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EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4339

EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON ESCHERICHIA COLI B/Rλ

by

Michael J. Dowler, CPT, MSC
Jack S. Wolpert, SP4

February 1970

DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Research Laboratories
Medical Research Laboratory
Edgewood Arsenal, Maryland 21010

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Experimental Medicine Department

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Task 1B562602AD1904

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FOREWORD

The work described in this report was authorized under Task 1B562602AD1904, Methods and Evaluation of Chemical Effects on Personnel, New Methods for Biological Assays (U). This work was started in January 1968 and completed in February 1969. The experimental data are contained in notebooks 1 and 2.

Acknowledgments

We are grateful to Dr. Bruno Papirmeister for his ideas and suggestions during the course of the research and his helpful criticisms of the manuscript. We would also like to thank Drs. W. W. Anderson, L. J. Arcement, J. K. Dorsey and W. B. Watt for the critical reading of the manuscript.

DIGEST

The effect of lysergic acid diethylamide (LSD) on *Escherichia coli* B/rλ was examined as a test system for damage to genetic material. The chemical depressed bacterial growth rate in a dose-dependent manner. This effect was not amplified by the DNA-repair inhibitor, caffeine. The lowering of the growth rate by LSD was completely reversible upon dilution. LSD caused a dose-dependent extension of the lag in recovery in growth rate after UV damage. Caffeine caused a much longer extension of this lag time. All of the above effects were noted only when extremely high doses of LSD were used. It should be noted that the relevance of these findings to the reported chromosome damage is questionable.

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EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON *Escherichia coli*, STRAIN B/rλ

I. INTRODUCTION.

Lysergic acid diethylamide (LSD) has been the subject of much recent controversy. A large portion of this controversy appeared to be a result of (a) an extremely high rise in the use of LSD and (b) a report by Cohen, Hirschhorn and Frosch[1] that LSD users had a higher incidence of chromosomal abnormalities than control subjects who had not taken the drug. The implications of this finding sparked many investigations on the possible effect of LSD on chromosomes *in vivo* and in cell culture, and possible teratogenic or mutagenic effects of this drug. Several investigators[1-6] reported an effect of LSD on chromosomes, while others[7-10] found no such occurrence in similar systems. A teratogenic effect due to LSD has been reported by some investigators,[11-14] however, this effect was not noted in the experiments of Warkany and Takacs.[15] Browning[16] reported an increased mutation rate after LSD treatment, but the study of Grace, Carlson, and Goodman[9] did not confirm this finding. Recently there have been two reports[17, 18] of LSD-induced damage in the meiotic chromosomes of mice. As can be seen, there is no clear consensus on the issue of LSD-induced chromosomal damage, teratogenesis, or mutagenesis. A review by Smart and Bateman[19] summarized and compared most of the findings in the area of LSD-induced chromosomal damage and teratogenesis. Clearly there is some unnoticed variable which is not constant among different laboratories. This could be something as simple as the chemical purity of the LSD used or as complex as the interaction of seemingly unimportant factors.

There have not been many published reports dealing with a possible molecular explanation of the chromosomal or teratogenic phenomena which were sometimes observed. However, Yielding and Sterglanz[20] have demonstrated the *in vitro* binding of LSD to deoxyribonucleic acid (DNA). This binding did not occur when the DNA was nonhelical nor did the binding occur with RNA. This demonstration of rather specific binding indicates that such chromosomal aberration could involve DNA damage.

DNA damage can be divided into three groups: (1) Direct damage or covalent alteration, such as that caused by UV light, X-ray, or alkylating agents; (2) indirect damage or interference with metabolism which culminates in DNA-bond alterations, such as that caused by 5-fluorodeoxyuridine;[21] and (3) changes caused by compounds which combine with DNA but do not, by themselves, produce covalent alteration. An example of the third type is the DNA-repair inhibitor caffeine. In low concentrations this compound, alone, does not cause covalent alteration of DNA. After irradiation by UV light, however, low concentrations prevent the repair of DNA.

Our study of these three types of DNA damage involved experimentation with the bacterium *Escherichia coli*, strain B/rλ*. The use of strain B/rλ provided several advantages for

*An enormous amount of work has been done on the *E. coli* B and K-12 systems, dealing with repair of DNA damage caused by UV light, X-rays and chemical alkylating agents. For background on these systems refer to an excellent review by Howard-Flanders[22] and the Brookhaven Symposia in Biology No. 20 (in particular a paper by Evelyn Witkin[23]).

studying DNA damage. Because the strain contained a λ prophage it was more sensitive to direct DNA damage (type 1) than was strain B/r. Also, because B/rλ had DNA-repair capability, repair-inhibitor properties of a compound (type 3) could be readily assessed.

