ANTICONVULSANT, BEHAVIORAL, TOXICOLOGICAL AND ANTIEPILEPTIC CHARACTERIZATION OF DIPHENYLSILANEDIOL

Dissertation for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
VERNE D. HULCE

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ANTICONVULSANT, BEHAVIORAL, TOXICOLOGICAL
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OF DIPHENYLSILANEDIOL

presented by

Verne D. Hulce

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ABSTRACT

ANTICONVULSANT, BEHAVIORAL, TOXICOLOGICAL
AND ANTIEPILEPTIC CHARACTERIZATION
OF DIPHENYLSILANEDIOL

By

Verne D. Hulce

The research discussed in this dissertation began as an investigation of the general pharmacology of organosilicon compounds that possessed some feature in their structures that made them structurally different from carbon compounds. Diphenylsilanediol is a geminal-diol. No analogue with carbon substituted for silicon exists for this compound.

Emphasis was placed on diphenylsilanediol as it became apparent that this compound was a broad spectrum anticonvulsant. Anticonvulsant activity appears in its ability to antagonize convulsions due to general electrical stimulation (maximal electroshock), general chemical stimulation (pentylenetetrazol, strychnine, and picrotoxin), and both electrical and chemical focal stimulation of the neocortex. These results were obtained with the rat, gerbil, and mouse as subjects. Depending on subject species, test procedure and route of administration, these anticonvulsant effects appeared in the dose range of from 2 mg./kg. to 100 mg./kg. Studies on probable anticonvulsant mechanisms for this compound showed that one of its actions is the inhibition of post-tetanic potentiation.

Behavioral evaluation revealed a behavioral toxicity, in terms of rotating rod performance depression, for rodents at doses ranging from 100 mg./kg. to 300 mg./kg. as effective doses--fiftieth percentile (ED-50). Operant behavioral test models identified an anti-anxiety effect beginning at 10 mg./kg. after intraperitoneal administration in the rat.

There was no significant tolerance development to the anti-convulsant effects after periods up to three months following chronic oral administration of diphenylsilanediol. Drug withdrawal hyperexcitability was observed only as a small change in seizure threshold after dosing with diphenylsilanediol was stopped. This occurred only after long term administration of a high (behaviorally toxic) dose.

Toxicity studies demonstrated a lethal dose (LD-50) of 2,500 mg./kg. after single dose administration. With chronic administration, a dose-dependent eosinophilia was detected. Other hematologic effects included a dose-dependent increase in reactive lymphocytes. A histopathologic observation of swollen liver cells was examined further using liver function tests and electron microscopic procedures. The liver was not functionally impaired and the cell changes could be attributed to a proliferation of smooth endoplasmic reticulum. This ultrastructural change is not considered to be a pathological change, but only an adaptation due to an increase in metabolic activity of these cells in processing a foreign substance for excretion.

Antiepileptic testing was done using the epileptic gerbil and the epileptic dog as test subjects. Seizure control was observed for

both of these species after the administration of diphenylsilanediol. The canine subjects were subjected to constant clinical laboratory monitoring which verified the hematological changes seen in the toxicity study done with mice. In addition, an increase in serum alkaline phosphatase levels was observed. There is no apparent explanation as to its origin and it appears that it is not associated with any impairment as shown by other routine determinants of liver function.

The compound diphenylsilanediol appears to be an effective anticonvulsant having pharmacological properties that make it similar to the range of anticonvulsant drugs currently in use but not entirely like any one single drug. It inhibits post-tetanic potentiation as does diphenylhydantoin, while being able to antagonize the effects of chemical convulsants as does phenobarbital. The compound also possesses anti-anxiety activity similar to diazepam yet appears to possess less motor depressant activity at anticonvulsant dose levels.

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By

Verne D. Hulce

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TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	viii
PREFACE	1
INTRODUCTION	2
Organosilicon Chemistry	2
Pharmacology of Organosilicon Compounds	6
Diphenylsilanediol--Chemistry, Properties	8
Plan for the Characterization of Diphenylsilanediol	14
ANTICONVULSANT CHARACTERIZATION	20
General Remarks	20
Maximal Electroshock Generalized Seizures	21
Methods	22
Results	24
Chemical Stimulation Models	31
Methods	31
Results	34
Focal Stimulation Models	34
General Remarks	34
Focal Electrical Stimulation of the Neocortex	36
Methods	37
Results	39
Post-Tetanic Potentiation Model	39
Methods	41
Results	43
Tolerance and Physical Dependence Studies	45
Methods	46
Results	50
Discussion of Anticonvulsant Effects	55
BEHAVIORAL CHARACTERIZATION	60
General Remarks	60
Rotating Rod Effects	62
Methods	63
Results	64

Page

Operant Behavioral Effects 64
Methods 66
Results 67
Discussion of Behavioral Effects 78

TOXICOLOGICAL STUDIES 87

General Remarks 87
Acute Toxicity 87
Methods 87
Results 88
Chronic Toxicity 91
Methods 91
Results 92
Gross and Microscopic 92
Hematological Observations 104
Supplementary Toxicity Studies 109
Electron Microscopic Examinations 109
Methods 109
Results 110
In Vivo Liver Function Tests 110
Methods 113
Results 114
Discussion of Toxicological Results 119

ANTIEPILEPTIC STUDIES IN SEIZURE DISORDER ANIMALS 125

Introduction 125
Epileptic Gerbil Model 128
Methods 129
Results 131
Epileptic Dog Model 131
Methods 134
Results 141
Discussion of Antiepileptic Studies 148

GENERAL DISCUSSION 150

Results of the Characterization Program 150
Some Considerations of Diphenylsilanediol as an Anticonvulsant Compound 154
Some Considerations of Diphenylsilanediol as an Antiepileptic Drug 160

RECOMMENDATIONS 165

BIBLIOGRAPHY 167

LIST OF TABLES

Table		Page
1.	Chemical Comparison of Silicon with Carbon	3
2.	Desirable Properties for an Antiepileptic Drug	16
3.	Plan for Characterization of Diphenylsilanediol (DPSD)	18
4.	Anticonvulsant Screen Results for DPSD Against Seizures in Mice (MES, MET)	26
5.	Time Course for the Anticonvulsant Action of DPSD	27
6.	Route of Administration Effects on the Antagonism of MES Seizures by DPSD in Mice	29
7.	Comparative Electroconvulsive Shock Seizure Antagonism	30
8.	Antagonism of Drug Induced Seizures in Rats by DPSD	35
9.	Inhibition of Cortical Electrical After-Discharge by DPSD (30 mg./kg. i.p.) in the Rat	40
10.	Inhibition of Post-Tetanic Potentiation by DPSD	44
11.	Design for Study to Evaluate Potential for Tolerance and Physical Dependence for DPSD	48
12.	Pentylenetetrazol "First Twitch" Threshold Changes Associated with DPSD Administration for 14 Days	51
13.	Pentylenetetrazol "First Twitch" Threshold Changes Associated with DPSD Administration for 90 Days	53
14.	Summary of Anticonvulsive Action of DPSD	56
15.	Comparison of the Anticonvulsive Action of Anticonvulsant Compounds	57
16.	Rotating Rod Performance Decrement Due to DPSD	65
17.	Two Hour FR-40 Response Totals After Dosing Rats with DPSD	68

Table		Page
18.	Two Hour FR-40 Response Totals After Dosing Rats with Diphenylhydantoin (DPH)	71
19.	Two Hour FR-40 Response Totals After Dosing Rats with Diazepam	73
20.	Two Hour Conditioned Emotional Suppression (CES) Response Totals After DPSD Dosing	76
21.	Two Hour Conditioned Emotional Suppression (CES) Response Totals After DPH Dosing	79
22.	Two Hour Conditioned Emotional Suppression (CES) Response Totals After Diazepam Dosing	81
23.	Results and Comparisons of Behavioral Effects	83
24.	Lethal Effects of DPSD (i.p., Mice)	89
25.	Lethal Effects of DPSD Administered Subacutely in Food	90
26.	Design for Chronic Toxicity Study in Mice	93
27.	Organ and Body Weight Changes After Chronic Dosing with DPSD for Three Months	94
28.	Summary of Gross and Microscopic Observations on Organs from Mice Dosed with DPSD (6.4 mg./gm. food) for 90 days	95
29.	Peripheral Leukocyte Changes After Chronic Dosing with DPSD for Three Months	105
30.	Dose-Dependent Leukocyte Changes After Dosing with DPSD for Three Months	106
31.	Plasma Level of BSP Calibration Data	115
32.	Plasma Levels of BSP in Control Mice--Time Course	116
33.	Plasma Levels of BSP in Treated vs. Control Mice	120
34.	Acute Lethality Comparisons of DPSD with Other Anticonvulsive Compounds	121
35.	Specific Toxicity Responses to the Chronic Administration of Anticonvulsant Compounds	123

Table		Page
36.	Animals, In Addition to Man, Displaying Idiopathic Seizures	126
37.	Seizure Characteristics for Gerbil Colony	130
38.	Effects of Anticonvulsant Drugs on the Idiopathic Seizure of Mongolian Gerbils	132
39.	Case Summary on Epileptic Dog A.G. Treated with DPSD	135
40.	Case Summary on Epileptic Dog R.D. Treated with DPSD	138
41.	Case Summary on Epileptic Dog B.S. Treated with DPSD	142
42.	Summary of Treatment Results with Epileptic Dogs Treated with DPSD	144
43.	Summary of Anticonvulsant Characterization Program for DPSD	151
44.	Acute Dose Safety Comparisons of DPSD with Other Anticonvulsant Compounds	162
45.	Spectrum of Action Comparisons of DPSD with Other Anticonvulsant Compounds	164

LIST OF FIGURES

Figure		Page
1.	Examples of Uniquely Stable Compounds of Silicon	5
2.	Synthesis of Diphenylsilanediol from Silicon	10
3.	60MHz. Nuclear Magnetic Resonance Spectrum of a Solution of Diphenylsilanediol in Dimethylsulfoxide-D_5 . .	11
4.	Infrared Absorption Spectrum of Diphenylsilanediol-- Sample Suspended in a Hydrocarbon/Halocarbon Mull	12
5.	Schematic of Shocker Used to Deliver Maximal Electroshock for Convulsive Seizures	23
6.	Time Course for the Anticonvulsant Action of Diphenylsilanediol	28
7.	Device for the Application of Penicillin to the Rat Neocortex	33
8.	Method for Cortical After-Discharge Studies	38
9.	Diagrammatic Summary of the Post-Tetanic Potentiation (PTP) Method and Data Collection	42
10.	Pentylenetetrazol (MET) Seizure Threshold During and After DPSD Administration (0.4 mg./gm. food) for 14 Days	52
11.	Pentylenetetrazol (MET) Seizure Threshold During and After DPSD Administration (6.4 mg./gm. food) for 90 Days	54
12.	Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diphenylsilanediol	69
13.	Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diphenylhydantoin	72
14.	Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diazepam	74

Figure Page

15a. Mechner Diagram for the Combined FR-40 and CES Behavioral Paradigm Used as an Anxiety State Model . . . 75

15b. Cumulative Record of a Rat Performing Under a Combined FR-40 and CES Paradigm for Behavior 75

16. Responding During Conditioned Emotional Suppression (CES) Periods After Dosing with Diphenylsilanediol . . . 77

17. Responding During Conditioned Emotional Suppression (CES) Periods After Dosing with Diphenylhydantoin . . . 80

18. Responding During Conditioned Emotional Suppression (CES) Periods After Dosing with Diazepam 82

19. Section of Spleen from a Mouse Treated with DPSD for 90 Days 97

20. Section of the Adrenal Gland from a Mouse Treated with DPSD for 90 Days 97

21. Section from Kidney of a Mouse Treated with DPSD for 90 Days 98

22. Bone Marrow Smear from the Femur of a Mouse Treated with DPSD for 90 Days 99

23. Section from Cerebellum of a Mouse Treated with DPSD for 90 Days (Original Magnification 180X) 100

24. Section from the Cerebellum of a Mouse Treated With DPSD for 90 Days (Original Magnification 1000X) 101

25. Section of Liver from a Mouse on the Control Diet for 90 Days 102

26. Section of Liver from a Mouse Treated with DPSD for 90 Days 103

27. Eosinophil and Lymphocyte from Peripheral Blood of Mouse Treated with DPSD for 90 Days 107

28. Toxic Neutrophil (with Doehle Body) from Blood of Mouse Treated with DPSD for 90 Days 107

Figure		Page
29.	Reactive Lymphocyte from Peripheral Blood of Mouse Treated with DPSD for 90 Days	108
30.	Electron Micrograph of Liver from Control Mouse	111
31.	Electron Micrograph of Liver from Mouse After Treatment with DPSD for Three Months	112
32.	Calibration Curve for Plasma Levels of BSP (phenoltetrabromophthalein sodium sulphonate)	117
33.	Disappearance of BSP from Mouse Plasma: Normal Control Group Subjects	118
34.	Structural Forms of Phenyl-Containing Anticonvulsant Drugs	155
35.	Comparison of Alcoholic Forms of Selected Diphenyl-Containing Anticonvulsant Compounds	157

PREFACE

Diphenylsilanediol is an organosilicon compound. The current biological investigation of this compound began as an attempt to examine the central nervous system pharmacology of organosilicon compounds selected on the basis of having a unique chemical structure which could not be duplicated with a carbon substituted analogue. Two other compounds, phenyl-(2,2',2"-Nitrilotriethoxy) silane and di-t-butylsilane, had received some preliminary attention in earlier studies by the author of this manuscript. These compounds had local anesthetic and possible anti-depressant properties respectively. Each might have equally satisfied my interest in the pharmacology of organosilicon compounds but diphenylsilanediol was selected because of its potential usefulness as an anticonvulsant and antiepileptic compound.

INTRODUCTION

Organosilicon Chemistry

Silicon and its compounds are ubiquitious constituents of the planet earth and the universe. Silicon composes 27.7% of the earth's crust making it the second most abundant element, whereas oxygen is first. Carbon, considered as the basis for life on this planet, is fourteenth and comprises 0.03% of the earth's crust (Weast, 1971). Like carbon, silicon is a group four element in the periodic table. As a third period element it has some important properties which make it different from carbon (Table 1).

The electronic ground state of silicon as $3s^2 3p^2$ resembles the carbon $2s^2 2p^2$ configuration on initial examination, but the atomic number of 14 instead of 6 for carbon provides the 3d atomic orbitals in an energetic position of easy availability. These d-orbitals play an important role in the chemistry of silicon. On the Pauling scale, silicon has an electronegativity of 1.8 compared with 2.5 for carbon and 2.1 for hydrogen. This makes silicon the more electropositive element in a carbon-silicon bond. With silicon-hydrogen bonding, the hydrogen appears in part as a hydride ion and reacts accordingly (Bazant et al., 1965). When hydrocarbon compounds are compared with analogous hydrogen-silicon compounds on reacting with oxygen, for example, the silicon compound is found to be more reactive, which

Table 1

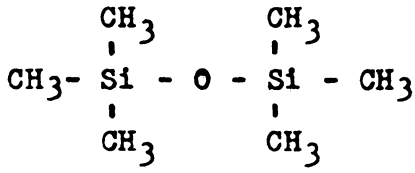
Chemical Comparison of Silicon With Carbon

Comparison Aspect	Carbon	Silicon
Group	IV	IV
Period	2	3
Atomic Number	6	14
Atomic Weight	12.01	28.09
Natural Isotopes	12, 13, 14	28, 29, 30
Electronic Configuration	$1s^2 2s^2 2p^2$	$1s^2 2s^2 2p^6 3s^2 3p^2$
Hybridization States	sp, sp^2, sp^3	sp^3, sp^3d, sp^3d^2
Electronegativity (Pauling Scale)	2.5	1.8
Bond Energies (Kcal./Mol.)	(C-C) 82.5	(Si-Si) 53
	(C-Si) 76	(Si-C) 76
	(C-O) 85.5	(Si-O) 108
	(C-H) 98.7	(Si-H) 76

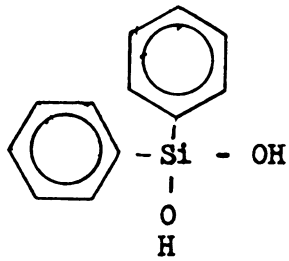
Sources: (Eaborn, 1966; Roberts and Caserio, 1964).

would be anticipated for metal hydrides (Roberts et al., 1965). A third factor which serves to differentiate silicon from carbon in its chemistry and compounds is physical size. Carbon has an atomic radius of 0.77 Å compared with 1.77 Å for silicon. Though the more common electronic hybridized state for both carbon and silicon is the tetrahedral sp^3, carbon can form double and triple bonds with carbon, oxygen, nitrogen, and sulfur commonly through the overlap of sp^2 or sp hybridized orbitals. For thermodynamic reasons, silicon is unable to assume these hybridization states and consequently does not form the more classical double bonds (Burger, 1973). Silicon is able to use its d-orbitals for back-bonding which imparts some multiple bond character to Si-O and Si-F bonds (Eaborn, 1960). Silicon is also able to utilize the trigonal-bipyramid, sp^3d, hybridization state for five coordinate compounds and the square-bipyramid sp^3d^2 for six coordinate bonding (Burger, 1973).

The implications of this discussion are graphically illustrated in Figure 1 where four compounds of silicon are listed which have markedly increased stability compared to any carbon analogues. Hexamethyldisiloxane is a stable compound due to the high bond energy of the silicon oxygen bonds. The carbon analogue, di-t-butyl ether is subject to rapid hydrolysis to t-butanol. Diphenylsilanediol exists as a geminal diol. The carbon analogue of this compound does not exist and if attempted to be synthesized would rapidly be converted to a diphenyl ketone with the loss of water. Compounds C and D in Figure 1 are five and six coordinate compounds of silicon, respectively. No carbon analogues are known or theoretically stable.



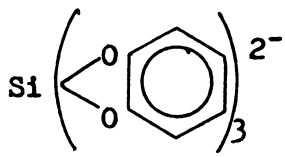
A. Hexamethyldisiloxane



B. Diphenylsilanediol



C. Phenyl-(2,2'2"-nitrilotriethoxy)-silane



D. Silicon tricatecholate

Figure 1. Examples of Uniquely Stable Compounds of Silicon

Pharmacology of Organosilicon Compounds

Though an extensive coverage of the world's literature on organosilicon pharmacology will not be attempted here, a brief review of recent developments in this field will hopefully provide a perspective for the examination of diphenylsilanediol pharmacology and toxicology which makes up the majority of this dissertation.

Despite the abundance of silicon in the earth's crust, only recent discoveries have implicated this element as being an essential element important to life in organisms more advanced than the diatoms. The verification of silicon as an essential element had eluded investigators due, principally, to its large abundance and the difficulty in producing silicon-free environments for laboratory research. The independent reporting by Carlisle (1970) and Schwarz and associates (Milne et al., 1972) showed that silicon-deficient diets in chicks and in the rat led to reduced growth rates and structural deformities. More recent studies (Schwarz, 1973; Carlisle, 1974) suggested that silicon participates in the formation of bone and connective tissue through the crosslinking of polysaccharides by siloxane bridges.

Our discussion of the pharmacology will begin with an examination of two organosilicon compounds, 1-phenylsilatrane and 2,6-cis-diphenylhexamethylcyclotetrasiloxane, which are beginning to be characterized as having well-defined pharmacological activity.

Some recent reviews (Fessenden and Fessenden, 1967; Garson and Kirchner, 1971; Voronkov et al., 1971; Voronkov, 1975) attest to a developing interest in the pharmacology of silicon compounds. The

Russian book, Kremnii i Zhizn, (Silicon and Life) by Voronkov, Zelchan, and Lukevits (1971) includes more than 5,000 references in its bibliography. Unfortunately, most of the data on organosilicon compounds are descriptive toxicological responses.

The compound 1-phenylsilatrane (see Figure 1.C), a trivial name of phenyl-(2,2',2"-nitrilotriethoxy) silane, was one of the earliest (Baltkais et al., 1964) organosilicon compounds reported to possess significant acute toxicity. The LD-50 was 0.3 mg./kg. This property was commercialized by M & T Chemicals, Inc., Rahway, New Jersey, as a rodenticide. This was based on the extremely toxic nature of this compound and on its susceptibility to hydrolysis in aqueous media (Greaves et al., 1974). An examination of this compound by Hulce and Rech (1973; 1974) identified this compound to be a potent local anesthetic. This activity appears to account for the acute toxicity of 1-phenylsilatrane.

Bennett, Gorzinski, and LeBeau (1972) and LeVier and Jankowiak (1972) isolated and characterized the compound 2,6-cis-diphenylhexamethylcyclotetrasiloxane as having anti-androgenic and estrogenic activity. More recent investigations by LeVier and Boley (1975) have attempted to localize this activity in the hypophysis and hypothalamus through the control of dopamine release at the level of the hypothalamic-median eminence pathways.

