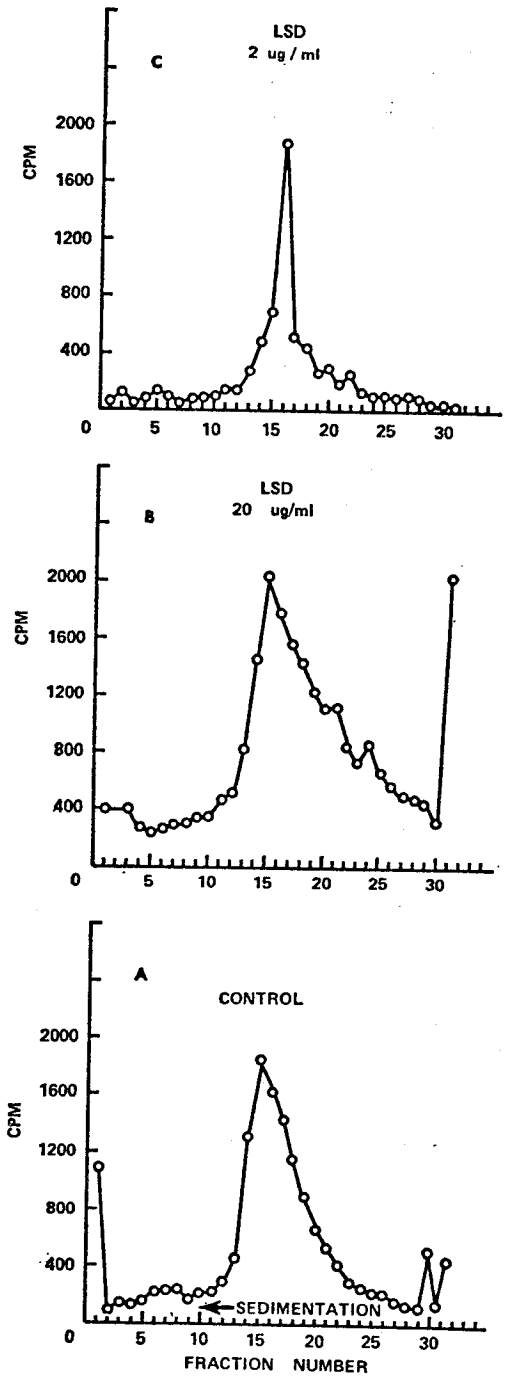

FIG. 2. Alkaline sucrose gradients of ^{3}H-thymidine material from human amnion cells which were treated with LSD. Estimated single-stranded molecular weight at peaks, using condition reported by Setlow *et al.*,[43] is greater than 10^{8} daltons.