The similarity of the DNA-repair processes in the well-studied bacterial system and in other cells is pointed out by Cleaver.[24] His evidence indicates that normal human skin fibroblasts can perform DNA repair replication after UV irradiation. Fibroblasts obtained from patients with the hereditary disease *xeroderma pigmentosum* showed a much reduced or absent repair replication of DNA. Thus, the bacterial test system involving DNA damage and repair may prove relevant to higher order systems.

II. MATERIALS AND METHODS.

A. Strains.

Experiments were carried out with *Escherichia coli*, B/r mal$^+$ (λ), obtained from W. Harm, Southwest Center for Advanced Studies, Dallas, Texas.

B. Growth and Dilution.

Bacteria were grown in 0.8% Difco nutrient broth containing 0.5% NaCl, in a 37°C shaking water bath, to a titer of 2 to 8 × 10^8 cells/ml as indicated by optical density at 650 mμ.

Bacteria grown for plating experiments were diluted 1 to 100 into room-temperature 0.12 *M* tris(hydroxymethyl)aminomethane hydrochloride buffer (tris-HCl) *p*H 7.8. The cells were agitated for 30 seconds and then set aside for 30 minutes at room temperature to allow cell clumps to disperse. The bacteria were again agitated for 60 seconds just prior to use.

Cells for liquid-growth experiments were taken in log phase, quickly chilled in an ice bath, and then centrifuged at 8000 g for 20 minutes at 4°C. The supernatant was discarded and the pellet was warmed in a 37°C water bath for 10 minutes to prevent a loss in cell viability due to pretreatment with ethylene diamine tetra acetate (EDTA) in tris-HCl at low temperatures (described later). The bacteria were resuspended in 25° tris-HCl.

C. UV Irradiation.

Bacteria were irradiated from a distance of 15 cm with a 9-watt, low-pressure, mercury-arc lamp* emitting chiefly at 2537 A. Cells were exposed while stirring, in an open petri dish, 3.5 cm in diameter, at a volume of 2 to 3 ml and a titer of 5.6 × 10^8 cells/ml for the plating experiments, and 1.6 × 10^8 to 6.5 × 10^9 cells/ml for the liquid growth experiments. UV doses were adjusted for changes in titer and volume during treatment. All postirradiation handling was confined to a darkened room to prevent photoreactivation.

*Model SL 2537. Black Light Corporation of America, Gabriel, California.

D. EDTA Treatment.

Prior to being placed in the growth medium, cells were routinely treated with EDTA, using the method of L. Leive[25] and L. Leive and V. Kollin.[26] This treatment insures cell permeability to LSD. Leive[25,27] has shown that actinomycin and a number of other compounds can enter cells only after EDTA treatment, such treatment not affecting normal growth rates or viability. Although we later found LSD to be effective in the liquid growth medium, without EDTA, we continued routine EDTA treatment to be consistent.

Cells suspended in tris-HCl at a concentration of 5×10^6 to 1×10^{10} cells/ml were exposed to 2×10^{-4} *M* EDTA for 2 minutes at 25° to 37°C and then diluted 10 fold with the appropriate solution. This dilution was then handled as indicated in the assay section.

E. Assay Methods.

1. Plating assay.

The diluting solution was 0.15 *M* NaCl at room temperature. The appropriate serial dilutions were plated to give 60 to 250 colonies per plate. Duplicate plates for each of the dilutions were prepared by pipetting 0.5 ml of cells into an empty plate and pouring in a measured amount of 45° to 47°C liquid nutrient agar. This agar contained 1.1% Bacto agar, 0.8% Difco nutrient broth, 0.5% NaCl, and the appropriate amount of LSD and/or caffeine where applicable. The time which elapsed from termination of EDTA exposure to pouring of the agar was approximately 10 minutes. After the agar hardened, plates were inverted, incubated in the dark at 37°C for about 60 hours and counted.

2. Liquid growth assay.

The diluting solution was nutrient broth medium containing LSD or caffeine where applicable. Cultures were grown while shaking in a 37°C water bath and optical density measurements at 650 mμ were taken at the indicated times. Figure 1 shows that, for untreated B/rλ, a doubling of absorbance corresponds to a doubling of titer and not simply an increase in cell size. This relationship was found to hold for the various treatments.