Some anticonvulsant organosilicon compounds have appeared in the literature, but, though interesting, are silicon-substituted analogues of known organic anticonvulsants. Belsky et al. (1968) were able to

demonstrate anticonvulsant activity in sila derivatives of acetylurea and barbiturate anticonvulsants. Fessenden and Coon (1965) showed the same anticonvulsant and muscle relaxant properties for silameprobamate as meprobamate. The sila derivative had a shorter duration of action after i.p. administration and was not effective p.o.

Diphenylsilanediol--Chemistry, Properties

Having current commercial use, diphenylsilanediol has also played a role in the history of organosilicon pharmacology. Originally synthesized in 1905 by Dilthey and Eduardoff (1904), the compound was not well characterized until 1912 when Kipping (1912) purified it as a crystalline solid from benzene solutions.

Today silicon compounds bearing methyl and phenyl groups comprise the majority of commercially available silicon compounds and still dominate the organosilicon chemistry literature. Diphenylsilanediol is a commonly produced intermediate in the silicones industry (Noll, 1968) with the hydrolysis of diphenyldichlorosilane being the most common synthesis. This latter compound is one of the products of phenyl-containing silicon compound production by the the direct process (Rochow, 1945) or Grignard (Kipping, 1904) synthetic routes. This makes it an industrial compound of modest production volume.

Most dichlorosilanes and dihydroxysilanes (diols) are used in the synthesis of silicones through polymerization (Eaborn, 1960; Noll, 1968). Diphenylsilanediol is difficult to polymerize (Tyler, 1952) due to the steric effects of the bulky phenyl groups, but does find

commercial use as a filler in making silicone elastomers where it serves as a structure control additive (Peter et al., 1953), preventing hardening in silicone rubber materials filled with a reinforcing silica.

Figure 2 summarizes a typical commercial synthesis of diphenylsilanediol from raw material and via the direct process (Rochow, 1945). Currently all organosilicon compounds are prepared from elemental silicon which is prepared by the reduction of sand by coke in an electric furnace--Reaction I. The direct process reactions (II in Figure 2) yield a mixture of phenyl-containing organosilicon compounds. Fractional distillation yields diphenyldichlorosilane which can be reacted with methanol, Reaction III, to yield diphenyldimethoxysilane. Hydrolysis of this product yields diphenylsilanediol which may be further purified by recrystallization (Noll, 1968).

Chemically, diphenylsilanediol is a white crystalline solid having an empirical formula of $C_{12}H_{12}O_2Si$. Its melting point is not well-defined as the compound has a tendency to change its hydration state and to polymerize at higher temperatures (Eaborn, 1960). Purity can be characterized by reference to standardized infrared absorption spectra (Anderson, 1974) or nuclear magnetic absorption spectra (Williams, 1974). Figure 3 shows the nuclear magnetic resonance spectrum for a pure sample of diphenylsilanediol. Figure 4 shows the infrared absorption spectra for the same sample which was obtained from D. R. Bennett at Dow Corning Corporation, Midland, Michigan and used as a reference standard in testing.

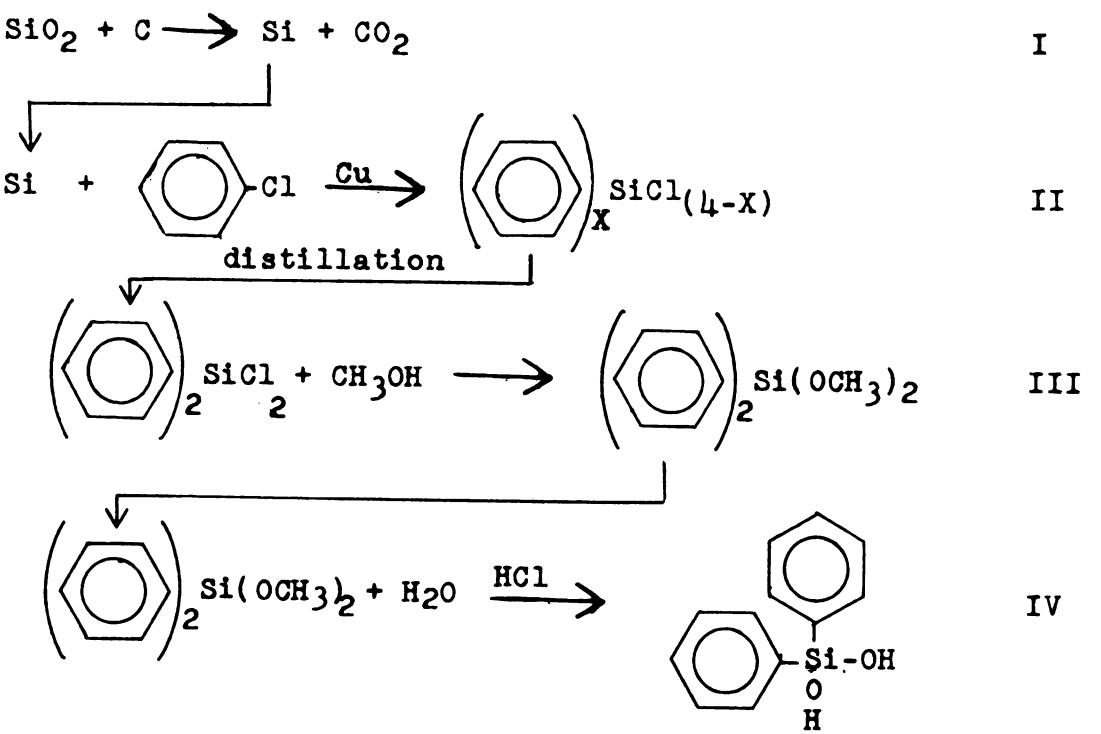


Figure 2. Synthesis of Diphenylsilanediol from Silicon.

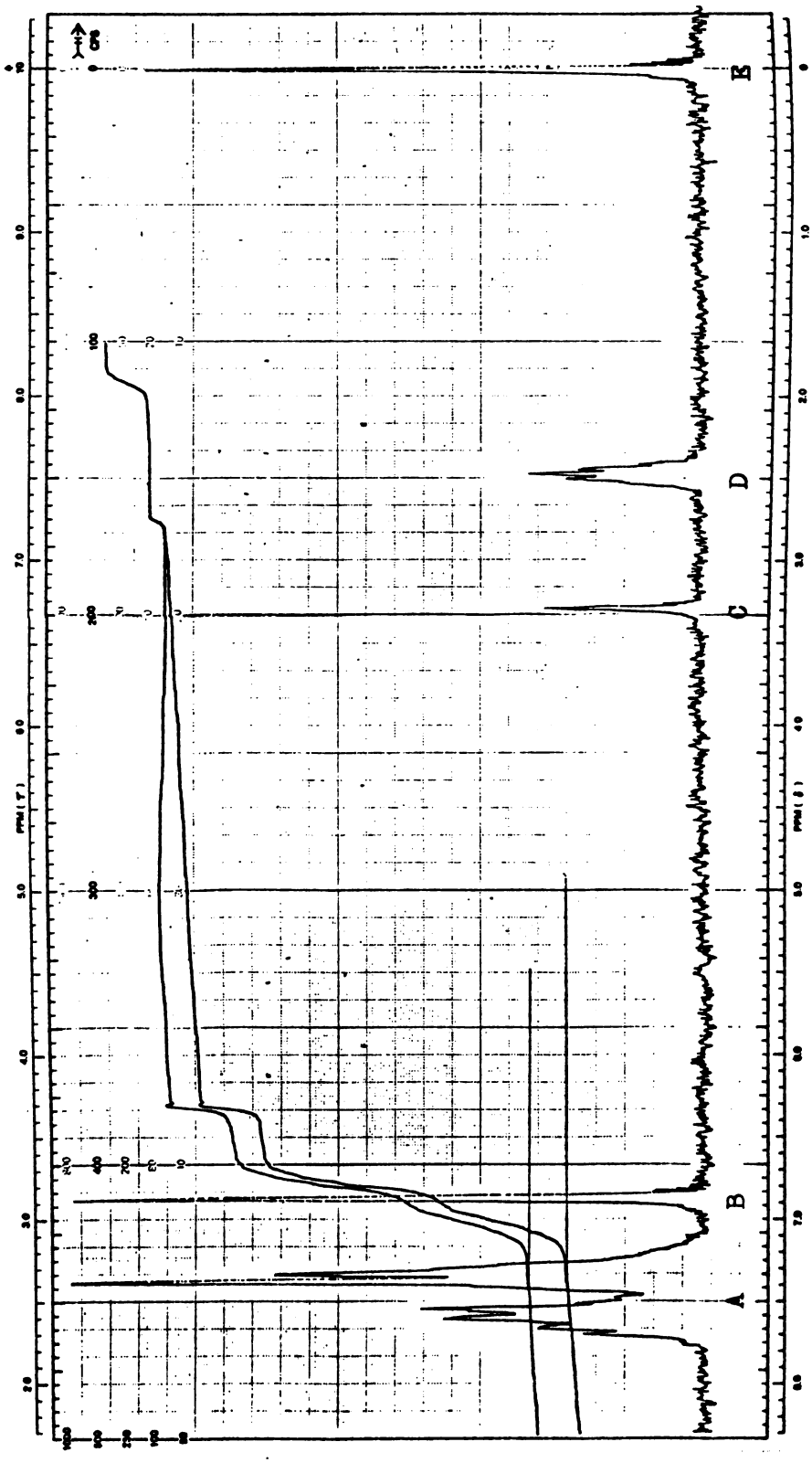


Figure 3. 60MHz Nuclear Magnetic Resonance Spectrum of a Solution of Diphenylsilanediol in Dimethylsulfoxide-D_5.

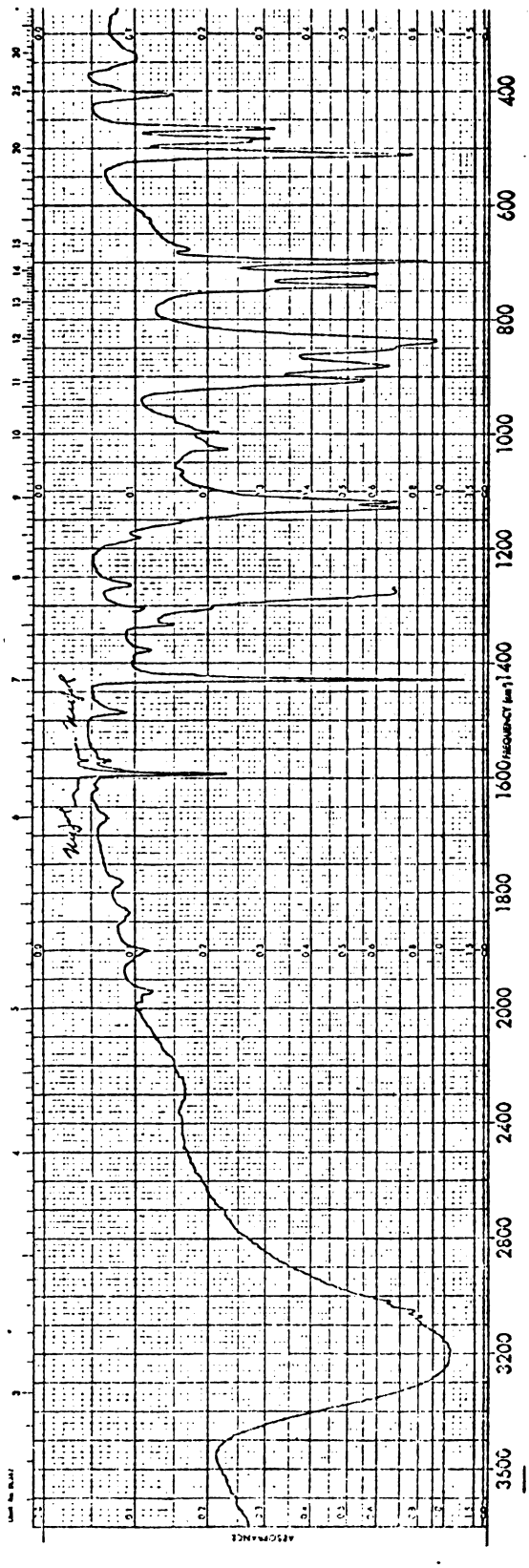


Figure 4. Infrared Absorption Spectrum of Diphenylsilanediol - Sample Suspended in a Hydrocarbon/ Halocarbon Mull.

The presence of both phenyl and hydroxy groups in diphenylsilanediol enables this compound to be soluble in both polar and non-polar solvents. Though poorly soluble in water, this compound is readily soluble in alcohols, hydrocarbons, ethers, etc. In both acid and alkali solutions, there is a tendency for this compound to undergo polymerization by silanol condensation.

Note that diphenylsilanediol is structurally a geminal diol. Carbon compounds with geminal diol structures tend to be unstable unless stabilized by strong electron withdrawing groups (i.e., as in chloral hydrate). The carbon compound tends to form a ketone, but, due to the inability of silicon to form stable double bonds, it is unable to make this rearrangement.

Three samples of diphenylsilanediol were obtained for use in the investigations discussed here. The reference sample, obtained from D. R. Bennett of the Dow Corning Corporation, Midland, Michigan, and identified as E-580-63-1, was labeled as being 99% pure. The material used for the majority of the tests discussed below was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, and identified as lot number 102717. A third sample, obtained as General Electric PC Powder, proved to be impure and was not used except occasionally in the epileptic dog study when the Aldrich material was not available.

The infrared absorption spectra of these three materials were similar except that the General Electric material showed some additional absorption bands. Nuclear magnetic resonance spectra for these materials was examined closely to estimate purity. In Figure 3 note that

the hydrogen absorption bands are labeled A, B, C, D, and E. The lines above the absorption spectra are the integrations for the absorption curve areas and are directly proportional to the number of hydrogen protons undergoing absorption state changes. The bottom line in this figure, labeled 8 to 0, is an index of the chemical shift of the absorbing proton nuclei with peak E being the standard (tetramethyl silane-TMS). Peak complex A is due to the phenyl groups on diphenylsilanediol; peak B, the silanol hydrogens; peak C, the water in the samples; and peak D attributed to the solvent which was deuterated DMSO (dimethylsulfoxide-D_5). Comparing the silanol hydrogen chemical shifts, the standard was 6.88 ppm; the Aldrich sample was 6.87; and the General Electric material was 6.80. The phenyl to silanol ratios (theoretically 5:1) were 5.1:1 for the standard; 5.2:1 for the Aldrich sample and 6.1:1 for the General Electric sample. The higher phenyl concentrations are probably attributable to the existence of polymeric forms of diphenylsilanediol being present.

Plan for the Characterization of Diphenylsilanediol

With the demonstration by Putnam and Merritt (1937) that diphenylhydantoin elevated the electroconvulsive shock threshold in the cat and their later (Merritt and Putnam, 1938) proof of its effectiveness in epileptic patients, the possibility of identifying new drugs in the laboratory for the treatment of epilepsy seemed apparent. In a review paper eight years later (Merritt and Putnam, 1945), these

authors presented a list of no less than 1,000 compounds that they had screened in the cat yielding 75 that showed exceptional seizure protection. The majority of these compounds contained phenyl groups, or were otherwise aromatic, which supported their 1943 postulate that phenyl-containing compounds had the best potential as anticonvulsant agents. Considering structure-activity relationships for clinically useful antiepileptic drugs, Toman and Goodman (1948) concluded that phenyl substitution was essential for drugs used to treat grand mal or psychomotor epilepsy but was also either non-essential or detrimental to drugs used in the treatment of petit mal seizure types. This position has been supported through the years in the structure activity reviews by Close and Spielman (1961), Spinks and Waring (1963), Delgado and Isaacson (1970), and Mercier (1973).

The properties of a compound which could disqualify it as a potential antiepileptic, even though it may appear to be an effective anticonvulsant, are its acute and chronic toxicity, its behavioral effects, and its duration of action.

Toman (1970) writing in Goodman and Gilman's textbook states, "The ideal antiepileptic drug should be capable of complete suppression of seizures at a dosage level that does not cause sedation or other undesired central toxicity. Since it must be used continuously for months or years, it should be well tolerated by the oral route, inexpensive, long acting, and incapable of inducing tolerance or withdrawal signs. It should be devoid of systemic toxicity. . . ."

From this definition one can extract 10 properties of an antiepileptic drug as shown in Table 2.

Table 2

Desirable Properties for an Antiepileptic Drug

Adapted From Toman (1970)
(Reference in List of References)

The Ideal Antiepileptic Drug:

1. suppresses all seizure types.
2. is effective orally.
3. is long acting.
4. shows no tolerance to its action.
5. shows no withdrawal effects.
6. has no sedation nor CNS toxic action.
7. shows no systemic toxicity
8. shows no idiosyncratic toxicity.
9. has a wide margin of safety.
10. is inexpensive to buy.

The procedures and programs outlined below, to evaluate the anticonvulsant action of diphenylsilanediol, are guided by Toman's definition of an ideal antiepileptic drug. This, of course, is evaluated in the light of data on currently available antiepileptic compounds. Suppression of seizures is to be evaluated with a variety of acute and chronic seizure models. Both dose-response and response-time data after intraperitoneal and per os doses will facilitate comparisons between drugs and with behavioral and toxicological parameters. Sedation and central toxicity will be evaluated by behavioral methods which provide sensitive and standardized measurements. Oral dosing will also be evaluated chronically to observe accumulation, tolerance, and physical dependence possibilities. Determination of organ and system toxicity was also planned as part of this research and will be incorporated with the chronic-dosing studies. It was anticipated that, at the completion of these studies, the anticonvulsant and supporting properties of diphenylsilanediol should be sufficiently well defined to permit a statement that this compound is similar to existing anticonvulsants or has its own unique spectrum of action.

Table 3 lists the major phases of this research and the objectives to be accomplished in each phase. Each of these five phases also represent the chronology for a systematic characterization of diphenylsilanediol. The primary concern is for the anticonvulsive action which, upon being sufficiently interesting, justifies the pursuit of the remaining phases.

Table 3

Plan for the Characterization of Diphenylsilanediol (DPSD)

I. Anticonvulsant Testing

Objectives: anticonvulsive action spectrum, comparisons with known compounds.

II. Behavioral Characterization

Objectives: neurotoxicity screen, behavioral aspects of general pharmacology.

III. Toxicological Studies:

Objectives: acute lethality dose range, organ system chronic toxicity.

IV. Antiepileptic Studies:

Objectives: establishment of efficacy results in clinical disease state.

V. General Pharmacology:

Objectives: pharmacokinetics, tolerance phenomena, organ system pharmacology.

Published toxicity data in the Soviet literature (Kelman et al., 1968) showed diphenylsilanediol to be relatively non-toxic when administered to rats and mice. The LD-50 was 2,200 mg./kg. During discussions with the then manager of biomedical research at Dow Corning Corporation, Dr. Donald R. Bennett, it was learned that primary screens conducted at the Dow Chemical Company, Zionsville, Indiana, and Gruppo Lepetit, Milano, Italy, suggested that this compound might show anti-convulsant activity. The potential for a new anticonvulsant agent having a novel chemical structure and a large therapeutic index coupled with the possibility of a potentially new mechanism of action created the interest leading to these investigations summarized herein.

ANTICONVULSANT CHARACTERIZATION

General Remarks

There are perhaps as many, if not more, techniques to evaluate anticonvulsant drugs as there are anticonvulsant drugs. One of the problems with this state of affairs is that few compounds have been evaluated by all procedures and few procedures have evaluated all compounds. Recent summary articles (Naquet and Lanior, 1973; Swinyard, 1973; Woodbury, 1972) attest to this difficulty. The procedures explained below which were adopted for use in characterizing the anticonvulsant properties of diphenylsilanediol (DPSD) have the advantages of either having been used widely, and thus permit comparisons of data from other compounds, or show some unique activity for a particular drug, thus justifying their use. Most of the procedures involve acute observations even though epilepsy itself is a chronic phenomenon. Acute studies are designed to cover a range of convulsant types. These are categorized as whole brain or focal mechanisms and involve both electrical and chemical stimulation. There are very few chronic models in use, and most of these involve focal lesions. One chronic model to be discussed later is the epileptic dog. Also part of this effort is the evaluation of the effects of chronic oral dosing on the anticonvulsant action observed for DPSD. The purpose of chronic administration is to evaluate accumulation of effect, tolerance, and

whether physical dependence develops. Details of rationale and methodologies are given below.

Maximal Electroshock Generalized Seizures

Earlier studies had revealed that DPSD was effective in antagonizing maximal electroshock induced seizures in the mouse (Bennett, 1972, personal communication). The ED-50 was reported to be 16.5 mg./kg. after i.p. administration in methyl cellulose. As a preliminary investigation, the re-determination of this ED-50 value was undertaken for further comparisons of data. Using maximal electroshock antagonism as a dependent variable, an estimate of some temporal parameters of the action of DPSD could also be determined. Time of peak effect, half-time and duration of action were explored for estimating doses and times for future experiments.