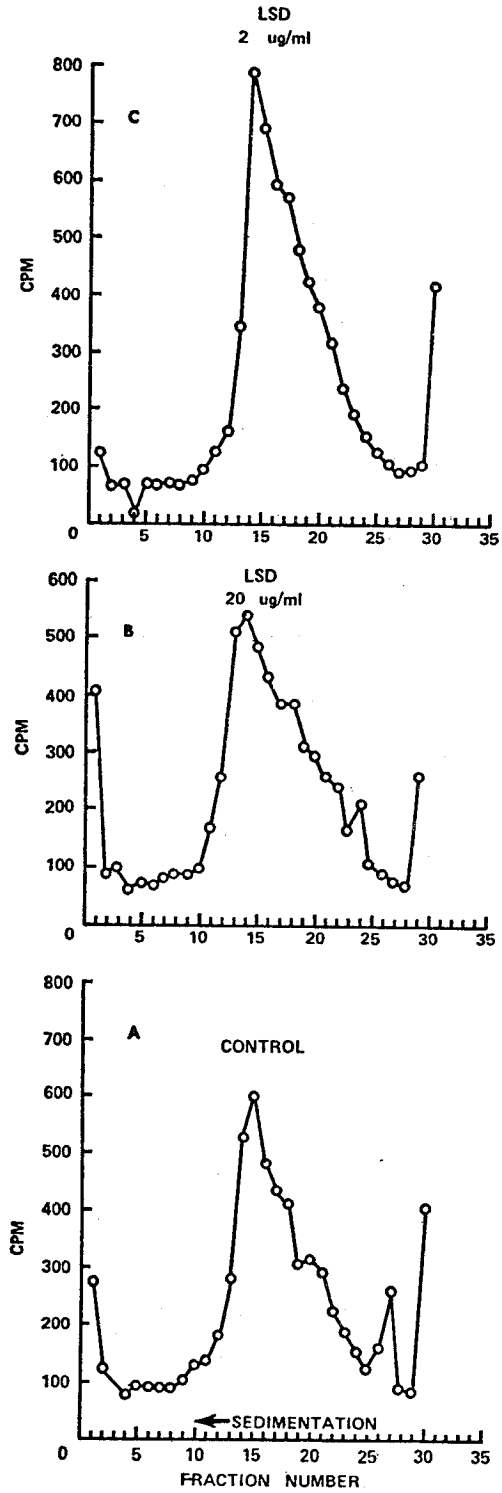

FIG. 3. Alkaline sucrose gradients of ^{3}H-thymidine material synthesized in LSD-treated and non-LSD-treated human amnion cells.

TABLE 1. FORMATION OF ULTRA-VIOLET LIGHT-INDUCED THYMINE-CONTAINING DIMERS IN THE DNA OF LSD-TREATED HUMAN AMNION CELLS

Treatment	$\widehat{XT}$/T*	% Thymine as dimer
No u.v.†, no LSD	0/685,900	0·00
u.v., no LSD	455/632,100	0·07
u.v. + 20 μg/ml LSD	400/481,500	0·08
u.v. + 0·002 μg/ml LSD	330/418,500	0·07

* $\widehat{XT}$ refers to both $\widehat{TT}$ and $\widehat{UT}$ dimers, since chromatography procedures used here do not separate them from each other.

† Cells were irradiated with 300 ergs/mm² of 2537 Å ultra-violet (u.v.) light.

The data in Table 1 indicate that, at the concentrations of LSD that were used and at the ultra-violet dose that was delivered to the cells, no detectable difference was found in the formation of ultra-violet-induced pyrimidine dimers in DNA. Also, in Table 2, it is apparent that excision of ultra-violet-induced pyrimidine dimers was not affected in LSD-treated cells.

TABLE 2. EXCISION OF ULTRA-VIOLET LIGHT-INDUCED THYMINE-CONTAINING DIMERS IN LSD-TREATED HUMAN AMNION CELLS

Treatment	Time after u.v. dimers analyzed (hr)	$\widehat{XT}$/T*	% Thymine as dimer
u.v.†	0	688/1,276,700	0·05
u.v.	24	300/860,900	0·03
u.v. + 20 μg/ml LSD	24	307/881,700	0·03
u.v. + 0·002 μg/ml LSD	24	310/1,023,700	0·03

* $\widehat{XT}$ refers to both $\widehat{TT}$ and $\widehat{UT}$ dimers, since chromatography procedures used here do not separate them from each other.

† Cells were irradiated with 200 ergs/mm² of 2537 Å ultra-violet (u.v.) light.

In spite of the fact that the number of chromosome breaks that were found in LSD-treated cells was small, it was hoped that, if LSD does induce breaks in the DNA molecule, one might detect a shift in the molecular weight of LSD-treated cells, such as those observed in cells treated with doses of ultra-violet light or X-rays, which are known to induce chromosome breaks.[42,43] No detectable shifts in the molecular weight were observed (Fig. 2).

Some compounds such as caffeine, which also breaks chromosomes,[44,45] have been shown to interfere with the linkage of small fragments of DNA into larger ones.[46] LSD, given to cells synthesizing DNA, does not appear to interfere with the synthesis of normal-sized DNA (Fig. 3).

In conclusion, with the results of these techniques for measuring DNA metabolism, we cannot determine whether LSD had no effect or little effect, or whether the techniques were not sensitive enough to pick up any effect.

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-treated and non-

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