F. Test Compounds.

The compounds were caffeine monohydrate* and the LSD maleate salt** (formula wt: 439). The LSD was twice recrystallized from methanol and characterized by its IR spectrum and UV absorption maximum.

All solutions were autoclaved prior to use, with the exception of the LSD and caffeine which were prepared by adding the compounds to presterilized solutions.

*Merck Chemical Division, Merck & Company, Inc., Rahway, New Jersey.
**Edgewood Arsenal Chemical Research Laboratory.

III. EXPERIMENTATION AND RESULTS.

The initial experiments were done using a plating technique, with LSD in the agar, to investigate the effects of this compound on bacterial viability. The observed results indicate that the effects of LSD are dose dependent (see figure 2).

Some studies modeled after those carried out with other agents were attempted in order to elucidate the mechanism of action of this effect. Harm[28] has shown that caffeine, by virtue of inhibiting dark repair, considerably potentiated killing of UV-irradiated bacteria. Were LSD to cause direct damage to DNA, as does UV light, a similar synergism might be expected to occur when caffeine is included in the plating agar. The result of this experiment is shown in figure 3. The fact that the two curves are not significantly different demonstrates that the LSD effect on *E. coli* B/rλ was not the result of direct DNA damage which was subject to normal dark repair. Concentrations of LSD higher than 1.25 mg/ml gave anomalous results because of poor colony formation.

To examine the possibility that LSD acted as a caffeine-like repair inhibitor, we assessed the effect of LSD on UV-irradiated bacteria. Bacteria were irradiated with varying doses of UV light and then plated in the presence of 1.0 mg LSD/ml of nutrient agar. Aliquots of the irradiated bacteria were also plated in unsupplemented agar and in agar containing 1.5 mg caffeine/ml. The results of this experiment are shown in figure 4. Whereas caffeine greatly accentuated the lethal effect of UV light, LSD was much less effective and produced a survival curve which deviated only slightly from the control. This indication of LSD causing a weak inhibition of repair was more rigorously examined by the spectrophotometric method. The results are discussed later.

During the course of the above experiments it was noticed that it took a much longer incubation time to obtain colonies of countable size on the LSD-treated plates than on the untreated controls. Also, there was a great variation in size of even the subsurface colonies on the LSD-treated plates. These results were further investigated by reexamining the dose-response curve. The plates were counted at the regular time, allowed to incubate an additional 24 hours, and then reexamined. There appeared to be many additional colonies not counted in the original scoring. These colonies were not contaminants from the first counting because they were morphologically identical to the original colonies and the majority were subsurface colonies. Control plates showed no contamination. These data suggested that LSD had an effect on bacterial growth rate or that catabolism of LSD was necessary for growth to occur.

By the use of a spectrophotometric growth assay, it was shown that LSD affected the growth rate of the bacteria. The optical density was followed at 650 mμ as an indication of doubling. A concomitant plating experiment revealed that the rise in optical density was due to actual doubling and not merely an increase in cell size (Turbidity would show both as an increase in optical density, figure 1). The highest concentration of LSD used (1.25 mg/ml) was bacteriostatic for 5 hours. However, the bacteria eventually grew to approximately the control growth limit. LSD at 1.0 mg/ml was examined first because it was used in our plating experiments. This bacteriostatic effect could be reversed by diluting out the LSD (see figure 5). After dilution there was no effect on the growth rate as indicated by comparison with the control curves. The growth rate was the same for LSD-treated bacteria diluted 1 to 20 into fresh media after brief or longer periods of exposure (curves B and E, respectively); control bacteria