The mouse and rat are standardized subjects for anticonvulsant testing of drugs (Swinyard, Brown, and Goodman, 1952) and, except for the oral administration of diphenylhydantoin (Swinyard, and Toman, 1950), have similar dose-response patterns. With the introduction of the gerbil as an epilepsy model (Loskota, Lomax, and Rich, 1974), and with the establishment of a colony of these rodents in our laboratory, it was felt important to have comparable dose-response data for this species.

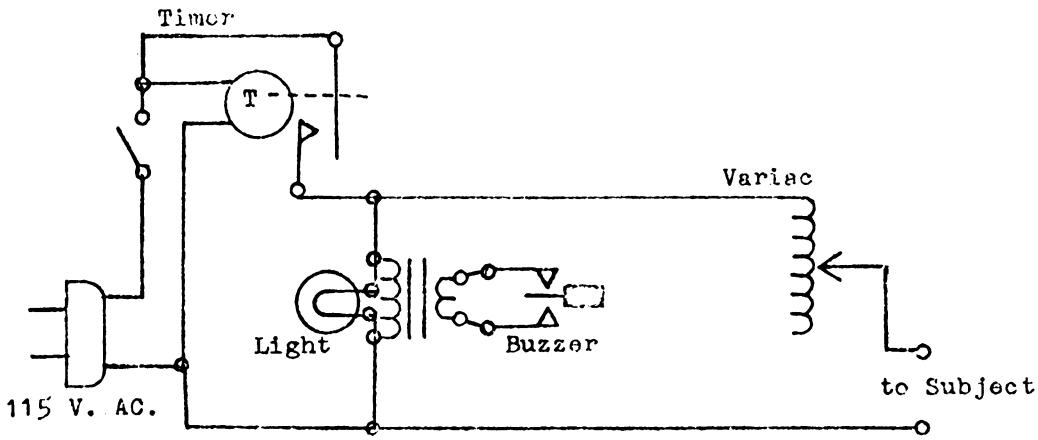
For direct comparison, the antagonism of maximal pentylenetetrazol seizures was also determined. The objectives of these early studies were to (1) compare the maximal seizure antagonism of DPSD

against maximal electroshock and maximal pentylenetetrazol seizures; (2) evaluate the time course for the anticonvulsant action of a single dose of DPSD; (3) establish the influence of route of administration on the anticonvulsant action of DPSD; and (4) examine the possibility of significant species variation in the anticonvulsant action of DPSD.

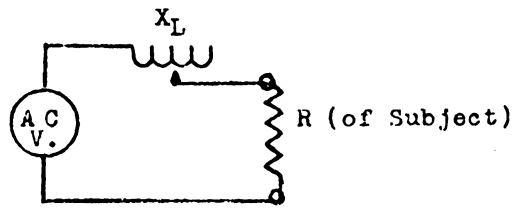
Methods

Subjects were rats, mice (Spartan Laboratory Animals, Haslett, Michigan), and gerbils (Meriones unguiculatus). The gerbils were obtained from a number of sources on the Michigan State University campus and maintained as a breeding colony in the Life Sciences Building. The mice, rats, and gerbils were adult females with weights of 20-30 grams, 200-250 grams, and 70-90 grams, respectively. Groups of six rodents were administered either 10, 30, 100, or 300 mg./kg. of DPSD in 0.5% methyl cellulose solution in distilled water. Control subjects received the methyl cellulose solution only. Electroshock was delivered with a Woodbury-Davenport Shocker, Figure 5 (Woodbury and Davenport, 1952) through corneal electrodes according to the method of Toman, Swinyard, and Goodman (1946). With the administration of 60 Hz. A.C. interrupted shocks of 0.2 second duration and 50 mA., all untreated mice demonstrated tonic extension of the hind limbs.

Electroconvulsive shock was administered for 0.2 seconds with a current level of 50 mA. for mice, 100 mA. for gerbils, and 150 mA. for rats. Groups of six subjects were scored as number protected per number treated. Seizure protection was scored as the inhibition of



Actual Circuit Diagram



Equivalent Circuit

Figure 5. Schematic for Shocker Used to Deliver Maximal Electroshock for Convulsive Seizures.

tonic extension (Swinyard, Brown, and Goodman, 1952) and, for time effects, groups of six mice were tested at repeated intervals. One group was tested at 0, 30, and 300 minutes, another at 7.5, 60, and 480 minutes, and a third at 15, 120, and 600 minutes after dosing. Groups of six mice were also run in triplicate for statistical reasons. Response time experiments were done with a single dose of 50 mg./kg.

The comparison with maximal pentylenetetrazol seizures used the same end point of the antagonism of tonic hind limb extension but here the seizures were caused by the administration of 95 mg./kg. pentylenetetrazol via the subcutaneous route in mice. This convulsant was administered 30 minutes after the DPSD administration and was expected to produce seizures in 15 minutes. Animals not displaying tonic hind limb extension by 30 minutes after pentylenetetrazol administration were scored as protected.

Routes of administration were i.p. injection, oral gavage with the methyl cellulose vehicle, and administration with a powdered food in the diet. After i.p. administration animals were tested between 30 minutes and one hour post dose; after oral gavage, the time was from 45 minutes to 105 minutes post dose; and the subjects with DPSD in their food were tested after they had been on the food and drug mixture for 24 hours.

Results

A direct comparison of the dose response antagonism by DPSD of tonic hind limb extension due to maximal electroshock or to

pentylenetetrazol in mice is shown in Table 4. The maximal electroshock ED-50 value is 20 mg./kg. i.p. while the pentylenetetrazol seizure antagonism ED-50 is 48 mg./kg. The time course data are reported in Table 5 and plotted in Figure 6. The Table 5 entries are the individual responses of treatment groups at the times indicated. The mean values for the three determinations at each time period are recorded at the lower portion of the table. From an examination of Figure 6, the peak time for anticonvulsant action is at 30 minutes post dose and there is some activity up to 600 minutes, or ten hours, post dose. An apparent half-time for the elimination phase of this action is on the order of four hours.

Table 6 summarizes the effects of differing routes of administration of the DPSD antagonism of maximal electroshock tonic hind limb extension. The i.p. route ED-50 is 20 mg./kg., the oral gavage ED-50 is 2.9 mg./kg., and the oral ED-50 for drug mixed with food is 0.41 mg./gm. food or estimated as 68.3 mg. (DPSD)/kg. (body weight)/day (24 hr.).

Table 7 gives comparative results for the rat, mouse, and gerbil. Each of these values are similar in that each of the 95% confidence intervals have extensive overlap. The calculated ED-50 values are 20 mg./kg. in the mouse, 42 mg./kg. in the gerbil, and 30 mg./kg. in the rat.

Table 4

Anticonvulsant Screen Results For DPSD Against

Seizures In Mice (MES, MET)

A. Maximal Electroshock Seizure (MES) Antagonism*

Doses of DPSD (i.p., mg./kg.):

10	15	30	50	ED-50	95% C.I.
0/6#	1/6	5/6	6/6	20 mg./kg.	(15.1-24.9)

B. Maximal Pentylenetetrazol Seizure Antagonism+

Dose of DPSD (i.p. mg./kg.):

10	15	30	50	100	200	300	ED-50	95% C.I.
0/6#	0/6	2/6	3/6	5/6	6/6	6/6	48 mg./kg.	(3.15-76.9)

Notes: *MES delivered as 60 Hz., 50 mA., 0.2 sec. train through corneal electrodes.

DPSD was administered as a suspension in 0.5% methyl cellulose solution.

#Table entries are mice protected form tonic hind limb extension/tested mice. Test results evaluated 30 min. to 60 min. after DPSD dosing.

+Pentylenetetrazol administered subcutaneously at 95 mg./kg. and 15 min. before seizure is to be observed.

Table 5

Time course For The Anticonvulsant Action of DPSD

Minutes After Dosing With 50 mg./kg. i.p.

Group	0	7.4	15	30	60	120	300	480	500
1	0/6*			6/6			2/6		
2		1/6			5/6			2/6	
3			5/6			4/6			0/6
4	0/6			6/6			3/6		
5		2/6			6/6			1/6	
6			4/6			4/6			0/6
7	0/6			6/6			3/6		
8		1/6			5/6			2/6	
9			6/6			5/6			1/6
Mean	0	1.3	5	6	5.3	4.3	2.7	1.7	0.3
S.E.	0	.2	.6	0	.2	.2	.2	.2	.2

Note: *Table entries are protected/treated mice against maximal electroconvulsive seizures (MES).

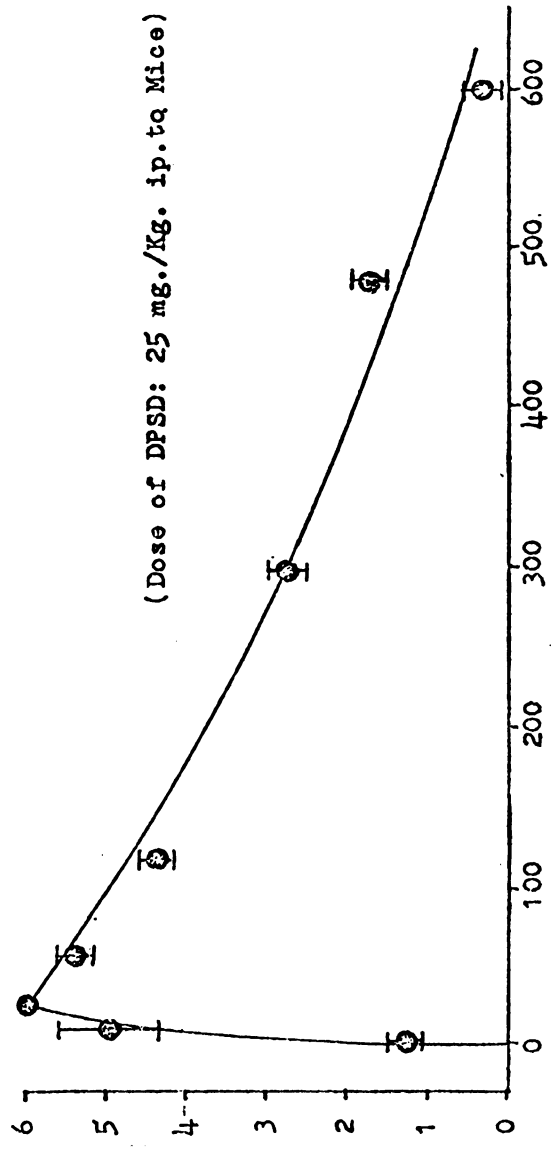


Figure 6. Time Course for the Anticonvulsant Action of Diphenylsilanediol. (After Intraperitoneal Administration to Mice and Tested Against Maximal Electroshock Seizures).

Table 6

Route Of Administration Effects On The Antagonism Of

MES Seizures By DPSD In Mice

A. Intraperitoneal (i.p.) Administration Tested 30 min. Post Dose

Dose of DPSD (mg./kg.):

10	15	30	50	ED-50	95% C.I.
0/6*	1.6	5/6	6/6	20 mg./kg.	(15.1-24.9)

B. Gavage (p.o.) Administration Tested at 1 Hour Post Dose

Dose of DPSD (mg./kg.):

1	2	4	6	10	ED-50	95% C.I.
0/6*	1/6	4/6	5/6	6/6	2.9 mg./kg.	(1.3-4.5)

C. Oral Administration in Food Tested After 24 Hours on Diet

Concentration of DPSD in Food (mg./gm.):#

0.1	0.2	0.4	0.8	1.6	3.2	6.4	ED-50	95% C.I.
0/6*	1/6	4/6	5/6	5/6	6/6	6/6	0.41 mg./gm.@	(0.23-0.70)

Notes: *Protected/treated mice; protection against tonic extension from electroconvulsive shock 50 mA. 0.2 sec.
#DPSD added to mouse food at indicated levels; food available ad libitum.

Table 7

Comparative Electroconvulsive Shock Seizure Antagonism

Species	Shock Level	Dose of DPSD@ (mg./kg.) i.p.: 10	30	100	300	EE-50	95% C.I.
Mouse	50 mA. 0.2 sec.	1/6*	4/6	6/6	6/6	20 mg./kg.	(4.6-35.4)
Gerbil	100 mA. 0.2 sec.	0/6	3/6	5/6	6/6	42 mg./kg.	(4.8-79.2)
Rat	150 mA. 0.2 sec.	0/6	3/6	6/6	6/6	30 mg./kg.	(11.2-48.2)

Notes: @ DPSD in 0.5% methyl cellulose suspension.

* Animals protected/tested (protection = no tonic extension).

Chemical Stimulation Models

According to Swinyard (1973) and Woodbury (1972), no single anticonvulsant test procedure is adequate to identify the spectrum of a potential antiepileptic drug by itself. Only through an examination of anticonvulsant action against a variety of seizure mechanisms can the spectrum of action for an anticonvulsant drug be determined.

Diphenylhydantoin is very poor at antagonizing chemical convulsions (Everett and Richards, 1944), contrasted with phenobarbital, which is very effective. The anticonvulsant action of DPSD against pentylenetetrazol, strychnine, and picrotoxin was thus determined.

Topical penicillin G to the brain neo-cortex of experimental animals produces a temporary lesion that discharges to produce convulsive seizures which occur repeatedly over a 24 hour period and then cease (Prince, 1972). Diphenylhydantoin (Lousi et al., 1968) and diazepam (Sharer and Kutt, 1971) effectively antagonize these focally-induced clonic seizures. Phenobarbital (Edmonds et al., 1974) apparently is ineffective. The effectiveness of DPSD against this seizure was also evaluated.

Methods

Subjects were female Sprague-Dawley rats (Spartan Laboratory Animals, Haslett, Michigan) weighing between 200 and 260 grams. Pentylenetetrazol, picrotoxin, and strychnine were obtained from Aldrich Chemical Company. Animals were treated with i.p. doses of DPSD suspended in 0.5% methyl cellulose one-half hour before injection

with one of the convulsant drugs via the subcutaneous (s.c.) route at an ED-99 dose. Pentylenetetrazol testing was done according to the method of Swinyard (1949), picrotoxin by the method of Hahn and Oberdorf (1962), and strychnine by the method of Everett and Richards (1944). The end points for scoring groups of six rats were no clonic seizures for the pentylenetetrazol tests during a 30-minute period post dose. This was also the criterion for scoring the strychnine test animals. With picrotoxin, the end point was protection from the seizures produced by this compound. ED-50 values and their 95% confidence intervals were determined using Finney's method (1952) for probit analysis.

Penicillin G, sodium salt, was applied to the cerebral cortex in the parietal lobe using an implanted cannula depicted in Figure 7. The inner cannula was packed with crystals of penicillin and inserted into the outer cannula for focal cortical stimulation with this topical convulsant drug. Only four rats were implanted with these cannulae which were fabricated from stainless steel hypodermic needles. Upon the insertion of the inner cannula continuing penicillin, each animal would begin having episodic seizures within 15 minutes. These seizures would decrease in frequency over a 12-hour period. For the purpose of anticonvulsant testing, DPSD was administered 30 minutes before the inner cannula was inserted. Animals not having seizures within 30 minutes after the cannula was inserted were scored as being protected by the anticonvulsant medication. Each rat received the dose vehicle (0.5% methyl cellulose solution), 30 mg./kg. DPSD and 100 mg./kg. DPSD

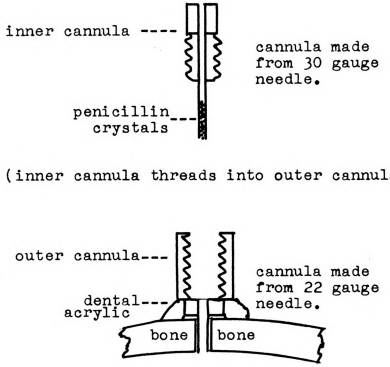


Figure 7. Device for the Application of Penicillin to the Rat Neocortex.

separated by a two-day inter-trial period. The order of dosing was permuted for the purposes of experimental design.

Results

Table 8 summarizes the data from the chemical-convulsant tests. Part A of this table shows that DPSD was able to antagonize seizures produced by pentylenetetrazol, strychnine, and picrotoxin. The ED-50 values for pentylenetetrazol and strychnine antagonism were very similar, being 58 mg./kg. and 60 mg./kg., respectively. The picrotoxin activity antagonism was produced at a higher ED-50 dose level of 150 mg./kg. An examination of Part B of Table 8 provides evidence that DPSD offers some protection from focal stimulation with penicillin. Both the number of seizures and the number of animals having seizures show a dose dependent reduction in penicillin convulsions due to DPSD. An ED-50 was not calculated as only two doses of DPSD were used. A reasonable estimate is that the ED-50 value lies between 30 and 100 mg./kg. With its ability to antagonize chemically-induced seizures, DPSD is unlike diphenylhydantoin and shows a broader spectrum of action.

Focal Electrical Stimulation Models

General Remarks

With the seizure models discussed above, the dependent variable was, in each case, a gross observation of the animal's behavior. With the focal electrical stimulation models discussed below, the attempt was not to produce gross seizure and convulsions, but to observe electrical phenomena in the nervous system in response to electrical stimulation.

Table 8

Antagonism Of Drug-Induced Seizures In Rats By DPSD

A. Protection From Systemic Convulsants

Convulsant (Dose, Route)	Dose of DPSD# (mg./kg.): 10	30	100	300	ED-50	95% C.I.
Pentylenetetrazol (80 mg./kg., i.p.)	0/6*	2/6	4/6	6/6	58 mg./kg.	(13-103)
Strychinine (1.5 mg./kg., s.c.)	1/6	3/6	3/6	5/6	60 mg./kg.	(7-113)
Picrotoxin (5 mg./kg., s.c.)	0/6	1/6	3/6	4/6	150 mg./kg.	(34-266)

Notes: *Table entries are protected/tested rats.

#DPSD given i.p. in 0.5% methyl cellulose.

B. Protection From Topical Penicillin To The Neocortex

Rat	Seizures	Dose of DPSD# (mg./kg.) 0	30	100	(Sequence)@
Bu	in 30 min.:	5	0	0	(0, 30, 100)
R	in 30 min.:	13	1	1	(100, 30, 0)
G	in 30 min.:	6	5	0	(30, 0, 100)
Bl	in 30 min.:	4	4	0	(0, 100, 30)
Total Seizures		28	10	1	
Quantal Score		0/4*	1/4	3/4	

Notes: #DPSD given i.p. in 0.5% methyl cellulose, 30 min. before penicillin.

@Order in which doses were administered separated by 2 days.

*Protected from seizures/treated.

These methods permitted observations on the effect of an anticonvulsant compound on the initiation and spread of an electrical disturbance to the nervous system.

Of the many procedures available (Purpura et al., 1972; Naquet and Lanoir, 1973), focal electrical stimulation of the neocortex (Penfield and Jasper, 1954) and post-tetanic potentiation (Esplin, 1957) of the superior sympathetic ganglion were selected.

Focal Electrical Stimulation of the Neocortex

Electrical stimulation of discrete sites in, and on, the brains of laboratory animals and humans (Penfield and Jasper, 1954) can evoke a sustained electrical discharge from cortical neurons that outlasts the stimulus duration by a wide margin and is not accompanied by gross motor seizures in the subject. These discharges can only be evoked by trains of high frequency stimulation above threshold values. Wilder, King, and Schmidt (1969) have shown that normal seizure propagation from an epileptic focal discharge has similar properties. This is strongly suggestive of the post-tetanic potentiation that Esplin (1957) demonstrated to be highly sensitive to the action of diphenylhydantoin. Most of these studies have been done in the cat (Schallek and Kuehn, 1963), the monkey (Delgado et al., 1956), or the human (Penfield and Jasper, 1954) to demonstrate drug effects. After-discharge is inhibited best by diphenylhydantoin, next by phenobarbital, and somewhat equivocally by diazepam. The rat has recently become a useful subject for post stimulus after-discharge phenomena (Marsan, 1972) and was selected for use in this study with DPSD.

Methods. Adult female Sprague-Dawley rats (Spartan Laboratory Animals) weighing 200-225 grams were implanted with epidural silver-silver chloride electrodes, according to the method of Pirch and Rech (1968), at least two weeks before testing. Additional electrodes made from a short section of stainless steel wire were placed 2 mm. anterior to the left-hand side recording electrode for stimulation of the cortex, and a stainless steel screw placed into the left side nasal bone to serve as a ground reference. Recording electrodes were located over parietal cortex using stereotaxic coordinates. Recording of the spontaneous electrocorticogram (ECoG) and evoked after-discharges were accomplished with a Grass Model 7 polygraph through a 7P5 wide band preamplifier. Biphasic stimulation was delivered at 60 Hz. with a Grass S-8 stimulator. Voltage and train duration were adjusted to evoke an after-discharge for a particular animal. Once these parameters were obtained, they reliably produced an after-discharge in a subject. Duration of the after-discharge was determined to the nearest 10 sec. by an examination of the ECoG record. Drugs were administered after a baseline response was obtained. Animals were retested 24 hours after the dose of DPSD was administered. DPSD was administered via the i.p. route in a 0.5% methyl cellulose solution 30 minutes before an after-discharge was evoked. Figure 8 shows the recording and stimulation techniques used in this study.