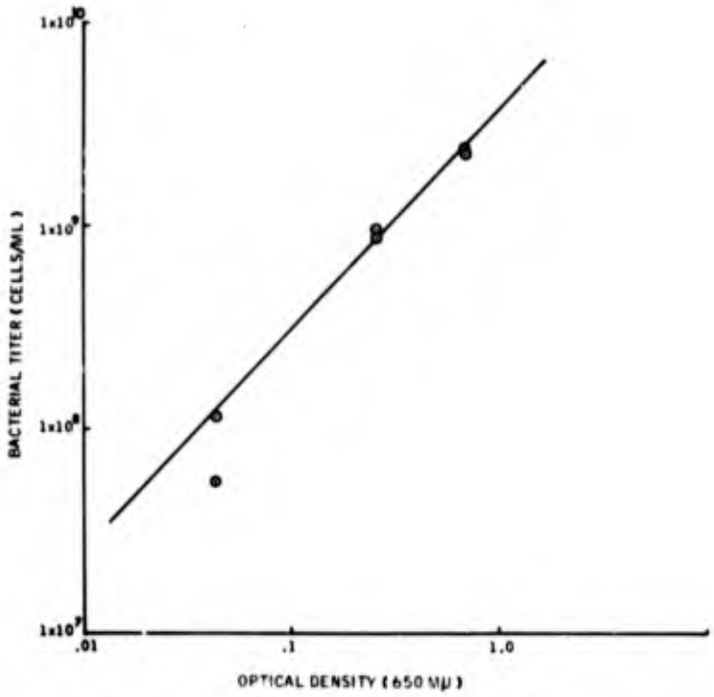

Figure 1. Bacterial Titer Versus Optical Density

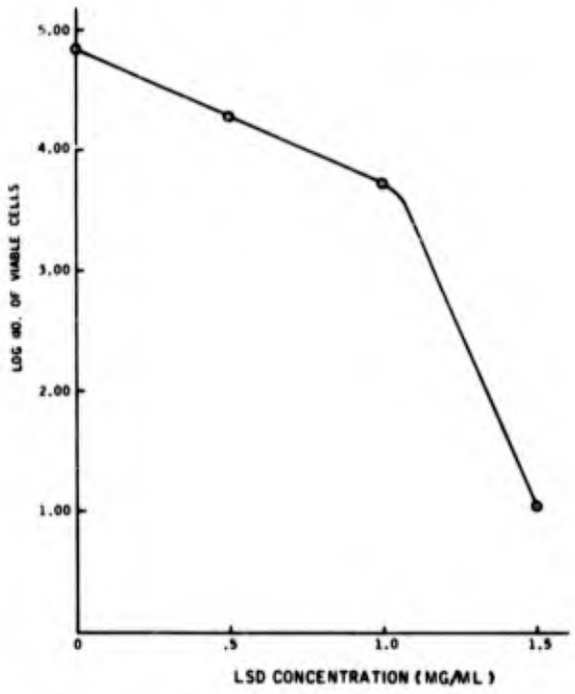

Figure 2. Effect of LSD on Bacterial Viability

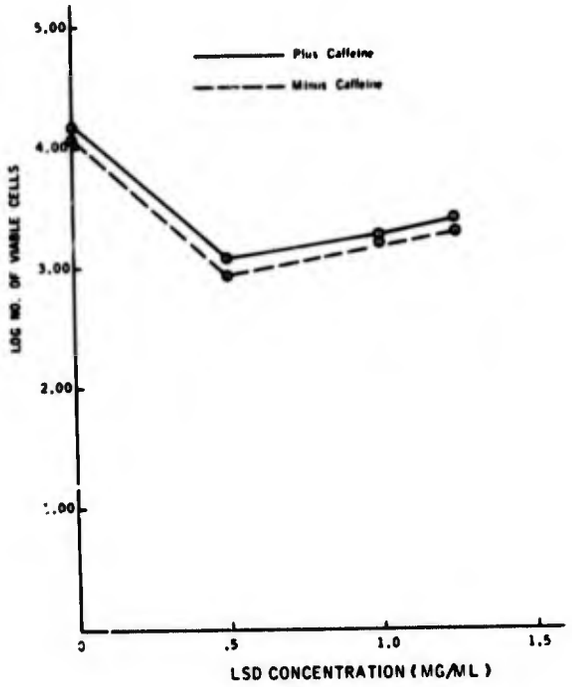

Figure 3. Comparison of the Effect of LSD on Bacterial Viability With and Without the Presence of Caffeine