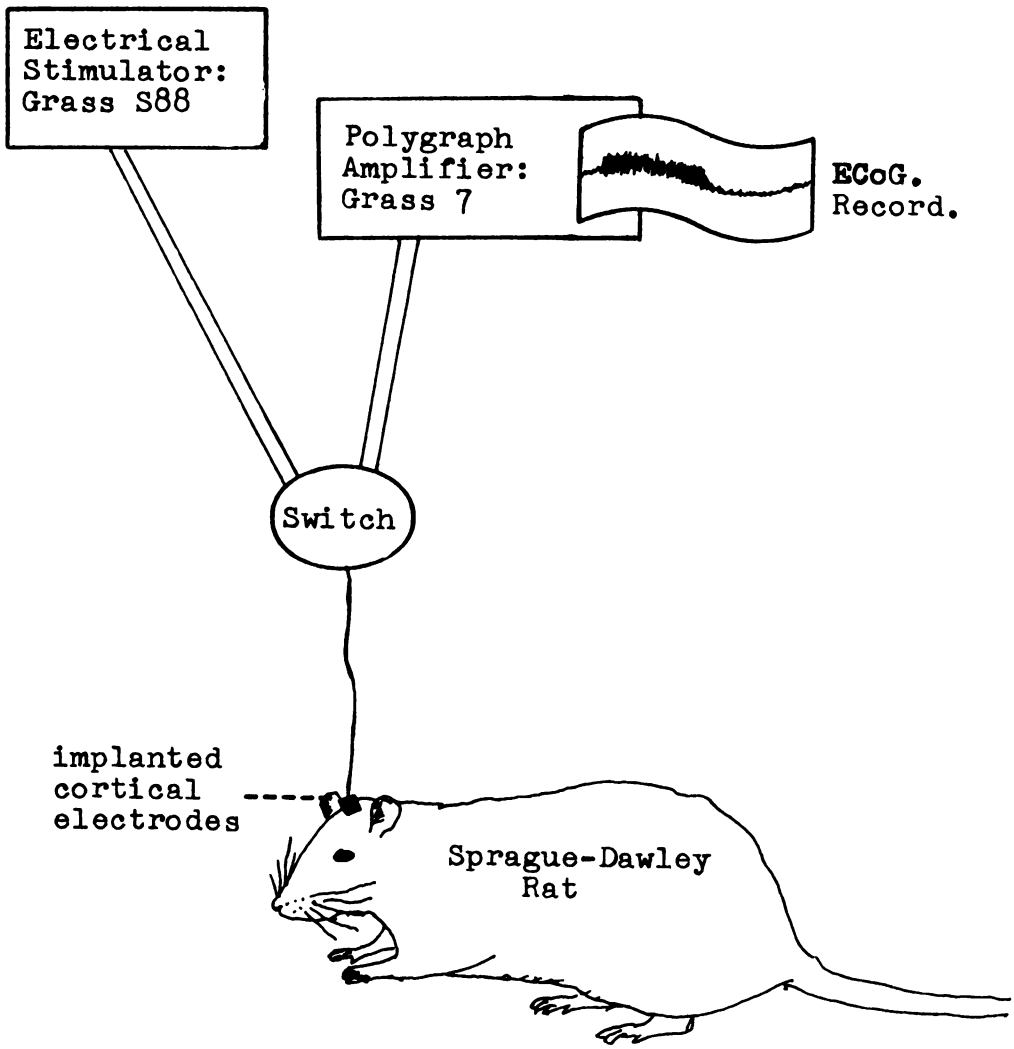


Figure 8. Method for Cortical After-Discharge Studies.

Results. Table 9 summarizes the results from the after-discharge experiments with three rats which received 30 mg./kg. of DPSD. There was a significant decrease in the duration of the after-discharge. Note that this drug was very dramatic in abolishing the after-discharge response. One subject also received a stimulus of twice the duration and twice the voltage, but this only served to evoke a tonic-clinic seizure in the animal and not a seizure-free, sustained discharge. These results strongly suggested that a more classical post-tetanic potentiation experiment be done.

Post-Tetanic Potentiation Effects

It was the purpose of the post-tetanic potentiation experiments using the cat sympathetic superior cervical ganglion preparation to test the hypothesis that DPSD, like diphenylhydantoin, acts to inhibit post-tetanic potentiation mechanisms.

With the demonstration by Esplin (1957) that post-tetanic potentiation in the spinal cord of the cat was markedly attenuated by anticonvulsant doses of diphenylhydantoin, an explanation for the action of this drug was developed. Toman (1970) notes that patients with epilepsy caused by a focal discharge still have the pre-seizure aura, but are without the seizure when treated with diphenylhydantoin. This, he surmised, is evidence that post-tetanic potentiation inhibition is an important clinical mechanism for diphenylhydantoin. Later studies showed that trimethadione (Esplin and Curto, 1957) and phenobarbital (Esplin, 1972) do not inhibit post-tetanic potentiation. The ability

Table 9

Inhibition Of Cortical Electrical After-Discharge

By DPSD (30 mg./kg. i.p.) in The Rat

	Stimulus Parameters			Discharge Duration		
Subject Ident.	Amplitude Volts	Frequency Hz	Duration Sec.	Control Time	Post - Dose 1 hr.	24 hrs.
G-1	5	60	10	100 sec.	15 sec.	90 sec.
B-2	8	60	10	120 sec.	10 sec.	160 sec.
C-2	6	60	10	180 sec.	20 sec.	130 sec.
		Means for 3 subjects:		133 sec.	15*sec.	127#sec.
		Standard Errors:		24 sec.	2.8 sec.	20 sec.

Notes: *Significant from control (0.05 level).

#Not significant from control.

of diphenylhydantoin to inhibit post-tetanic potentiation is seen at the neuromuscular junction (Raines and Standaert, 1969) and the various sympathetic ganglia (Esplin and Zablocka, 1969; Esplin, 1972) in addition to the spinal cord.

Methods. Because Esplin and Zablocka (1969) had shown that diphenylhydantoin blocks post-tetanic potentiation in cat sympathetic ganglia, and because of the relative simplicity of this procedure (over a spinal root preparation), it was employed using the technique of Gebber and Volle (1966); see also Volle (1962). Cats were surgically prepared under diallylbarbiturate anesthesia with the superior cervical ganglion isolated with its afferent sympathetic trunk and efferent sympathetic nerves intact. Stimulating electrodes were placed on the ascending sympathetic trunk and recording electrodes on one of the sympathetic branches (e.g., the external carotid nerve). The evoked monosynaptic potential was displayed on an oscilloscope and recorded on a Grass polygraph for analysis.

Figure 9 depicts the recording and stimulation procedures used to elicit post-tetanic potentiation in the cat superior cervical ganglion. An idealized output record is also shown describing the peak amplitude and tetanization duration measures discussed below. The peak amplitude of the tetanization record is identified and measured in millivolts. With a prior determination of baseline amplitude for reference, the time for the return of the potentiated response to baseline is also noted. These three parameters of baseline amplitude, peak potentiated amplitude, and the time for the potentiation to

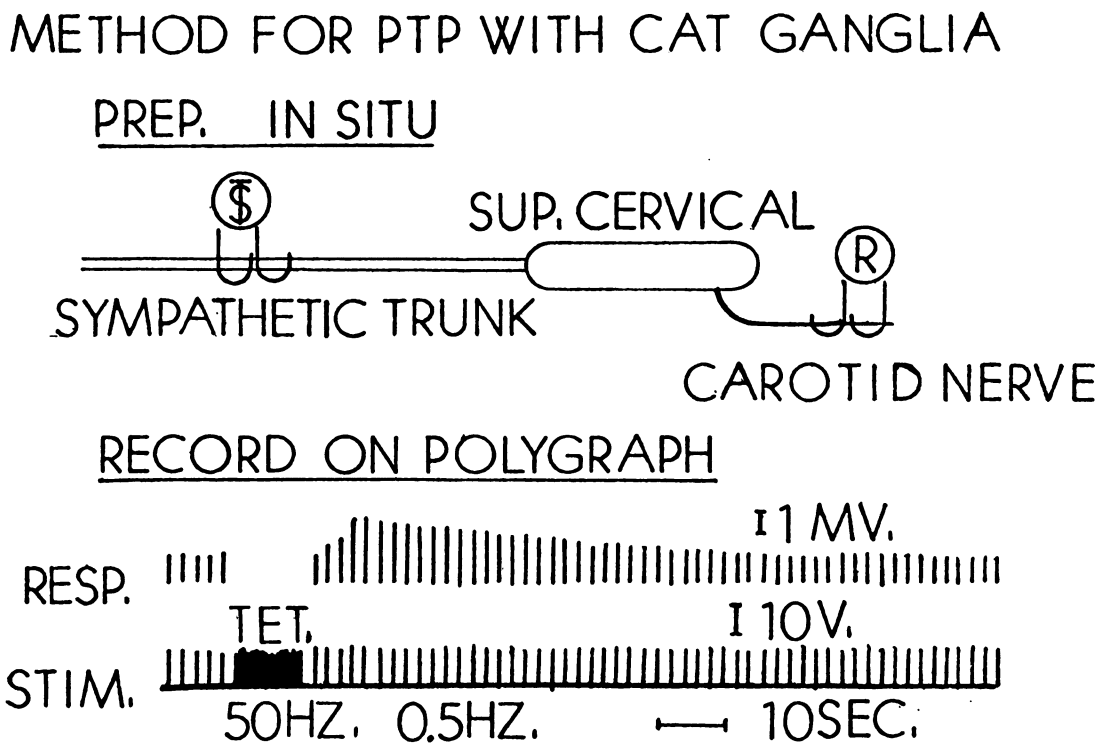


Figure 9. Diagrammatic Summary of the Post Tetanic Potentiation (PTP) Method and Data Collection.

return to baseline amplitude are recorded for each subject both before and after drug administration. Each datum point consists of the mean of three successive determinations. Administered drug was suspended in a 0.5% methylcellulose vehicle at doses of 0.0 mg./kg., 25 mg./kg., and 50 mg./kg. of DPSD via the i.p. route. Three animals were used as control subjects, three as low-dose subjects, and three as high-dose subjects. The initial dose selected was 50 mg./kg. of DPSD as this represented a high anticonvulsant dose in mice and rats. A total of sixteen cats were prepared surgically for post-tetanic potentiation experiments. Only those animals showing consistent, repeatable, evoked-responses between 500 and 1,500 microvolts and tetanization durations close to one minute were included in this study.

Results. Table 10 is a compilation of the post-tetanic potentiation results with nine cats both before and after dosing with vehicle alone, 25 mg./kg. of DPSD, and 50 mg./kg. of DPSD. At the bottom of this table the means and standard error values for each dose level are presented. In order to facilitate examination of this data, the differences in before-dosing and after-dosing amplitudes of the maximum post-tetanic potentiation amplitudes in millivolts are examined separately. The mean values for these parameters show a significant difference between the 25 mg./kg. and the 50 mg./kg. values when compared with the control (vehicle) values. The overall F statistic for a completely randomized analysis of variance design was not

Table 10

Inhibition Of Post-Tetanic Potentiation By DPSD

mg./kg. Dose	Subject	Baseline Values Amplitude Base	Tet.	(mV.) Diff.	Duration Of Tet.	After Dosing Values Amplitude Base	Tet.	(mV.) Diff.	Duration Of Tet.
0	2	1.3	3.4	2.1	96 sec.	1.1	2.7	1.6	88 sec.
0	3	0.9	1.7	0.8	72 sec.	0.8	1.5	0.7	87 sec.
0	11	1.0	1.8	0.8	53 sec.	1.1	2.6	1.5	49 sec.
25	4	1.9	3.3	1.4	90 sec.	1.9	2.4	0.5	51 sec.
25	6	0.5	1.1	0.6	82 sec.	0.6	0.9	0.3	45 sec.
25	7	0.8	2.0	1.2	37 sec.	0.8	1.5	0.7	23 sec.
50	9	1.1	2.7	1.6	74 sec.	0.9	1.3	0.4	12 sec.
50	10	1.7	2.4	0.7	56 sec.	1.2	2.0	0.8	15 sec.
50	12	0.9	1.6	0.9	57 sec.	0.9	1.1	0.2	17 sec.
0	Mean	1.1	2.3	1.2	74 sec.	1.0	2.3	1.2	75 sec.
0	S.E.*	.09	.55	.43	12 sec.	.10	.38	.28	13 sec.
25	Mean	1.1	2.1	1.1	70 sec.	1.1	1.6	0.5	40 sec.
25	S.E.	.42	.64	.24	16 sec.	.40	.43	.11	8 sec.
50	Mean	1.2	2.2	1.1	62 sec.	1.0	1.5	0.47	15 sec.
50	S.E.	.24	.33	.27	6 sec.	.10	.27	.18	1.4 sec.

* standard error of the mean.

significant at the $p < .05$ level; but an application of Dunnett's modified t-test showed both values to be significantly different from the control value at the above alpha level. Notice, however, that the two amplitude values are not different from each other. In addition, there is a dose-related decrease in post-tetanic potentiation duration due to DPSD. Here all values were found to be significantly different using a combination of F-test and Dunnett's t-test procedures.

Thus, DPSD inhibits post-tetanic potentiation as part of its action.

Tolerance and Physical Dependence Studies

The wide range of anticonvulsant activity observed for DPSD, as discussed above, suggests a therapeutic potential for this compound as an anticonvulsant or antiepileptic agent. The latter therapeutic indication demands long term administration. Under these conditions, the possibility of tolerance development to the DPSD anticonvulsant action needs to be examined. In addition, the potential for physical dependence and a resulting withdrawal syndrome upon cessation of the DPSD administration also needs to be seriously considered.

In this section an attempt was made to design a study which would give some indication of the potential for both tolerance and physical dependence associated with DPSD administration. McQuarrie and Fingl (1958) were able to demonstrate a lowering of seizure threshold to low-frequency electroshock seizures and to pentylene-tetrazol administered intravenously after withdrawal from alcohol

administration for as little as one day. Using these techniques, or modifications of them, some degree of physical dependence, as evidenced by lowered seizure threshold, has been demonstrated for a number of anticonvulsant drugs (Dzhagatspanyan and Klygul, 1971; Rumke, 1967; Swinyard et al., 1957). These articles also show an associated tolerance development evidenced in a decreasing anticonvulsant action with repeated dosing of mice with meprobamate (Swinyard et al., 1957), phenobarbital, primidone, trimethadione, diazepam, chlordiazepoxide, and diphenylhydantoin (Dzhagatspanyan and Klygul, 1971; Rumke, 1967). There are, of course, variations in the degrees of both tolerance development and physical dependence with each of the individual anticonvulsant drugs. (A discussion of these variations is considered below in the light of results from the DPSD tolerance and dependence studies.)

Methods

The procedures used here were modeled after a study by Dzhagatspanyan and Klygul (1971) in which tolerance and physical dependence were shown for diazepam and chlordiazepoxide in comparison with phenobarbital. The seizure threshold method used there, and below, is the timed intravenous infusion of pentylenetetrazol technique of Orloff et al. (1949). Threshold dose of pentylenetetrazol to produce convulsive seizures is determined before treatment with an anticonvulsant test drug, at various times during anticonvulsant drug treatment, and for a number of days after the anticonvulsant drug treatment has been stopped. Three types of information are gained from this type of

experimental design. An elevation of the seizure-threshold dose of pentylenetetrazol, when control periods are compared with the treatment periods, indicates an anticonvulsant action against pentylenetetrazol for the test compound. The second type of information is tolerance development. During the daily dosing period, a drop in seizure threshold dose for pentylenetetrazol during the dosing period is evidence that tolerance to the drug's anticonvulsant action has occurred. An increasing threshold during this same time period is indicative of either accumulation of the drug in the organism or sensitization ("negative tolerance") to the action of the drug. The third type of information focuses on the change in threshold after the compound has been withdrawn. A decrease in threshold dose for pentylenetetrazol would be indicative of physical dependence if the threshold level dropped below the control values established before dosing with the anticonvulsant drug. By following the time course of this decrease in seizure threshold, the onset and duration of a physically dependent state can be characterized. Table 11 summarizes these observation criteria for tolerance and physical dependence.

Subjects were female Swiss Webster mice (Spartan strain), housed in groups of six animals, each weighing from 25-30 grams, and fed ad libitum on ground rat food (Purina Rat Chow, Ralston Purina Co., St. Louis, Missouri) and water. The rat food also served as a dosing vehicle for the DPSD. Two doses of DPSD were administered to the mice. The lower dose was the ED-50 anticonvulsant dose that antagonized maximal electroshock induced tonic hind-limb extension (see Table 6 above)

Table 11

Design For Study To Evaluate Potential For

Tolerance And Physical Dependence For

DPSD

Method: - From McQuarrie and Fingl (1958) and Dzhagatspanyan and Klygul (1971).

Tolerance: - Decrease in elevated pentylenetetrazol threshold values from the increase established after acute test drug with repeated administration of the test drug.

Physical Dependence: - Decrease in pentylenetetrazol threshold values below pre-dosing values after the test drug is abruptly discontinued.

Pentylenetetrazol Threshold: - First twitch value (Orloff et al. 1949).

Subjects: - Mice (Swiss Webster, Spartan Strain): females; 25-30 gm.

Test Drug: - Diphenylsilanediol (DPSD).

Route of Administration: - Dispersed in food.

Dose of DPSD: - 0.4 mg./gm. food; (MES ED-50 - 0.41 mg./gm. food)

6.4 mg./gm. food; (RotaRod ED-50 = 6.62 mg./gm. food)

Duration of Dosing: - 14 days and 90 days.

Test Times: - Before-Dosing period: 1 day before
During-Dosing period: days 2, 5, 14, 30, 90
After-Dosing period: days 1, 2, 3.

when administered in the food for 24 hours. The higher dose was the rotating rod depression ED-50 value (see Table 16 below). Food was prepared once a week by grinding the DPSD into the powdered food on a milligram of DPSD per gram of food basis. The lower dose was 0.4 mg. DPSD/gm. food and the higher dose was 6.4 mg. DPSD/gm. food. The food was placed into porcelain feeding dishes which were filled daily with the ground food and placed in the cages with groups of six mice. Eighty-four mice were used in this experiment with forty-two in the low dose group and the other forty-two in the high dose group.

Six animals maintained in one cage were tested together for seizure threshold during intravenous infusion of pentylenetetrazol (Sigma Chemical Co., St. Louis, Missouri). The pentylenetetrazol was dissolved in 0.9% saline solution and administered via the lateral tail vein through a 27 gauge hypodermic needle connected to a syringe driven by a syringe pump (Harvard Apparatus Co., Inc., Millis, Massachusetts). The 1% solution of pentylenetetrazol was administered at a rate of 19.4 ml./hr. At the start of the infusion, a stopwatch was started and the time to the first twitch (Orloff et al., 1949) was recorded. The dose of pentylenetetrazol to produce this event was calculated from infusion time, infusion rate, and body weight for individual animals.

The low dose groups were tested on the day that DPSD was to be added to their food, at the end of the first full day of dosing, day 2, after seven days of dosing, after fourteen days of dosing, and one, two, and three days after dosing with DPSD was discontinued. At

any particular test time, the individual mice from a cage of six were tested using the timed intravenous infusion of pentylenetetrazol technique. The mice were restrained in a clear plastic holder (No. 2, rat holder, Narco Biosystems, Inc., Houston, Texas) during these observations which permitted limited movements of the mice and clear observation of the seizure activity.

The high dose groups were tested on the day that DPSD was added to their food; at the end of their first full day of dosing, on the fifth day, fourteenth day, thirtieth day, and on the ninetieth day of dosing; and again on the first (91st.) and third (93rd.) days after dosing with DPSD had been discontinued. As above, one cage of six mice was tested at any one time period and the time (i.e., dose) for the first twitch after pentylenetetrazol administration was observed for each animal.

Table 11 summarizes the methods design as discussed above in this section.

Results

Data on the pentylenetetrazol threshold changes associated with administration and withdrawal of 0.4 mg./gm. food are listed in Table 12 and plotted in Figure 10. Though there was an elevation in pentylenetetrazol threshold associated with DPSD administration, there is no evidence of tolerance or physical dependence. The more severe dosing conditions of 6.4 mg./gm. food for 90 days produced the results listed in Table 13 and plotted in Figure 11. The threshold for pentylenetetrazol threshold seizures was elevated to 126 mg./kg. i.v.

Table 12

Pentylenetetrazol "First Twitch" Threshold

Changes Associated With DPSD Administration For 14 Days

(Dosing started at 0.4 mg./gm. food on day one and stopped on day 14.)

Test Day	Number Tested@	Infusion Time#	Total MET*	Dose Level+	Standard Error
1	6	21.7	1.17	30	4.6
2	5	53.6	2.88	103	11.6
7	4	51.2	2.76	95	18.3
14	5	58.3	3.28	106	17.3
15	6	26.7	1.46	49	4.2
16	5	25.4	1.37	44	2.9

Notes: @Number of mice in group on test day.