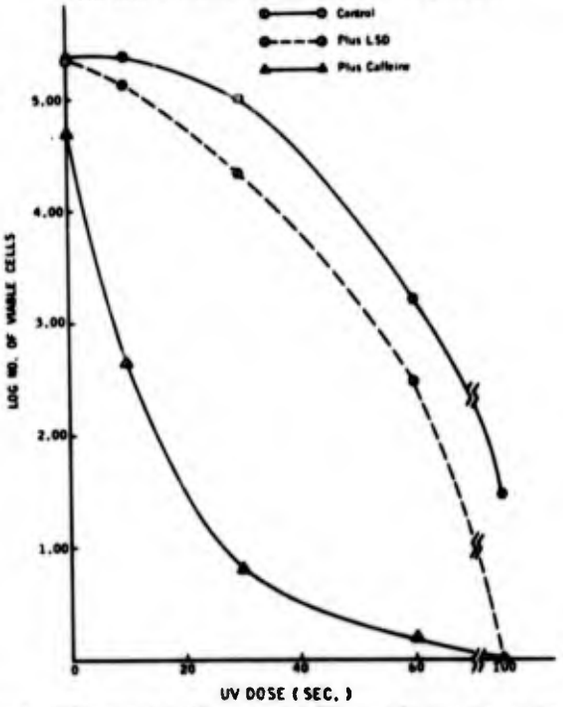

Figure 4. Effects of Caffeine and LSD on UV-Irradiated Bacteria

diluted 1 to 20 into fresh media that contained 0.05 mg LSD/ml, a concentration 1/20th that of the initial concentration (curve C); and untreated control cells placed in fresh, unaltered media (curve D). The small lag period appearing in curves B and E might have been the time necessary for the LSD to diffuse across the membrane. (The bacteria themselves should contain LSD at approximately 1.0 mg/ml initially.) This suggested that our EDTA treatment (see materials and methods) to insure permeability to LSD was unnecessary. This was verified by a check on growth rate with and without EDTA treatment (figure 6).

When cells were exposed to lower doses of LSD, a dose-dependent decrease in the growth rate was observed (figure 7). This would indicate that LSD altered the growth rate and that the observed recovery (eventual growth to limit in the presence of LSD) probably does not involve detoxification by the cell but rather normal growth at a slower rate.

It was possible to verify experimentally that there was neither detoxification nor significant breakdown of LSD within 4 hours after initiation of our experiments. After incubation with LSD (1.0 mg/ml) for 4 hours the culture was centrifuged at 15,500 g for 20 minutes. The supernatant was reinoculated with fresh bacteria and their growth was followed spectrophotometrically (figure 8). Control bacteria grew normally. The bacteria in the LSD supernatant grew at the same reduced rate as the originally exposed culture. Because the effect of LSD on the growth rate of *E. coli* B/rλ was shown to be dose dependent (see figure 7), this would indicate that the original concentration of LSD had not been effectively reduced.

The effect on growth rate of *E. coli* B/rλ by four compounds with varying properties was examined (figure 9). The final concentration of each compound was 1.0 mg/ml. The reasons for choosing these compounds included (a) molecular weight similar to LSD, (b) charged compound like LSD, or (c) heterocyclic-nitrogenous-ring system like LSD. At the levels tested none of these compounds had any effect on the growth rate of the bacteria.

As we mentioned earlier, there is some evidence to indicate that LSD is an extremely weak inhibitor of DNA repair. Caffeine, a known DNA dark repair inhibitor, increases the lag in recovery of growth rate after UV damage (see figure 10). Six hours after UV treatment the caffeine-treated cells had not grown. The growth of cells in the medium that did not contain caffeine was delayed from 90 to 120 minutes, depending upon the UV dose, and then the cells grew at the same rate as the controls. When LSD was used in place of caffeine (figure 11) a dose-dependent extension of the lag period was observed, but it was not nearly as long as the extension caused by caffeine. The cells exposed to 0.5 mg of LSD/ml lagged about 120 minutes and those exposed to 0.75 mg/ml lagged about 190 minutes. This evidence indicates that LSD is a very weak inhibitor of repair. The mechanism of this inhibition is unknown.

IV. DISCUSSION.

There is ample evidence that LSD causes effects which are genetic as well as psychotomimetic. The reported genetic effects include enhanced mutation rate,[16] teratogenesis[11-14] and increased chromosomal aberrations.[1-6, 17, 18] These effects by LSD all seem to involve some interference with the normal functioning of genetic DNA, although not necessarily via the same molecular mechanism. Because the reported effects have not always been corroborated by other investigators and have resulted in considerable controversy, a thorough investigation of LSD's effect on cellular DNA is indicated.

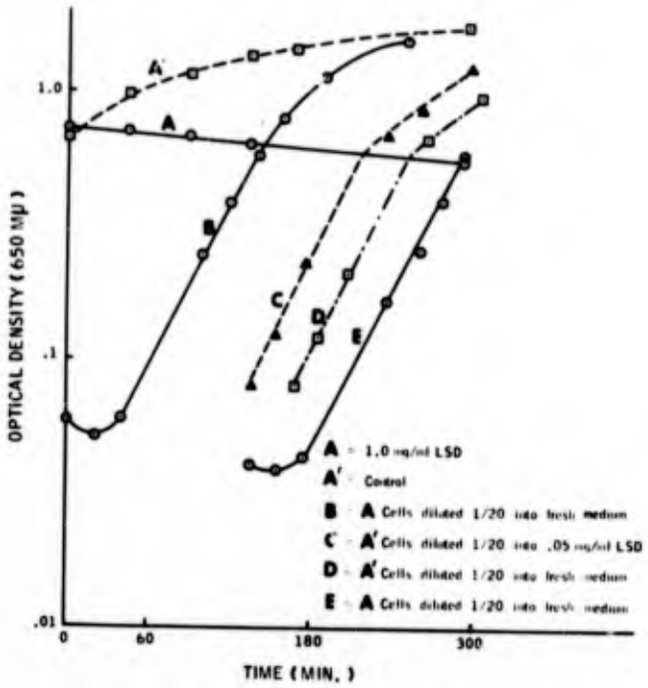

Figure 5. Bacterial Growth Rates in Concentrated and Diluted Solutions of LSD

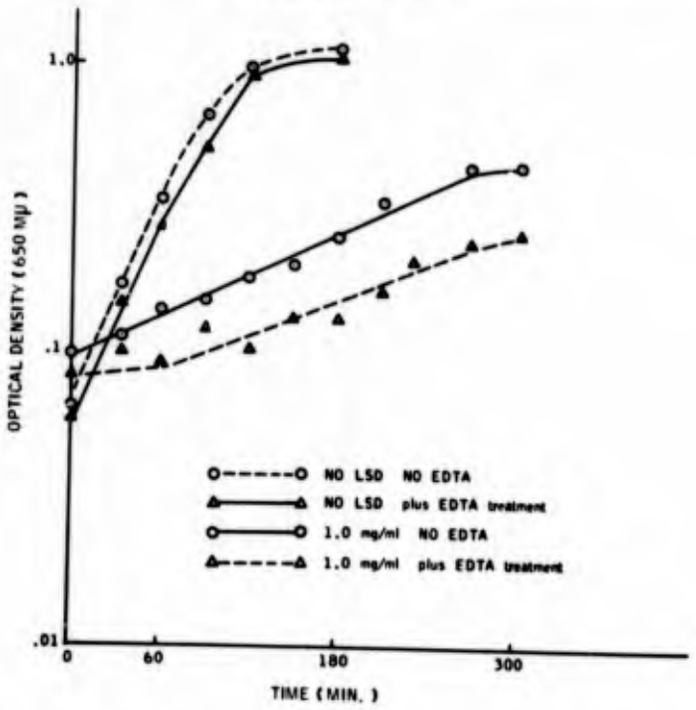

Figure 6. Bacterial Growth Rates as Affected by LSD and EDTA

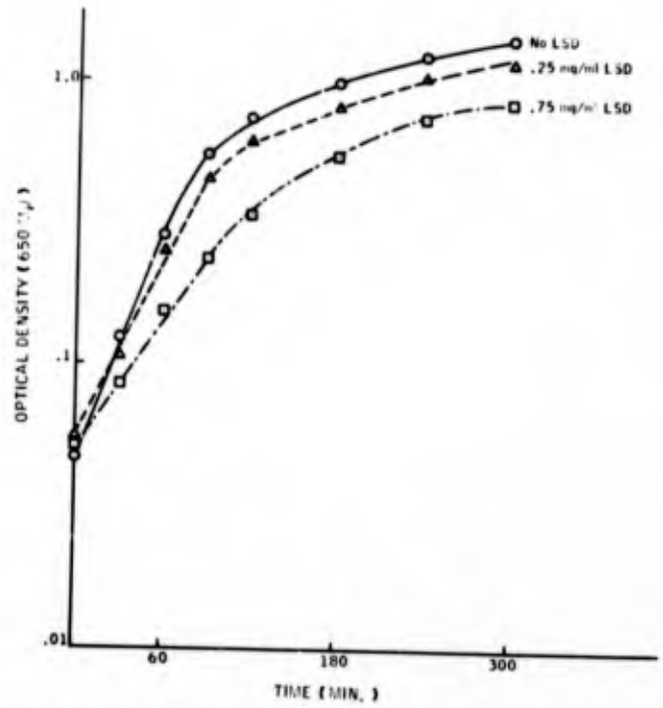