#Time to first twitch observation in seconds with i.v. infusion of 1% pentylenetetrazol solution to the lateral tail vein.

*Calculated total dose of pentylenetetrazol (MET) in mg.

+Calculated mg./kg. dose of MET admisistered i.v.

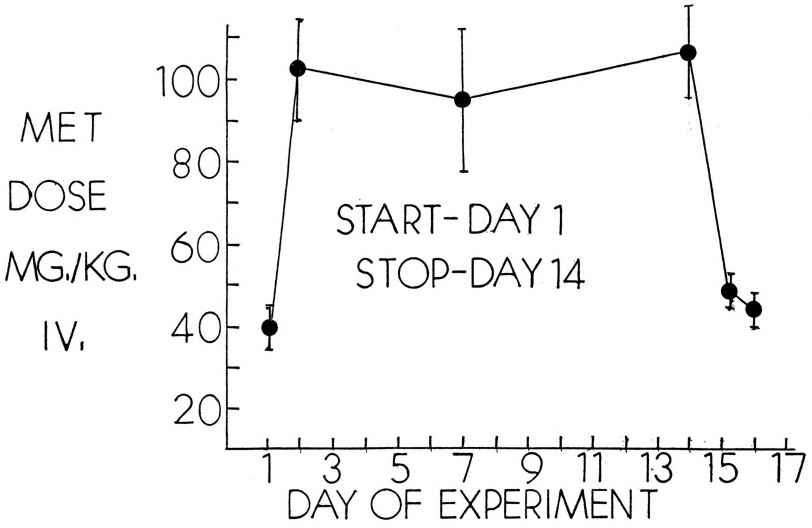


Figure 10. Pentylenetetrazol(MET) Seizure Threshold During and After DPSD Administration (0.1mg./g. food) for 14 Days. (Tolerance and Physical Dependence Study).

Table 13

Pentylenetetrazol "First Twitch" Threshold

Changes Associated With DPSD Administration

For 90 Days
(Dosing started at 6.4 mg./gm. food on day one and stopped on day 90.)

Test Day	Number Tested@	Infusion Time#	Total MET$	Dose Level+	Standard Error
1	5	21.1	1.13	40	5.1
5	4	64.7	3.48	134	10.8
14	4	70.4	3.79	126	17.5
30	5	69.7	3.75	121	14.7
90	4	70.1	3.77	125	16.2
91	5	16.2	0.87	29	4.9
93	5	15.4	0.82	27	6.0

Notes: @Number of mice in group on test day.

#Time in seconds to first twitch observation with i.v. infusion of 1% pentylenetetrazol (MET) solution at 0.3233 ml./min. to the lateral tail vein.

$Calculated total dose of pentylenetetrazol (MET) in mg.

+Calculated mg./kg. dose of MET administered i.v.

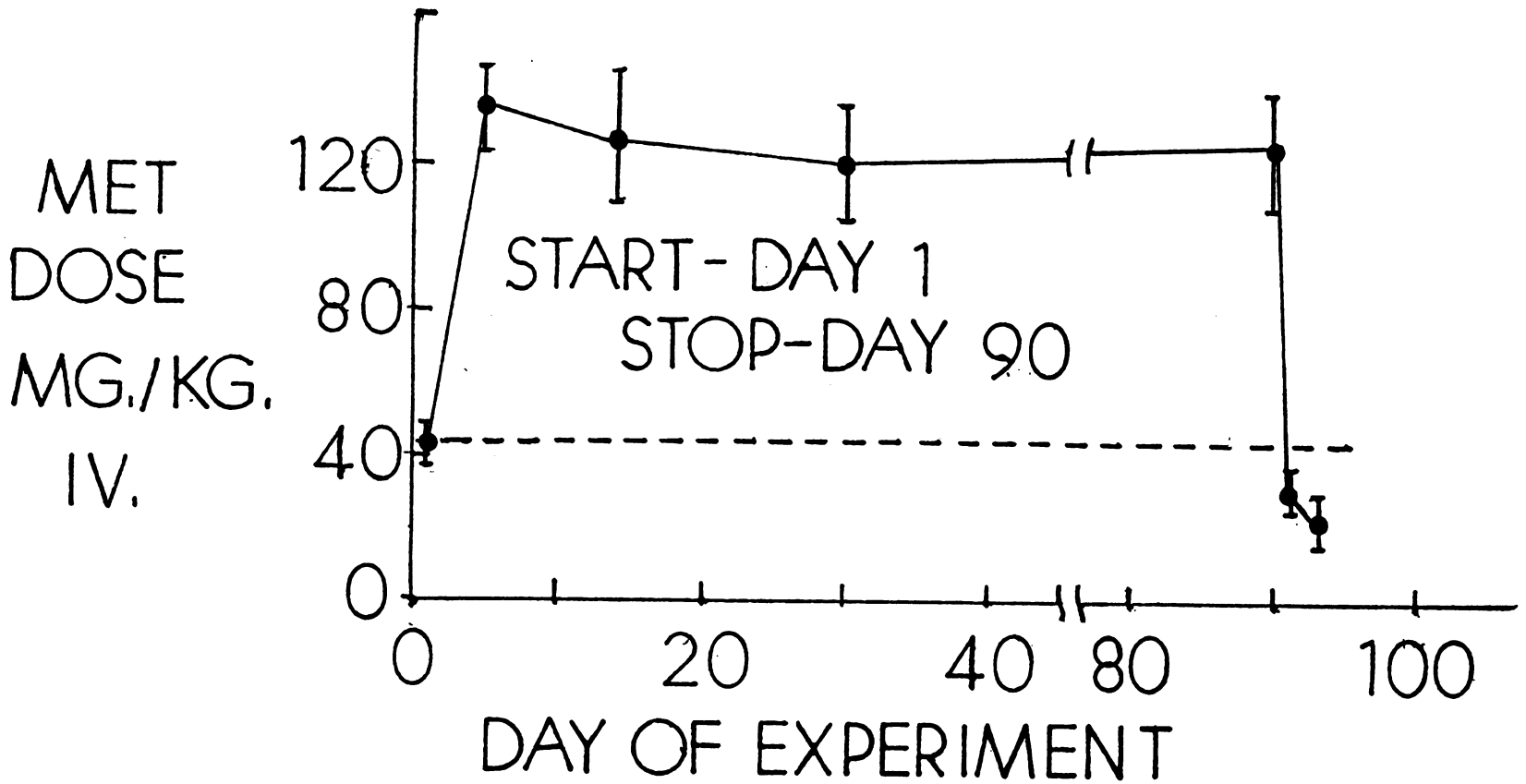


Figure 11. Pentylenetetrazol(MET) Seizure Threshold During and After DPSD Administration (6.4 mg./g. food) for 90 Days. (Tolerance and Physical Dependence Study).

and can be compared with the elevation to 103 mg./kg. i.v. with the lower dose of DPSD as it appears in Table 12. In both cases the control (before test drug) values were approximately 40 mg./kg. Again there is little evidence of tolerance development on this dosage regimen. The decrease in seizure threshold to 29 mg./kg. and 27 mg./kg. at one and three days after the DPSD is withdrawn is evidence for some physical dependence activity. There were, however, signs of spontaneous increases in excitation level or seizures.

Discussion of Anticonvulsive Effects

As a result of these anticonvulsive activity studies, one can conclude that DPSD has a broad spectrum of anticonvulsant action. This compound is able to protect rodents from the tonic seizures produced by electroshock and pentylenetetrazol and the clonic seizures from pentylenetetrazol, strychnine, picrotoxin, and penicillin. It is also able to elevate the threshold for seizures produced by pentylenetetrazol and for focal electrical stimulation of the brain. In addition to suppressing neocortical after-discharges to electrical stimulation, it inhibits post-tetanic potentiation in sympathetic ganglia. Furthermore, DPSD is able to suppress seizure phenomena with chronic dosing showing little evidence suggestive of rapid tolerance development and no major withdrawal effects. These results and associated doses are summarized in Table 14. A comparison of these results for DPSD with the published results for some clinically useful anticonfulsive agents is summarized in Table 15 and discussed below.

Table 14

Summary Of Anticonvulsant Action Of DPSD

Test Method	Results
(Seizure Type)	(Seizure Suppression Dose Range)
Maximal Seizures (Tonic-Clonic)	
Electroshock	ED-50, 20 mg./kg. i.p. ED-50, 2.9 mg./kg. p.o.
Pentylenetetrazol	ED-50, 48 mg./kg. i.p.
Threshold Seizures (Clonic Generalized)	
Pentylenetetrazol	ED-50, 58 mg./kg. i.p.
Strychnine	ED-50, 60 mg./kg. i.p.
Picrotoxin	ED-50, 150 mg./kg. i.p.
(Clonic-Focal)	
Penicillin	(30-100 mg./kg. i.p.)
(Clonic-Threshold)	
Pentylenetetrazol	(Threshold Elevation-66.6-1066 mg./kg./day)
Non-Convulsive Seizures	
Neo-Cortical After-Discharge	(Threshold elevation, duration decrease-30 mg./kg. i.p.)
Post-Tetanic Potentiation	(Inhibition of Amplitude and duration 25-50 mg./kg. i.p.)
Chronic DPSD Administration	(Minimal tolerance and withdrawal effects-66.6-1066 mg./kg./day)

Table 15

Comparisons of the Anticonvulsant Action of Anticonvulsive Compounds

Compounds found to be Active (+) or Inactive (-)

Seizure Model	Diphenylhydantoin	Phenobarbital	Diazepam	Diphenylsilanediol
Maximal electrochock (tonic phase)	+	+	+	+
Pentylenetetrazol (tonic phase)	±	+	+	+
(clonic phase)	-	+	+	+
(threshold effects)	-	+	+	+
Strychnine (clonic phase)	-	+	+	+
Picrotoxin (clonic phase)	-	+	+	+
Pencillin (focal lesion)	-	+	±	+
After-discharge (decrease duration)	+	-	±	+
Post-tetanic Potentiation (inhibition)	+	-	±	+

Comparisons are made with three phenyl-containing anticonvulsive drugs--diphenylhydantoin, phenobarbital, and diazepam. An examination of the activity profiles for these four compounds, as shown in Table 15, reveals a strong similarity between DPSD and phenobarbital except for the post-tetanic potentiation effects and the apparent minimal withdrawal effects of DPSD.

Since Esplin (1957) demonstrated the inhibition of post-tetanic potentiation by diphenylhydantoin, many other compounds have been tested for their effects on this model (Esplin, 1972; Suria and Costa, 1974; Polc et al., 1974). In addition to diphenylhydantoin, only diazepam showed some degree of post-tetanic potentiation inhibition. One could speculate about what similarities these three compounds DPSD, diphenylhydantoin, and diazepam might have in common in terms of molecular size, electronic properties, etc., but a simple observation is that they are each diphenyl-containing compounds (see GENERAL DISCUSSION, p. 154, for more elaborations). Increased seizure susceptibility is a common phenomenon associated with the withdrawal of anticonvulsant medication (Rumke, 1967; Swinyard et al., 1957; Dzhagatspanyan and Klygul, 1971). The small effect seen with DPSD is encouraging as it implies the possibility of minimizing or eliminating this potential hazard of anticonvulsant medication.

The inhibition of after-discharge phenomena in the brain is a common property of many anticonvulsants (Shallek and Kuehn, 1963; Racine et al., 1974; Wise and Chineraman, 1974). DPSD as with diazepam, the barbiturates, trimethadione, and diphenylhydantoin, also has this

property. Unlike diphenylhydantoin, DPSD also elevates this seizure threshold.

The inhibition of the clonic portion of seizures produced by chemical convulsants (Stone, 1972) has been used to predict muscle relaxant (Roszkowski, 1964), antianxiety (Hanson and Stone, 1964) and petit mal antiepileptic activity (Swinyard et al., 1974). This activity for DPSD gives support for a wide spectrum of anticonvulsant action.

Elevation of pentylenetetrazol seizure threshold (Jenny and Pfeiffer, 1956) is another action that differentiates anticonvulsant drugs. DPSD, unlike diphenylhydantoin, elevates this threshold.

BEHAVIORAL CHARACTERIZATION

General Remarks

Another important area of consideration for anticonvulsant drugs is their effect on behavior. This evaluation was conducted for two reasons. One is to determine any behavioral toxicity that may be associated with the use of an anticonvulsant. The second is to identify any behavioral activity that could be therapeutically beneficial.

Diazepam, originally developed as a minor tranquilizer and hypnotic, has found recent use as an anticonvulsant drug (Brown and Penry, 1973), both in the treatment of status epilepticus and as an adjunct to antiepileptic therapy. On the other hand, diphenylhydantoin originally introduced as an anticonvulsant drug (Merritt and Putnam, 1938), has found current use as an anti-anxiety drug to treat psychoneurotic symptoms (Uhlenhuth and Stephens, 1972). The majority of anticonvulsant drug evaluations usually rely only on the inclined screen or rota-rod performance as a measure of behavioral effects (Swinyard, 1949; Toman, 1964; Hudson and Wolpert, 1970). As early as 1952, Swinyard, Brown, and Goodman had recommended that other behavioral measures be used in testing anticonvulsant drugs as the current techniques were sometimes inadequate. Laboratory and clinical assessments of the behavioral effects of diphenylhydantoin have been few and far

between. Most of these have shown mild tranquilizer or sedative-hypnotic effects for this drug. Early operant studies with the rat (Gordon, 1968; Doty and Dalman, 1969) were aimed at demonstrating that diphenylhydantoin could improve learning in animals impaired by age or other disability. On performance measures, this drug has been found to decrease fighting in mice (Tedeschi et al., 1959) decrease the toxicity of amphetamine with aggregated mice (Fink and Swinyard, 1962), reduce motor activity levels (Millichap and Boldrey, 1967), and to depress shuttlebox performance (Izquierdo and Nasello, 1973). Human studies have centered around the cerebellar ataxia produced by this drug (Stephens et al., 1974). Weinreich and Clark (1970) and Domino and Olds (1972) have also demonstrated that diphenylhydantoin inhibits responding under intracranial self-stimulation when tested on rats having electrodes placed in the medial forebrain bundle.

There are elaborate and lengthy schemes for the identification of psychotropic drug action (Nodine and Siegler, 1964; Siegler and Moyer, 1967) but selected procedures should be adequate to evaluate anticonvulsant drugs. Primidone and trimethadione (Woodbury, 1972), as well as the minor tranquilizer anticonvulsants, including methaqualone (Swift and Becker, 1959), have general depressant and anti-anxiety behavioral activity. With diphenylhydantoin there appears to be general depression at high doses while lower doses produce ataxia and cerebellar dysfunction syndromes (Kutt and McDowell, 1968). Compounds can be evaluated for neuromuscular relaxation and general depressant properties using rota-rod techniques (Moore and Rech, 1967).

Operant conditioning techniques, such as conditioned suppression procedures, introduced by Estes and Skinner (1941), provide convenient methods for detecting anti-anxiety properties of drugs that do not produce profound stimulation or depression in the disinhibiting dose range.

Rotating Rod Effects

The disruption of rotating rod performance in the mouse and rat is a widely used measure of neurotoxicity with anticonvulsant and sedative drugs (Raines et al., 1973; Tislow, 1968). Gross, Tripod, and Meier (1955) suggested that this measure is the best predictor of the dose level at which humans appear drowsy and ataxic. The three species used in the characterization studies of DPSD are the mouse, gerbil, and rat. As a measure of the neurotoxic effects of this drug and, also, for a simple behavioral comparison in these three species, the dose dependent effects of DPSD on rota-rod performance was evaluated. Two routes of administration were used. The intraperitoneal route is consistent with the majority of the anticonvulsant procedures discussed above. An oral route, involving administering the drug in the diet as a powdered suspension, provides a comparison for the anticonvulsant (MES) data after oral administration above, and an oral dosage level comparison for a toxicity study in mice (see below).

Methods

Subjects were 20-30 gram mice, 200-250 gram rats, and 70-90 gram gerbils. Mice (Swiss-Webster) and rats (Sprague-Dawley) were obtained from Spartan Laboratory Animals (Haslett, Michigan). Gerbils, Meriones unquiculatus, (random bred) were from the Michigan State University colony located in the Life Sciences Building. Rota-rod was run according to the method of Moore and Rech (1967) on a 4 inch diameter, sand-paper covered rod that was rotated at 8 revolutions per minute. Animals were trained to a criterion of three minutes on the rod without falling off for each of three consecutive trial periods before drug testing. Groups of six animals were dosed with i.p. DPSD, suspended in 0.5% methyl cellulose, one hour before rotating rod testing. For the special tests after powdered food administration in mice, the food-DPSD mixture was administered for 24 hours before rotating rod testing (see anticonvulsant section for the food composition and preparation procedures).

Subjects were scored with a one or zero if they completed or failed to complete three minutes on the rota-rod. A particular dose score would then be the number of subjects falling per the number tested in a group. These quantal data were evaluated using Finney's probit analysis method (Finney, 1952), thus giving ED-50 values together with their confidence intervals.

Results

Table 16 shows the dose response data for rota-rod depression with these rodents. ED-50 values and their 95% confidence intervals were calculated using Finney's method. Section A of this table (Table 16A) shows the rotating rod response values for the rat, gerbil, and mouse after i.p. administration of DPSD. The gerbil was the most sensitive to the depressant effects of DPSD, i.e., ED-50 of 80 mg./kg. vs. 170 and 200 for the rat and mouse, respectively. The gerbil also had the most difficulty in learning this task. A likely explanation is that the gerbil, having large flat hind feet, is ill-prepared to climb on moving surfaces unlike the rat and mouse. The mouse and rat data show essentially identical dose-response values.

An examination of Table 16B shows 6.62 mg. DPSD/gm. of food was the calculated value for the rotating rod ED-50 utilizing this oral route of administration. One can estimate the equivalent total daily dose for this food level by using a daily food consumption value of 16.7% of body weight for the mouse (Bell, 1962) to calculate a daily drug dosage of 1,105 mg./kg./day for DPSD.

Operant Behavioral Effects

As a further examination of the behavioral toxicity of DPSD the performance effect of this compound on an ongoing operant behavior was evaluated. Performance baselines under fixed ratio (FR) operants are relatively resistant to depression (Thompson and Schuster, 1968) until highly sedative doses of depressant drugs are reached.

Table 16

Rotating Rod Performance Decrement Due to DPSD

A. Comparative Effects after i.p. Administration (1 hr. post dose)

	Dose of DPSD (mg./kg.):						
Species	10	30	100	300	1000	ED-50	95% C.I.#
Rat	0/6*	0/6	1/6	5/6	6/6	170	(105-235)
Gerbil	0/6	1/6	4/6	6/6		80	(36-124)
Mouse	0/6	0/6	1/6	4/6	6/6	200	(72-328)

Notes: *Number falling off in less than 3 min./tested.
#95% confidence interval for the ED-50 value.

B. Effects After 24 Hour Oral Administration to Mice in Food

Food Concentration (mg./gm. food)	Depressed/ Treated*	Calculated@ Total Daily Dose (mg./kg.)
0.8	0/6	133
1.6	0/6	267
3.2	2/6	533
6.4	2/6	1067
12.8	4/6	2133

Notes: *Number falling off in less than 3 min./number tested.
@Calculated dose based on 166 mg. food/gm. body weight daily food consumption. ED-50 value: 6.62 mg. DPSD/gm. food (3.95-15.38).

Concomitant with this performance, a superimposed measure of experimental anxiety is highly sensitive to anti-anxiety drug effects (Estes and Skinner, 1941; Geller and Seifter, 1962; Blum, 1970). Anti-anxiety agents such as diazepam produce a release of conditioned emotional suppression (CES) at doses that are widely separated from those that produce a depression of response rate on a fixed ratio performance baseline. The barbiturates, on the other hand, produce both effects in similar dose ranges.

Methods

Adult female Sprague-Dawley rats were obtained from Spartan Laboratory Animals (Haslett, Michigan) and trained to bar press for food using standard procedures (Thompson and Schuster, 1968). They were stabilized at a FR-40 schedule, one food pellet for 40 bar presses, and then trained on an Estes and Skinner (1941) type of CES paradigm. On an average of once every two minutes, a tone was sounded in the animal chamber for 15 seconds; this was followed by a brief, 0.5 sec. shock of 0.6 mA. delivered to the feet of the rats through a shock scrambler. After training, subjects made very few responses while the tone was on. Animals were tested for two hours per day in a continuous run with these two paradigms, FR-40 and CES, superimposed. Electro-mechanical counters kept track of the total number of responses made by the animal during the two-hour background period and the total number of responses made while the tone was on. A depression of FR responding was determined as a statistically significant change from baseline after a given dose. CES effects were measured as significant

increases in the number of bar presses made during the "tone-on" period in drug-treated subjects compared with non-treated levels. Doses of DPSD were administered via the i.p. route at 15 minutes before testing. The drugs were suspended in a 0.5% methyl cellulose solution. Diazepam and diphenylhydantoin were also suspended in a methyl cellulose solution and administered via the i.p. route. Two hour response data were evaluated for significance using analysis of variance techniques (Guenther, 1964) and followed, if ANOVA was significant, by an examination of the individual treatment means using Duncan's Multiple Range Test (Brunning and Kintz, 1968). There were three rats in each group.