Figure 7. Bacterial Growth Rates in Low Concentrations of LSD

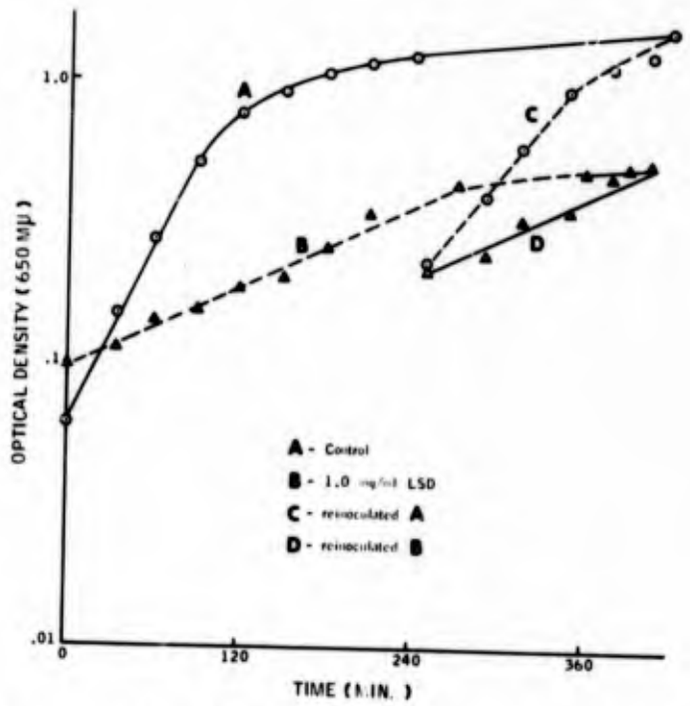

Figure 8. Bacterial Growth Rates in Original and Reinoculated Growth Media

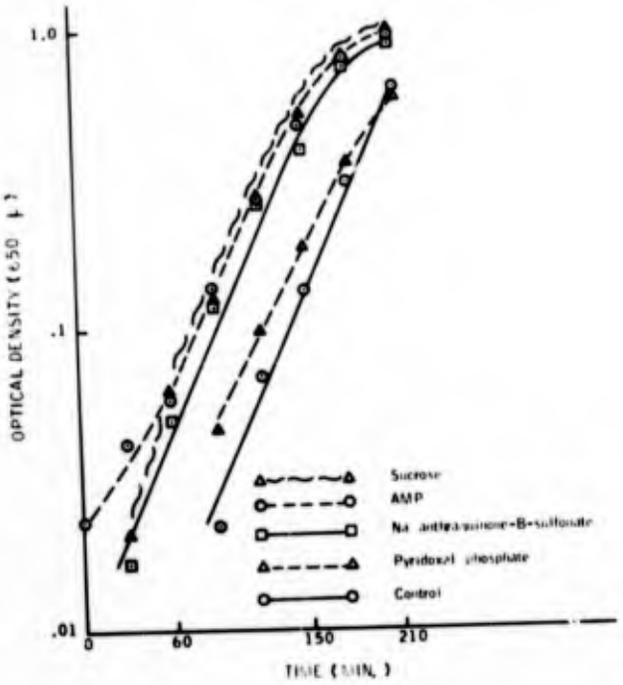

Figure 9. Bacterial Growth Rates in the Presence of Compounds Having Properties Similar to Those of LSD