Figure 15a depicts a Mechner diagram (Mechner, 1959) which uniquely and clearly describes both the FR-40 and CES behavioral paradigms. Figure 15b shows the response pattern of a typical control rat responding under this combined schedule. CES responses are those made during the "tone-on" period. The tone-on periods are shown as downward excursions of the bottom tracing shown in this figure. The cumulative record is standard with upward excursion indicating responses and diagonal excursions indicating reinforcements.

Results

Table 17 gives the two hour response totals for the FR-40 responding part of the paradigm for DPSD. The actual two hour counts are expressed together with the means of these values calculated as a percentage of the control group totals. These normalized values are plotted in Figure 12 to show the dose response trend. In a similar

Table 17

Two Hour FR-40 Response Totals After Dosing Rats With DPSD

	Dose of DPSD (mg./kg. i.p.)#:				
	(control) 0	10	30	100	300
	10101+	8510	8668	7891	4261
	7594	12184	11330	7301	3236
	8782	10608	9768	7599	2706
Mean	8826	10434	9922	7597	3401
S.E.	714	1074	767	179	462
% Mean*	100	118	112	86	38
% S.E.*	8.1	12.2	8.7	2.1	5.2

Notes: #Subjects dosed 15 min. before test run.

+Total response count for an individual animal.

*Calculated values expressed as percent of control.

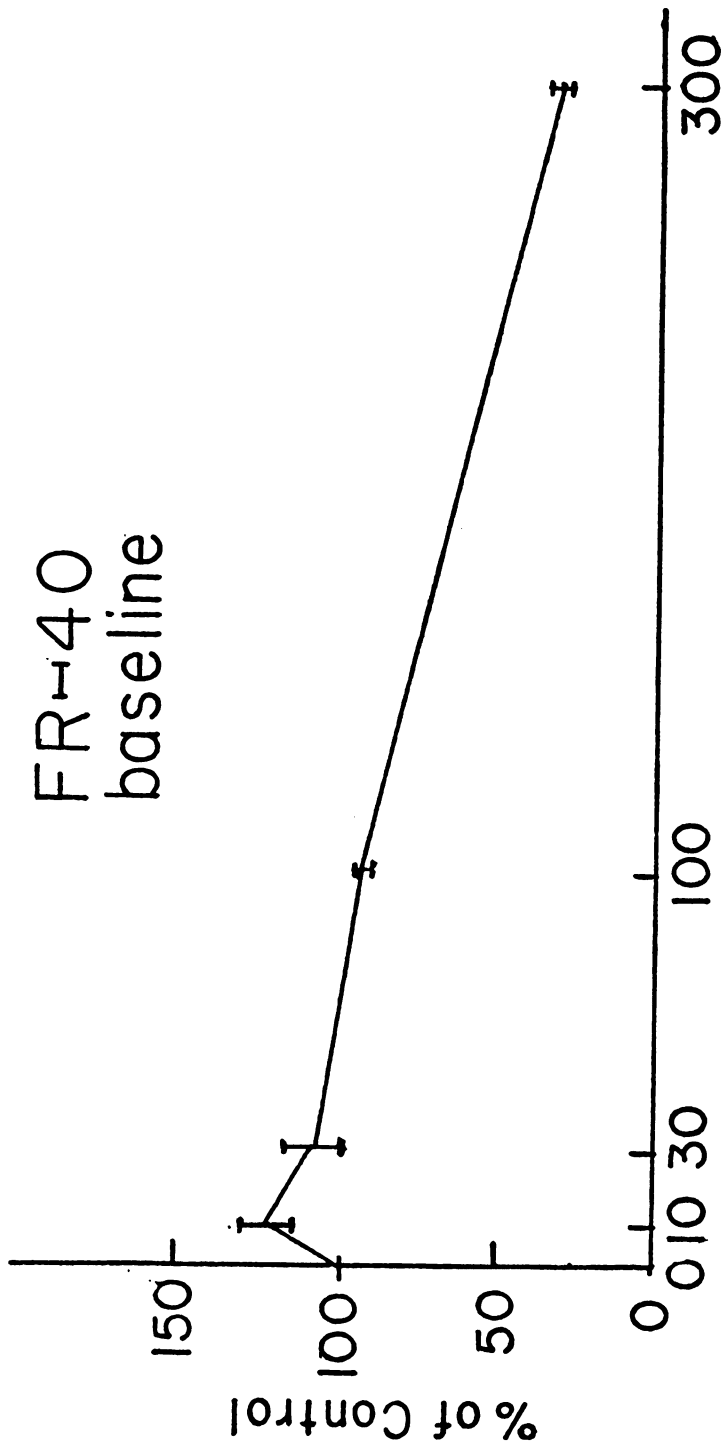


Figure 12. Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diphenylsilanediol.

manner the FR-40 results for diphenylhydantoin are shown in Table 18 and plotted in Figure 13. Also, the diazepam results appear in Table 19 and Figure 14.

For the DPSD data an F ratio of 15.8 was calculated, using a completely randomized design which was significant at the $p < 0.05$ level. Duncan's test revealed that only the high dose, 300 mg./kg., was significantly different. There appears to be a small stimulation in responding at the 10 mg./kg. dose level but it is not statistically significant. The values for 0, 10, 30, and 100 mg./kg. are statistically identical and represent no detectable behavioral impairment over this dose range. Discussion of the behavioral results for diphenylhydantoin and diazepam will be deferred to the next section, "Discussion of Behavioral Effects."

Table 20 summarizes the conditioned emotional suppression (CES) responses during a two-hour period after DPSD administration. The number of responses during the tone-on periods for the individual animals are presented, as well as these values normalized to the control group responding. An increase in CES responding is interpreted as an anti-anxiety effect according to the Estes-Skinner models (Estes and Skinner, 1941). The normalized means and standard error values for CES responses are plotted in Figure 16. Analysis of variance on these data produced an F value of 22.3 which was significant at the $p < 0.05$ level. Subsequent testing with Dunnett's t showed that all CES responses at greater than 10 mg./kg. of DPSD were significantly different from the control values with an alpha level of $p < 0.05$.

Table 18

Two Hour FR-40 Response Totals After Dosing Rats With Diphenylhydantoin (DPH).

	Dose of Diphenylhydantoin (mg./kg. i.p.)#:							
	0@	5	10	20	27	32	40	50
	6779+	7596	3804	8016	1903	1941	55	5
	3538	2303	7718	3800	1141	1410	1	115
	5838	5221	5539	5635	4608	4139	2	83
Mean	5392	5040	5687	5817	2550	2496	19	68
S.E.	967	1531	1132	1221	1051	835	17	32
% Mean*	100	93	105	108	47	46	0.35	1.26
% S.E.*	18	28	21	23	19	15	.31	.59

Notes: #Subjects dosed 15 min. before test run.

@Control group.

+Total response count for an individual rat.

*Calculated values expressed as percent of control.

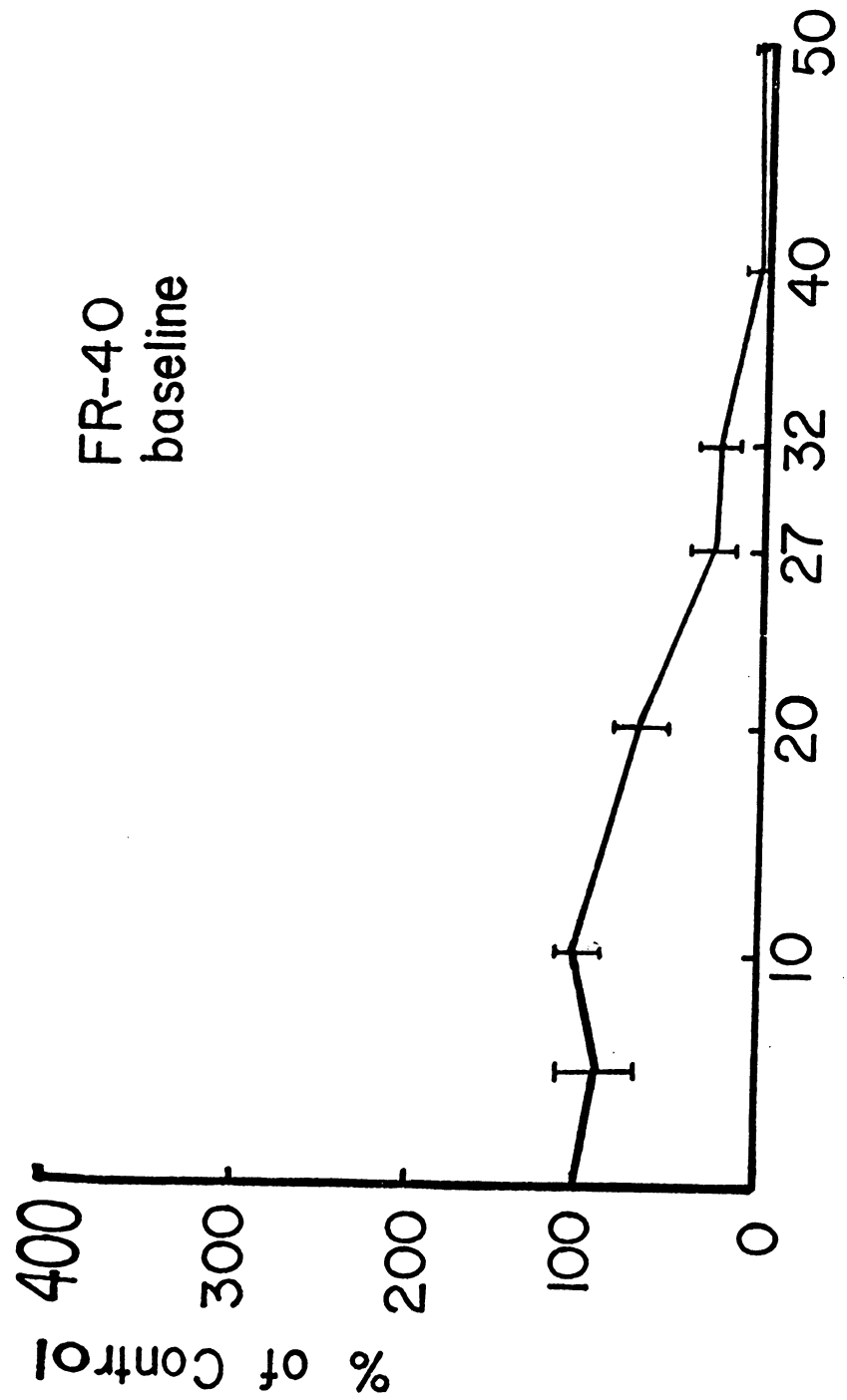


Figure 13. Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diphenylhydantoin (DPH).

Table 19

Two Hour FR-40 Response Totals After Dosing Rats With Diazepam.

	Dose of Diazepam (mg./kg. i.p.)#:					
	(control) 0	0.3	0.6	1.0	2.5	5.0
	2715+	6228	5686	6226	5481	1032
	3065	10007	8876	10917	6558	133
	8065	11634	10594	10095	7038	1220
Mean	4615	9289	8385	7079	6359	795
S.E.	1728	1501	1429	1446	460	338
% Mean*	100	201	182	197	138	17.2
% S.E.*	37	35	31	31	9.9	7.3

Notes: #Subjects dosed 15 min. before test run.

+Total response count for an individual rat.

*Calculated values expressed as a percent of control.

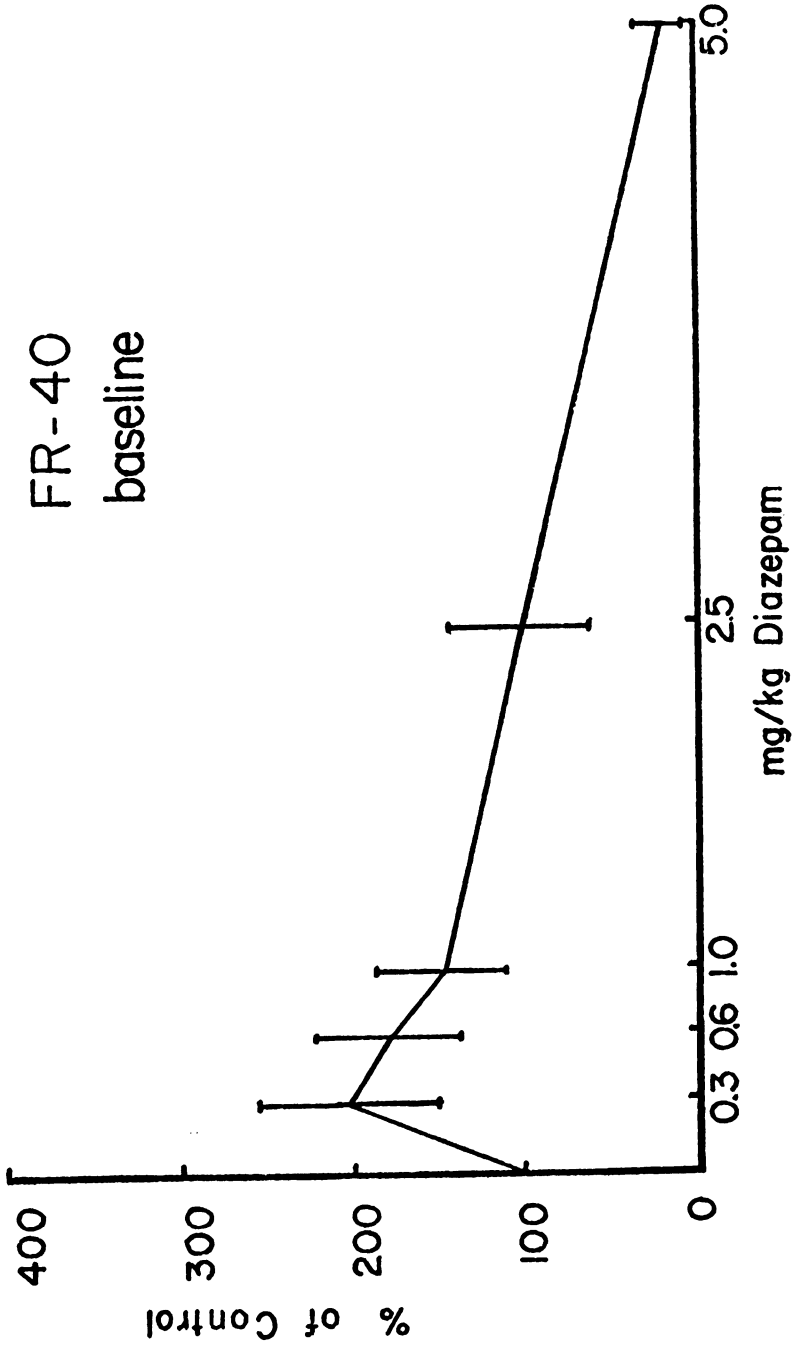


Figure 14. Dose Response on Two Hour Operant FR-40 Performance with Rats After Dosing with Diazepam.

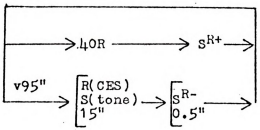


Figure 15a. Mechner Diagram for the Combined FR-40 and CES Behavior Paradigm Used as an Anxiety State Model.

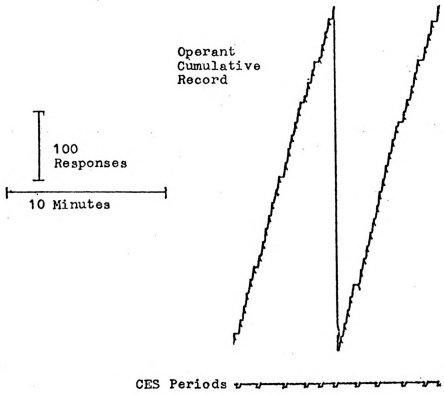


Figure 15b. Cumulative Record of a Rat Performing Under a Combined FR-40 and CES Paradigm for Behavior.

Table 20

Two Hour Conditioned Emotional Suppression (CES) Response Totals After DPSD Dosing.

	Dose of DPSD (mg./kg. i.p.)#:				
	(control) 0	10	30	100	300
	21+	106	394	785	231
	73	94	611	626	477
	19	173	539	921	364
Mean	37	124	498	767	357
S.E.	17.7	24.6	77.8	85.2	71.4
% Mean*	100	335	1346	2156	964
% S.E.*	47.8	66.6	210	230	193

Notes: #Subjects dosed 15 min. before test run.

+Total response count for an individual animal.

*Calculated values expressed as percent of control.

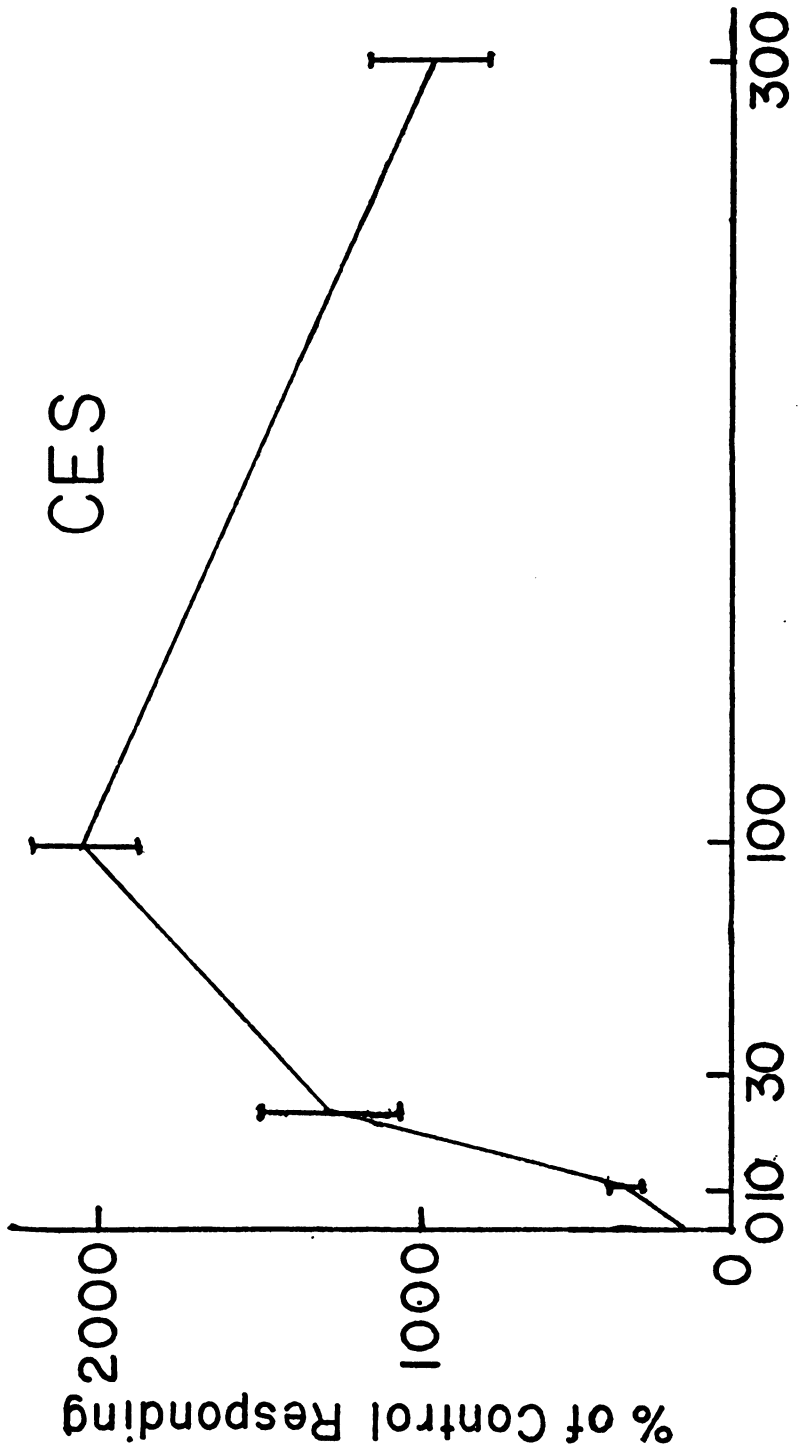


Figure 16. Responding During Conditioned Emotional Suppression (CES) Periods After Dosing with Diphenylsilanediol.

Thus at doses as low as 30 this compound is a very active anti-anxiety agent.

Conditioned emotional suppression (CES) results for diphenylhydantoin and diazepam are shown in Tables 21 and 22 and plotted in Figures 17 and 18, respectively. The discussion and comparison of these data are, also, deferred to "Discussion of Behavioral Effects" section below.

Discussion of Behavioral Effects

With the introduction of the rotating rod apparatus (Dunham and Miya, 1957), a simple and easily standardized device was made available to quantitate observations concerning the neurotoxicity of anticonvulsant and other central nervous system depressant drugs and compounds. The rota-rod data for DPSD produced an ED-50 value of 200 mg./kg. with the mouse and 170 mg./kg. with the rat. Literature values for phenobarbital and diphenylhydantoin (Raines et al., 1973) in the mouse are similar in magnitude. For phenobarbital an ED-50 of 126 mg./kg. is given and for diphenylhydantoin the value is 133 mg./kg. Table 23, part A, summarizes these results with their 95% confidence intervals. Mice data for diazepam yield an anticonvulsant ED-50 of 3.4 mg./kg. and a rotating rod depression ED-50 of 3.9 mg./kg., giving a therapeutic index of only 1.1 for this compound. The sedative-ataxic effects of diazepam are definitely overlapping with its anticonvulsive effects. This is observed in clinical practice too (Hollister, 1973) where diazepam finds use in treating status epilepticus but only at

Table 21

Two Hour Conditioned Emotional Suppression (CES)

Response Totals After Diphenylhydantoin Dosing.

	Dose of Diphenylhydantoin (mg./kg. i.p.)#:							
	0@	5	10	20	27	32	40	50
	19+	5	1	10	6	2	1	0
	11	1	28	12	6	8	0	16
	37	5	2	7	1	9	0	3
Mean	22.3	3.7	10.3	9.6	4.3	6.3	0.3	6.3
S.E.	7.7	1.3	8.8	1.4	1.7	2.2	0.3	4.9
% Mean*	100	16	46	43	19	28	1	28
% S.E.*	34	6	39	6	8	10	1	21

Notes: #Subjects dosed 15 min. before test run.

@Control group.

+Total response count for an individual rat.

*Calculated values expressed as percent of control.

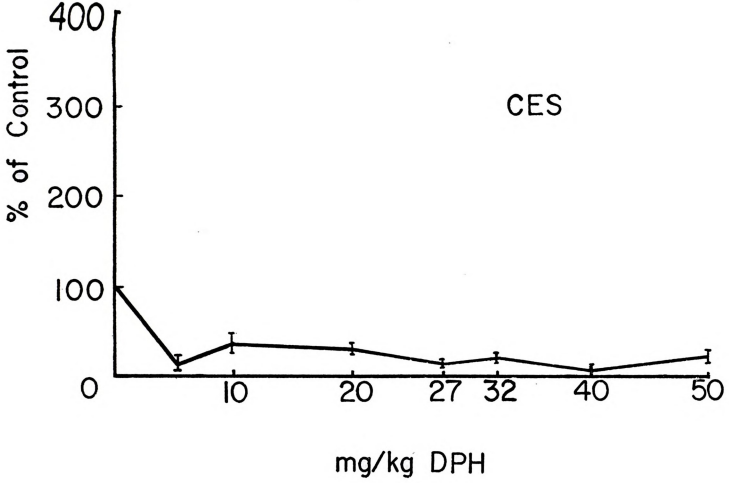


Figure 17. Responding During Conditioned Emotional Suppression Periods (CES) After Dosing with Diphenylhydantoin (DPH).

Table 22

Two Hour Conditioned Emotional Suppression (CES)

Response Totals After Diazepam Dosing.

Dose of Diazepam (mg./kg. i.p.)#:

	(control) 0	0.3	0.6	1.0	2.5	5.0
	5+	4	21	8	49	15
	2	7	5	43	46	13
	6	13	8	59	19	7
Mean	4.3	8	11	36.7	38	11.6
S.E.	1.2	2.6	4.8	15.1	9.5	2.4
% Mean*	100	185	261	847	877	267
% S.E.*	28	61	113	349	220	55.4

Notes: #Subjects dosed 15 min. before test run.

+Total response count for an individual rat.

*Calculated values expressed as a percent of control.

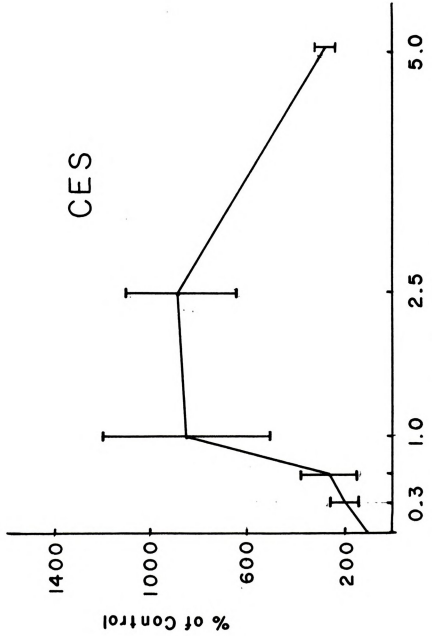


Figure 18. Responding During Conditioned Emotional Suppression (CES) Periods After Dosing with Diazepam.

Table 23

Results and Comparisons of Behavioral Effects

A. Rotating rod data from mice after i.p. administration.

Compound	Rotating Rod ED-50 and 95% C.I.	
DPSD	200 mg./kg.	(72-328)
Phenobarbital	126 mg./kg.	(78-201.6)
DPH	133 mg./kg.	(81.5-216.8)
Diazepam	3.9 mg./kg.	(2.3-5.8)

B. Operant performance on FR-40 with rats.

Compound	Dose for Performance	
	Increase	Decrease
DPSD	10 mg./kg.	300 mg./kg.
DPH	not seen	40 mg./kg.
Diazepam	0.3 mg./kg.	5.0 mg./kg.

C. Conditioned emotional suppression (CES) results

Compound	Antianxiety Responses		
	Initial Dose	Peak Dose	Peak Amplitude
DPSD	10 mg./kg.	100 mg./kg.	2156% of control
DPH	none	none	none
Diazepam	0.3 mg./kg.	2.5 mg./kg.	877% of control

doses that produce drowsiness and ataxia in human patients. DPSD appears to have the neurotoxic dose range well-isolated from the anticonvulsive range, making its therapeutic index more akin to diphenylhydantoin than to diazepam or phenobarbital (i.e., a therapeutic index of about 10).

For discussion of the behavioral characterization results using operant techniques it will be necessary to more closely examine the figures in the results portion of this section above. The comparisons to be made are summarized in Table 23, part B.

Baseline performance on FR-40 reinforcement paradigms is presented as a dose-response curve for DPSD in Figure 12, diphenylhydantoin in Figure 13, and diazepam in Figure 14. The overall shapes of these three curves are similar. The diazepam and DPSD curves each show an initial response increase before the decrease. The doses at which these increases and decreases occur are summarized in Table 23, part B. DPSD showed an increase in responding at 10 mg./kg., while a decrease became statistically significant ($p < 0.05$ level) at 300 mg./kg. Corresponding data for the diphenylhydantoin showed no increase and a decrease at 40 mg./kg. For diazepam the increase occurred at 0.3 mg./kg. and the decrease at 5.0 mg./kg. The overall similarity of DPSD to diazepam is apparent from these figures. Of more interest is the conditioned emotional suppression data.

Results on the conditioned emotional suppression studies for DPSD, diphenylhydantoin, and diazepam are shown in Figures 16, 17, and 18, respectively. Numerical comparisons are listed in Figure 23, part

C. An initial comparison of the shapes of these three figures reveals the similarities between the diazepam and DPSD dose-response data and the dissimilarity in these two with respect to the diphenylhydantoin data. The diphenylhydantoin data, Table 21 and Figure 17, show no conditioned emotional suppression rate increase, thus indicating no anti-anxiety properties for this compound.

Both DPSD and diazepam serve to facilitate the performance of rats under the conditions of experimental anxiety, making their classification as anti-anxiety compounds clear.

The magnitude of this effect can be seen through a comparison between the initial (threshold) increase of operant responding and the maximal (peak) observed increase in operant responding. These data, summarized in Table 23, have been extracted from Tables 20 and 22. For diazepam the first increase is observed at 0.3 mg./kg., reaching a peak at 2.5 mg./kg. with an increase to 877% above the control level. For DPSD, the first increase is at 10 mg./kg., the peak occurring at 100 mg./kg., having increased to 2,156% above the control level. For both of these compounds, the portion of the dose-response curves up to the peak responding on the conditioned emotional suppression paradigm shows no overlap with the baseline FR-40 depressant effects shown in Figures 12 and 14.

In conclusion, the behavioral evaluation of DPSD reveals no significant neurotoxicity or behavioral impairment in the anticonvulsant dose range of 10 to 100 mg./kg. on intraperitoneal administration. There is an additional pharmacological activity that occurs in the

anticonvulsant dose range. This anti-anxiety effect is not to be considered as a neurotoxic or behavioral impairment. Both the rotating rod apparatus and the operant FR-40 response rate measures show varying levels of impairment at doses above 100 mg./kg. These results tend to indicate that DPSD has behavioral properties that make it appear more like diazepam than other anticonvulsant drug types. An anticonvulsant action is obtained in DPSD with an improved therapeutic index and an associated anti-anxiety response. These statements are, of course, based on comparisons with the few anticonvulsant compounds for which there are behavioral data (Millenson and Leslie, 1974; Raines et al., Swinyard et al., 1952; Zbinden and Randall, 1967). Further examination of these comparisons will be made in the general discussion section at the end of this thesis.

TOXICOLOGICAL STUDIES

General Remarks

The assessment of potential toxicity for a chemical compound having pharmacological activity provides both an indication of the safety, or potential risk factors, associated with its use and perhaps some insight into the mechanism of action of the compound as a therapeutic agent. There are standardized procedures for the assessment of toxicity in the initial stages (Abrams et al., 1964; Nelson, 1965). An attempt to identify these basic data is reported in this section. Only simple observations on acute toxicity and the observations on a chronic (90 day) feeding study are reported below.

Acute Toxicity

The primary objective here is to assess the LD-50, or lethal dose, fiftieth percentile, and to make some observations on the relative toxicity of DPSD when it is compared with other anticonvulsants.

Methods

Groups of six Swiss Webster mice (Spartan Laboratory Animals, Haslett, Michigan) were treated via i.p. injection with 0.5% methyl cellulose suspensions of DPSD. Animals were treated with freshly prepared solutions (injection within 30 minutes after preparation).

With each dose and each group of six mice the number surviving after eight hours was noted. A particular dose-treatment group was scored as the number dead per number injected. As a variation on this procedure, groups of six mice received DPSD in their diet for a six day period to assess a basic lethality on very short term administration. Here the compound was mixed with ground rat food (Rat Chow, Ralston Purina Co., St. Louis, Missouri) and a small amount of chocolate flavoring added (Carnation Hot Cocoa Mix, Carnation Food Co., Kansas City, Missouri) to facilitate its consumption by the mice (pilot observations showed that the mice would not eat the higher dose mixtures unless a flavoring agent was added).

Results

Table 24 shows the results from an acute i.p. administration of DPSD to mice. The LD-50 was calculated to be 2,599 mg./kg. using the method of Finney (1952). The slope of this curve is evidenced in the values for the LD-10 at 1,068 mg./kg. and the LD-90 at 6,319 mg./kg. which suggest a wide range of values for the dose of DPSD which produce lethal effects. Animals that died did so without any signs of a stimulation phase; only general depression of central nervous system activity was observed.

Table 25 shows values of the food administration levels of DPSD and the observed deaths over six day period with mice on these diets. The far right column lists the last day in the six day observation period in which a mouse was found dead. A calculated LD-50 of 32.4 mg. DPSD/gm. of food was found using Finney's probit analysis

Table 24

Lethal Effects Of DPSD (i.p., Mice)

Dose (mg./kg. i.p.)	Number Dosed	Number Dead#	Percent Dead@
Vehicle* Alone	6	0	0.0
500	6	0	0.0
1,000	6	1	16.7
1,500	6	1	16.7
3,000	6	3	50.0
5,000	6	5	83.3
10,000	6	6	100.0

Notes: *Methyl cellulose 0.5% in saline.

#Dead animals counted 8 hours post dose.

@Calculated lethal dose values (mg./kg.):

LD Value	Lower 95% CI	Upper 95% CI
LD-10 = 1068	351	1657
LD-50 = 2599	1681	4167
LD-90 = 6319	3992	21139

Table 25

Lethal Effects Of DPSD Administered Subacutely In Food

Food Concentration@	Daily Dose#	Number Dosed	Number Dead*	Day+
6.4	1067	6	0	-
12.8	2133	6	0	-
25.6	4267	6	2	5
51.2	8533	6	5	3
102.4	17067	6	6	2

Notes: @Food formulation: x.x mg. DPSD/gm. food
50 mg. Carnation Hot Cocoa Mix
To make 1 gm. Purina Rat Chow.

#Calculated dose (mg./kg.) based on 166 mg. food/gm. body weight daily food consumption.

* Total number dead animals during 5 day dosing period.
Calculated LD-50: 32.4 mg./gm. food

+Day post-dose that last animal died.

techniques. This corresponds to an ingested dose of 5,410 mg./kg./day based on an estimated food consumption of 16.7% of body weight (Bell, 1962).

Chronic Toxicity Studies

As potential drugs to control epileptic seizures are generally administered on a chronic basis, the importance of evaluating the toxicity associated with longer term administration of a drug is apparent. The toxicological responses for diphenylhydantoin, primidone, phenobarbital, and diazepam (Woodbury et al., 1972) are well known. Standardization procedures (Hayes, 1972) recommend that a chronic dosing schedule continue for at least 10% of the animal's lifetime. Drug administration for a three month period in mice meet these criteria and objectives.

Methods

Subjects were Swiss Webster mice from Spartan Laboratory Animals, Haslett, Michigan. DPSD was administered as a mixture with pulverized rat chow (Ralston Purina Co., St. Louis, Missouri) at 1.6 and 6.4 mg./gm. of food. These doses were selected to represent an acute ED-90 for anticonvulsant action (the 1.6 mg./gm. food), and an ED-50 for rota-rod depression (the 6.4 mg./gm. food). This food was available on an ad libitum basis and was replenished once a day. During the dosing period the subjects were periodically examined for signs of gross toxicity in terms of appearance, mobility, and food and water consumption. During the last day of the drug administration

the subjects were weighed, euthanized by cervical dislocation, peripheral blood smears obtained after cutting off the tip of the tail, autopsied according to standardized techniques, and histological specimens prepared. At autopsy gross observations were made and organ weights determined. Organ weights on brain, liver, kidney, spleen, and adrenals were determined after blotting the tissue on a piece of filter paper to remove excess moisture. Organ samples were taken from the absorptive organs (stomach, small intestine, large intestine), the metabolic organs (liver, kidney, lung), and other organs including blood, bone marrow, spleen, lymph nodes, adrenal glands, gonads, cerebrum, and cerebellum. These were fixed in formalin except for the brain tissues which were fixed in buffered glutaraldehyde. Staining was with hematoxolin and eosin according to standard histological procedures (Humason, 1966). Blood and bone marrow smears were stained with Wright's stain. Table 26 summarizes the experimental design for this 90 day toxicity study.

Results

Gross and microscopic. The high dose group receiving 6.4 mg./kg. food was examined thoroughly in comparison with the control group not receiving DPSD in their diets. Table 27 lists the organ weights of selected organs for both treated and control groups. Only the adrenal and spleen weights were statistically different between these two groups. The smaller size of these organs was not a very great difference but was observable. As listed in Table 28 these differences in weight did not reflect any histological differences.

Table 26

Design For Chronic Toxicity Study In Mice

Compound: Diphenylsilanediol (DPSD)

Subject: Adult Mice, 20-30 gram body weights, 12 per group (6 male, 6 female), 3 groups (36 mice).

Doses and Dosing Schedule:

Compound in food (ad lib.); food replenished daily; food batches prepared weekly

Three Doses: Control Diet (no compound)
MES ED-90 Diet (1.6 mg./gm. food)
Rota-Rod ED-50 Diet (6.4 mg./gm. food)

Duration of Dosing: Three months (90 days)

Planned Observations:

During Dosing -

Body weight, general health, deaths

At End of Dosing Period -

Whole body and specific organ weights gross and microscopic examination of:

a. Absorptive organs: stomach, small intestine, large intestine.

b. Metabolic organs: liver, kidney, lung.

c. Hematopoietic organs: blood, bone marrow, spleen, lymph nodes.

d. Endocrine and reproductive organs: adrenal, gonads.

e. Brain: cerebrum, cerebellum

f. Electron microscopic examination of selected organs.

Table 27

Organ And Body Weight Changes After Chronic Dosing

With DPSD For Three Months

Body and Organ Weights in Grams

Treatment	Subject	Body	Liver	Kidney	Adrenal	Spleen	Brain
Control Diet	CC-1	35	1.810	0.435	0.020	0.220	0.479
	CC-2	39	1.572	0.500	0.057	0.135	0.519
	CC-3	34	1.545	0.448	0.021	0.215	-----
	CC-4	33	1.504	0.459	0.020	0.125	0.436
	CC-5	31	1.920	0.376	0.018	0.241	0.472
	CC-6	32	1.466	0.460	0.028	0.137	0.461
Mean Values		34	1.619	0.446	0.022	0.179	0.472
Std. Error		1.1	.079	.016	.003	.021	.013
Treatment treated diet 6.4 mg./gm. food#	TH-7	31	1.684	0.435	0.014	0.116	0.470
	TH-8	33	1.887	0.449	0.019	0.132	0.492
	TH-9	30	1.482	0.408	0.016	0.081	0.415
	TH-0	30	1.578	0.426	0.010	0.170	0.486
	TH-1	31	1.493	0.443	0.018	0.077	0.483
	TH-2	32	1.541	0.411	0.023	0.112	0.396
Mean Values		31*	1.611	0.429	0.017*	0.115*	0.457
Std. Error		1.2	.063	.007	.002	.014	.017

Notes: # - Average drug consumption 1067 mg./kg./day.

* - Significant difference from control ($p < 0.05$ level).

Table 28

Summary Of Gross And Microscopic Observations On

Organs From Mice Dosed With DPSD (6.4 mg./gm. food)

For 90 Days

Organ or Tissue	Gross	Microscopic
Absorptive Organs:		
Stomach	Normal	Normal
Duodenum	Normal	Normal
Jejunum	Normal	Normal
Ileum	Normal	Enlarged Lymphatics
Cecum	Normal	Normal
Colon	Normal	Normal
Metabolic Organs:		
Liver	Gall Bladder Large	Light: Congestion E.M.: Smooth ER. mild proliferation
Kidney	Normal	mild Congestion
Lung	Normal	Normal
Hematopoietic:		
Blood	Normal	Eosinophilia Reactive Lymphs.
Bone Marrow	Normal	Normal Ratios
Spleen	Slightly Smaller	Normal
Lymph Nodes	Normal	Normal
Endocrine and Reproductive:		
Adrenal	Slightly Smaller	Normal
Gonads Male	Normal	Normal
Gonads Female	Normal	Normal
Other Tissues:		
Visceral Fat	Normal	Normal
Skeletal Muscle	Normal	Normal
Cardiac Muscle	Normal	Normal
Brain	Normal	Normal

Figure 19 shows the spleen from a representative animal in the high dose DPSD group. There is no apparent histological change to account for the smaller mass. Figure 20 in a similar manner shows the adrenal gland for this treated animal. The kidney weights were normal, Table 27, and there was no major gross or microscopic (Figure 21) abnormalities observed except for a mild congestion. Bone marrow smears appeared normal with a balance of red and white cells in blast, immature, and mature stages as shown in Figure 22. The brains were examined closely with particular attention to the cerebellum area. Del Cerro et al. (1967) demonstrated that diphenylhydantoin had a disorganizing effect on the cerebellum caused by a depletion of the cerebellar Purkinje cells. Figure 23 shows a low magnification section of the cerebellum. The boxed-in region is enlarged in Figure 24 showing the Purkinje cells clearly. There is no apparent abnormality in either of these slides.

The only readily apparent abnormality observed on gross examination of the mouse was the enlarged gall bladders seen in 30% of the animals treated at the high dose. The enlargements appeared to be due to increased bile storage. Two of these gall bladders appeared hemorrhagic. The livers in these animals all appeared to be congested mildly and to have some cloudy appearance in their cellular structures.

Figure 25 shows a liver section representative of the tissues observed in the control group. By comparison, the treated group slides, Figure 26, showed enlarged sinusoids and swollen cloudy cells.

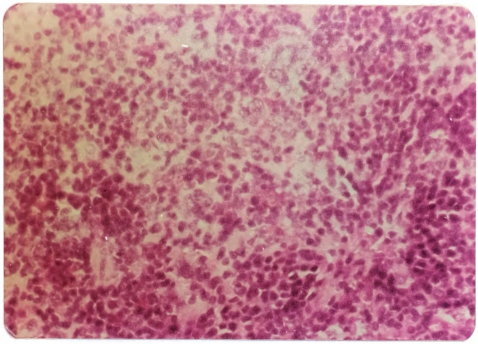


Figure 19. Section of Spleen from a Mouse Treated with DPSD for 90 Days. (Original Magnification 200 X).