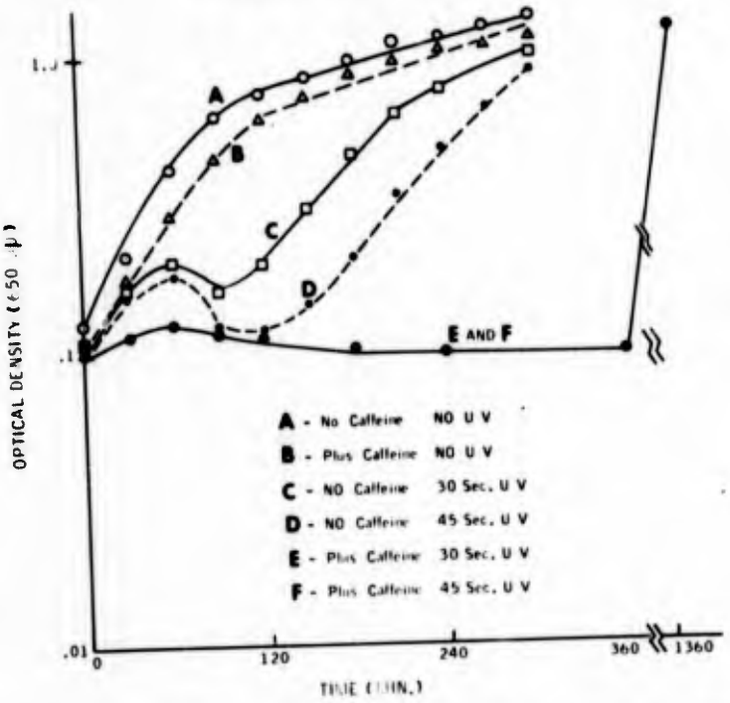

Figure 10. Growth Rates of UV-Irradiated Bacteria in the Presence of Caffeine

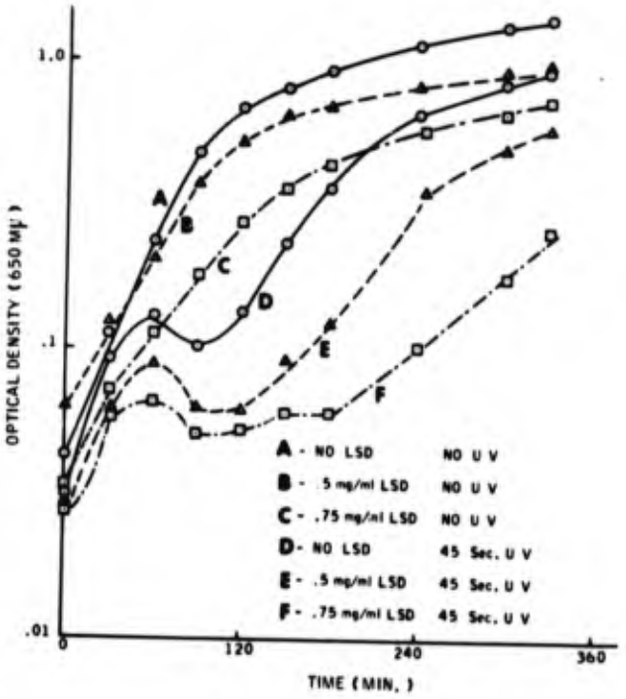

Figure 11. Growth Rates of UV-Irradiated Bacteria in the Presence of LSD

We used a bacterial system, *E. coli* B/rλ, which would be sensitive to unrepaired DNA damage, as a test system for damage to genetic material. In doing so, we have eliminated many properties of mammalian chromosomes such as the protein-DNA complex and the mitotic apparatus. However, if the effects of LSD on bacterial DNA can be well characterized, this may be an important foundation on which to base the study of the much more complex mammalian system.

Harm[28] has shown the sensitivity of these bacteria to DNA damage by UV light and repair inhibitors. Our investigation did not reveal that LSD alone had any of the properties of these "classical DNA damagers." However, recent experiments in our laboratory indicate that LSD can cause repair-related photodynamic inactivation of *E. coli* Bs-1 and T_1 bacteriophage.[29]

LSD did appear to act as a very weak repair inhibitor, the degree of inhibition being extremely small when compared with that of caffeine. It should be pointed out that no experiments were presented to show if LSD inhibits repair in the same manner as caffeine. It is not known whether repair inhibition could account for the chromosomal damage observed, but caffeine and other repair inhibitors have been reported to cause chromosomal abnormalities.[30-32]

LSD caused a dose-dependent lowering of the bacterial growth rate. This effect was shown to be completely reversible when the LSD was diluted. The molecular mechanism of this phenomenon would be an interesting problem for future study.

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13. ABSTRACT

The Effect of LSD on E. coli B/rλ was examined as a test system for damage to genetic material. LSD had a dose-dependent effect on bacterial growth rate. This effect was not amplified by the DNA-repair inhibitor caffeine. The lowering of the growth rate by LSD was shown to be completely reversible upon dilution. LSD was shown to cause a dose dependent extension of the growth recovery lag due to UV damage. Caffeine caused a much larger extension of this lag time.

14. KEY WORDS.

Lysergic acid diethylamide
Growth inhibition
DNA-repair inhibitor
Caffeine
Chromosomal aberrations
DNA damage

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