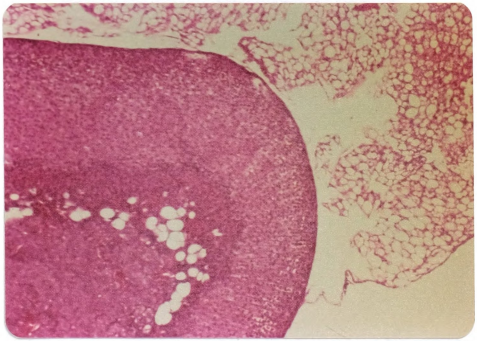


Figure 20. Section of the Adrenal Gland from a Mouse Treated with DPSD for 90 Days. (Original Magnification 40 X).

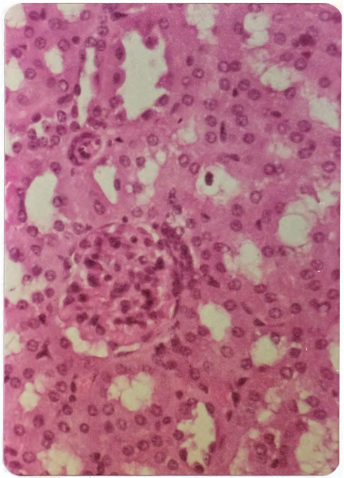


Figure 21. Section from Kidney of a Mouse Treated with DPSD for 90 Days. (Original Magnification 200 X).

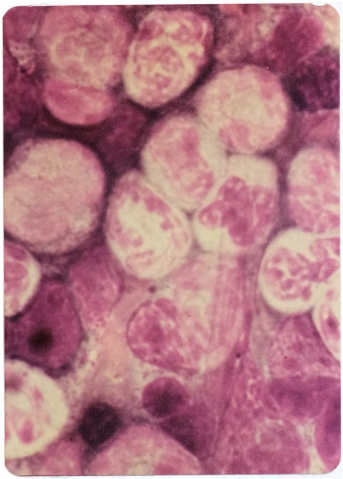


Figure 22. Bone Marrow Smear from the Femur of a Mouse Treated with DPSD for 90 Days. (Original Magnification 1000 X).

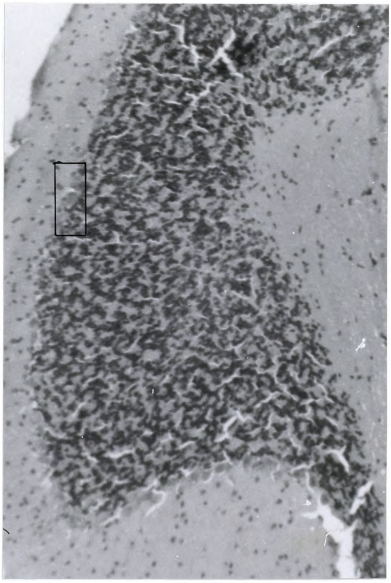


Figure 23. Section from Cerebellum of a Mouse Treated with DPSD for 90 Days. (Original Magnification 160 X).

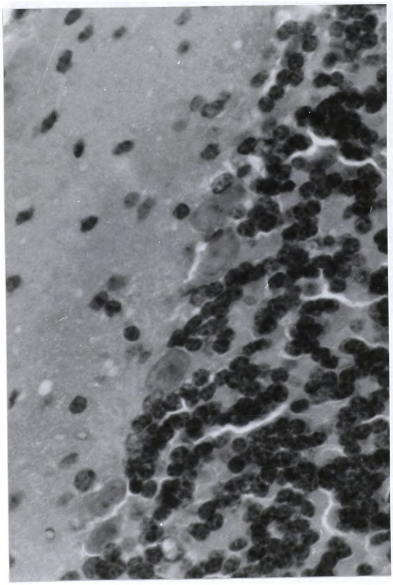


Figure 24 Section from Cerebellum of a Mouse Treated with DPSD for 90 Days.(Original Magnification 1000 X).

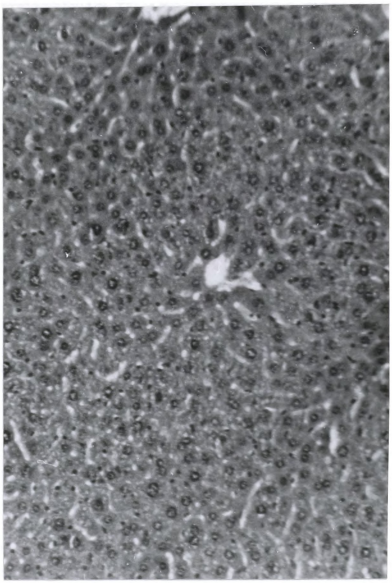


Figure 25. Section of Liver from a Mouse on the Control Diet for 90 Days.(Original Magnification 200 X).

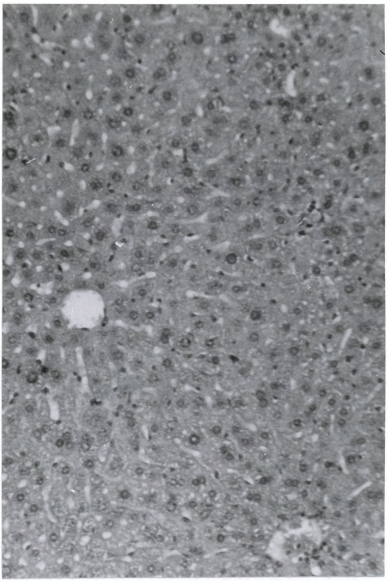


Figure 26. Section of Liver from a Mouse Treated with DPSD for 90 Days.(Original Magnification 200 X).

Hematological observations. The other major histological abnormalities observed, listed in Table 28, were in the blood. Table 29 lists the white blood cell differential counts for the control, low dose DPSD, and high dose DPSD treated mice. The reactive (appearing immunologically activated) lymphocytes are listed as a percentage of the lymphocytes for comparison purposes. These differential counts are summarized in Table 30. Representative blood cells from the high dose treated animals are shown in Figures 27, 28, and 29.

Two parameters increased in the treated groups. There is an increase in the percentage of reactive lymphocytes. These differences are statistically significant also (ANOVA and Dunnett's t-test). They both appear to be dose dependent in that the eosinophil count increases from 3.6% in the control group, to 4.8% in the low dose treated group, and 8.5% in the high dose treated group. Similarly, the reactive lymphocyte levels increase from 0.5% to 2.8% to 3.7%.

In the high dose treated group, some of the neutrophils contained cytoplasmic inclusion bodies (Doehle Body) as shown in Figure 28 and none were seen in the control group. A typical reactive lymphocyte is shown in Figure 29. This can be compared with a more normal appearing lymphocyte in Figure 27.

Table 29

Peripheral Leukocyte Changes After Chronic Dosing

With DPSD For Three Months

Treatment mg. DPSD/gm. Food	Subject Ident.	Leukocyte Differential Counts (Percent): Lymph.	Mono.	Neut.	Eos.	React. Lymph.
0	CC-1	70	2	20	8	1
0	CC-2	77	4	17	2	0
0	CC-3	83	3	10	4	0
0	CC-4	77	4	16	3	1
0	CC-5	75	10	13	2	1
0	CC-6	61	8	28	3	0
1.6	TL-1	75	1	17	7	5
1.6	TL-2	79	2	16	2	2
1.6	TL-3	82	0	16	3	4
1.6	TL-4	75	0	21	4	1
1.6	TL-5	89	0	6	5	1
1.6	TL-6	81	0	11	8	4
6.4	TH-7	69	3	18	10	7
6.4	TH-8	80	3	7	10	1
6.4	TH-9	79	2	12	7	3
6.4	TH-0	75	5	15	5	4
6.4	TH-1	78	2	14	6	3
6.4	TH-2	61	6	19	13	4

Table 30

Dose Dependent Leukocyte Changes After Dosing

With DPSD For Three Months

Food Level:	0 mg./g. food	1.6 mg./g. food	6.4 mg./g. food
Dose Level:	0 mg./kg./day	267 mg./kg./day	1067 mg./kg./day
Cell Types:			
Lymphocyte	73.8 S.E.3.1	80.2 S.E.2.1	73.6 S.E.3.0
Monocyte	5.1 S.E.1.3	0.5 S.E.0.3	3.5 S.E.0.7
Neutrophil	17.3 S.E.2.5	14.5 S.E.2.1	14.3 S.E.1.9
Eosinophil	3.6 S.E.0.9	4.8 S.E.0.9	8.5 S.E.1.2
Reactive Lymphocytes	0.5 S.E.0.2	2.8 S.E.0.7	3.7 S.E.0.8

Mean Values and Standard Errors On Samples Of Size n = 6

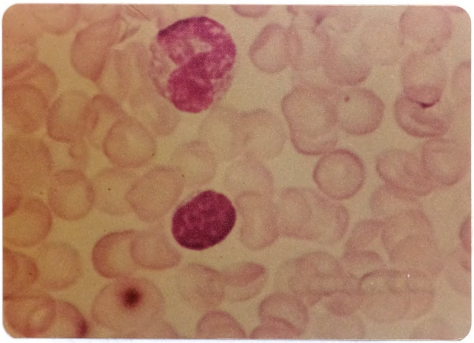


Figure 27. Eosinophil and Lymphocyte from Peripheral Blood of Mouse Treated with DPSD for 90 Days. (Original Magnification 1000 X).

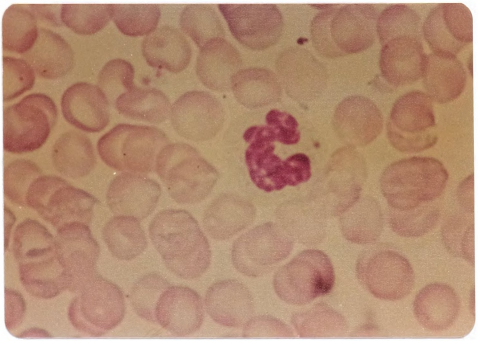


Figure 28. Toxic Neutrophil (with Doehle Body) from Blood of Mouse Treated with DPSD for 90 Days. (Original Magnification 1000 X).

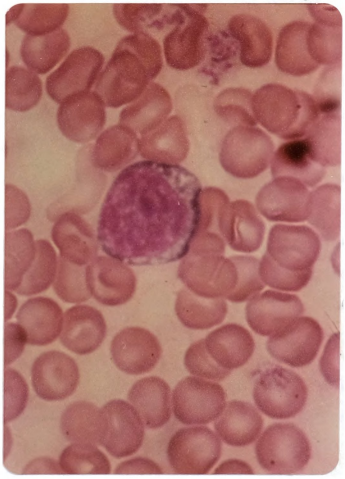


Figure 29. Reactive Lymphocyte from Peripheral Blood of Mouse Treated with DPSD for 90 Days. (Original Magnification 1000 X).

Supplementary Toxicity Studies

Electron Microscopic Examination

Observed changes in the livers of mice treated with DPSD for three months were not well-defined after light microscopy examination of liver tissue taken from these same mice at autopsy. Swollen cloudy cells can be ultrastructurally characterized further as having enlarged mitochondria, being vacuolated, having a disorganized rough endoplasmic reticulum, possessing fat droplets, having a proliferated smooth endoplasmic reticulum, or some combination of these primary changes (Sandwritter and Wartman, 1973). The clarification of these potentially toxic changes is attempted below.

Methods. Liver specimens were obtained from animals treated as discussed above and outlined in Table 26. Tissues were prepared using standard electron microscopic preparation techniques. Immediately after removal, small specimens were minced in a buffered 6% glutaraldehyde solution for primary fixation, washed with the phosphate buffer, post-fixed with a 1% osmium tetroxide solution, and dehydrated with increasing concentrations of ethyl alcohol. After dehydration, tissues were embedded in an epoxy resin (Epon 812) after clearing the propylene oxide mixed with increasing concentrations of the epoxy resin. After trimming the epoxy resin-embedded tissues, they were sectioned with a Porter-Blum MT-2 ultramicrotome using freshly prepared glass knives. These sections were transferred to carbon-collodion coated copper electron microscopy grids, stained with lead citrate and uranyl acetate,

and examined using a Hitachi HS-7S electron microscope. Three liver specimens from each of six treated and six control mice were examined. Only the high dose (6.4 mg./gm. food) treated animals were examined in the treated group. Electron micrographs were taken of representative liver areas in both the treated and control groups for further comparisons.

Results. A careful examination of both treated and control liver sections under the electron microscope revealed that there was only one major, consistent, and recurring difference between tissues in these two groups. The smooth endoplasmic reticulum was found to be more abundant in the cells of liver obtained from treated animals. Figures 30 and 31 compare electron micrographs of the control and treated liver sections. In Figure 31, note the abundance of smooth endoplasmic reticulum. Even close to the nucleus, where smooth E.R. rarely occurs normally, the smooth endoplasmic reticulum is clearly seen.

In Vivo Liver Function Tests

With the observation, discussed in the previous section, that chronic administration of high doses of DPSD tends to produce liver congestion and maybe some gall bladder enlargement, an examination of liver function was considered appropriate. Of the available test methods and procedures (Hoe, 1968) commonly used, the bromsulphalein (BSP) excretion method was chosen as it is both relatively easy to perform and has been shown to correlate with histopathological

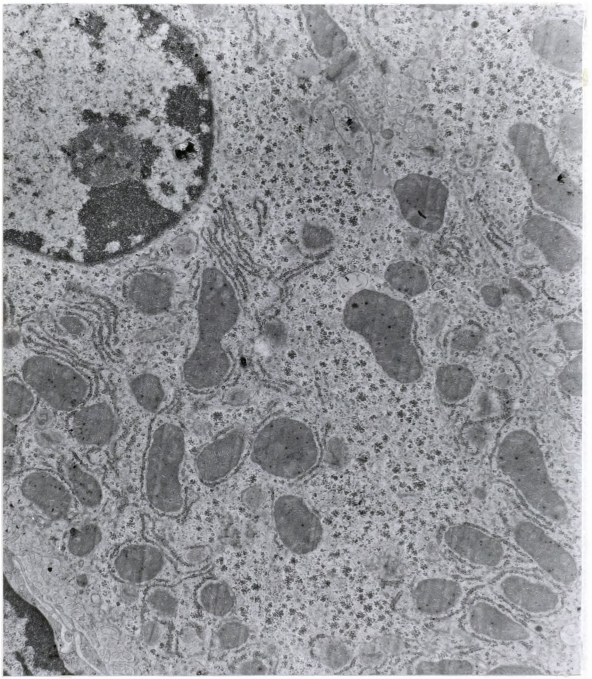


Figure 30. Electron Micrograph of Liver from Control Mouse - Control Diet for three Months. (13,800 X).

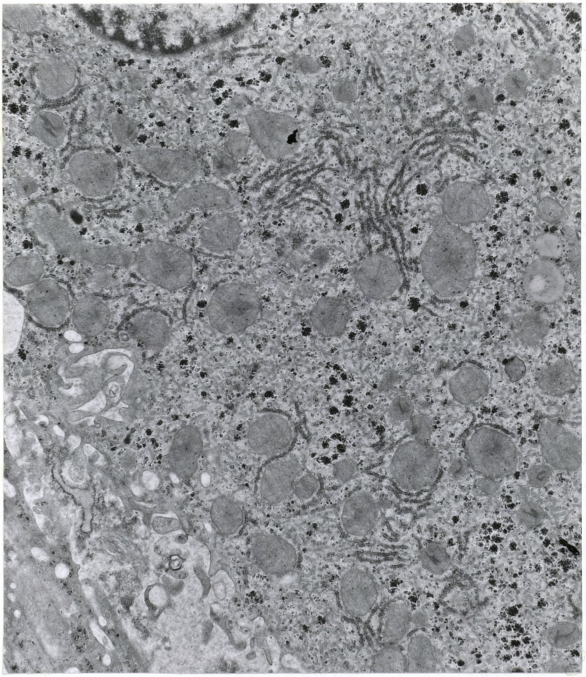


Figure 31. Electron Micrograph of Liver from Mouse after Treatment with DPSD for three Months. (13,800 X).

examination of the liver (Kutob and Plaa, 1962). The procedure involves the administration of this dye to a test animal and determining the time course of its elimination. As this compound is almost totally excreted via the liver into the bile (Hoe, 1968), its elimination at a control rate indicates some competency of the hepatobiliary system.

Methods. The treated subjects were six mice which had received 6.4 mg. DPSD/gm. food as their diet. The fifteen control mice had been on the same diet without the DPSD. The dye, phenoltetrabromophthalein (Bromsulphalein BSP, Hynson, Westcott and Dunning, Inc., Baltimore, Maryland), is supplied as a sodium sulphonate salt and was dissolved in physiological saline for intravenous administration (via the lateral tail vein in the mouse). A standard dose of 100 mg./kg. BSP was administered i.v. and blood samples drawn from groups of mice by cardiac puncture with the animals lightly anesthetized with diethyl ether. Blood samples were collected into heparinized test tubes and then spun on a laboratory centrifuge (10 min. at 2,000 rpm) to provide plasma samples. Analysis of BSP content was done by measuring the 580 nm. absorptance of alkalinized plasma samples on a Beckman DB-GT Grating Spectrophotometer in quartz cuvettes. A calibration curve was prepared based on 0.1 ml. of plasma alkalinized with 0.5 ml. of 0.1N NaOH and then diluted to 2 ml. with physiological saline. A plasma sample from an untreated mouse was used as a blank in the spectrophotometer and received the same regents. First, a calibration curve was constructed that permitted the calculation of BSP levels in mg./100 plasma. Next the control mice were treated with BSP and, using groups of three mice

each, an elimination curve for BSP from plasma was plotted. From this curve a decision was made to test the plasma content of BSP in the DPSD treated mice after 30 minutes post-administration of the BSP. These results would then be compared with the control group data at 30 minutes using a t-statistic at an alpha level of 0.05 probability.

Results. Table 31 shows the absorptance data for the calibration samples of BSP in plasma. These data are plotted in Figure 32. A regression equation was calculated as BSP (mg./100 ml.) = 32.45 O.D. - 1.249 to be used in calculating BSP levels for other samples.

In Table 32, Part B, the optical density (O.D.) readings for the plasma samples for the 15 control mice are presented. Blood was drawn from three mice at each time period of 5, 10, 15, 20, and 30 minutes post dose. The actual concentration of BSP was calculated via the equation given above. The calculated values, means, and standard errors are shown in Table 32, Part B; these are also tabulated in Figure 33. In this figure note that the BSP concentration is expressed on a logarithmic scale against a linear time scale. The logarithmic data points fit a straight line which becomes reasonable to fit to a linear regression model. The logarithmic numbers are found on Table 2 together with a linear regression equation calculated from these data. This equation is log (BSP) = -0.0465 (Time) + 1.7734.

Using the above time course as a guide, six mice that had received 6.4 mg. DPSD/gm. food (or 1,267 mg./gm. food) were injected with BSP and their blood examined for BSP levels as a function of time.

Table 31

Plasma Level of BSP Calibration@ Data

BSP* mg./100 ml. Plasma	Absorptance# (O.D.)
10	0.341
20	0.663
30	0.966
40	1.270
50	1.570
60	1.893

Notes: *BSP = phenoltetrabromophthalein sodium sulphonate.

#Plasma samples are alkalinized (0.1 ml. plasma, 0.5 ml. of 0.1N NaOH, diluted to 2 ml. with physiological saline) and the absorptance at 580 nm. measured on a Beckman DB-GT Grating Spectrophotometer.

@Calculated calibration:
BSP (mg./100 ml.) = 32.45 X O.D. = 1.249.

Table 32

Plasma Levels of BSP in Control Mice - Time Course

A. Concentration Calculated from O.D. Values

	Time After BSP* (Minutes)				
	5	10	15	20	30
Data Values	43.3	19.1	10.3	1.8	4.0
Values	47.0	27.6	12.4	17.0	2.4
mg. BSP per 100 ml./plasma	33.3	16.6	10.4	4.5	2.7
Mean	41.2	21.1	11.0	7.8	0.5
S.E. of Mean	4.1	3.3	0.7	4.7	0.5

B. Optical Density Measurements (O.D.) at 580 nm.

	Time After BSP* (Minutes)				
	5	10	15	20	30
Individual	1.37	0.63	0.35	0.09	0.16
O.D.	1.48	0.89	0.42	0.56	0.11
Values	1.06	0.55	0.36	0.18	0.12

C. Calculated Log (BSP) Values for Time Calculations

Time After BSP* (Minutes)				
5	10	15	20	30
1.637	1.281	1.013	0.255	0.602
1.762	1.441	1.093	1.230	0.380
1.522	1.220	1.017	0.653	0.431

These numbers provide the equation

$$\log(BSP) = -0.0465 \times (Time) + 1.773$$

Note: *100 mg./kg. BSP given i.v. at time zero.

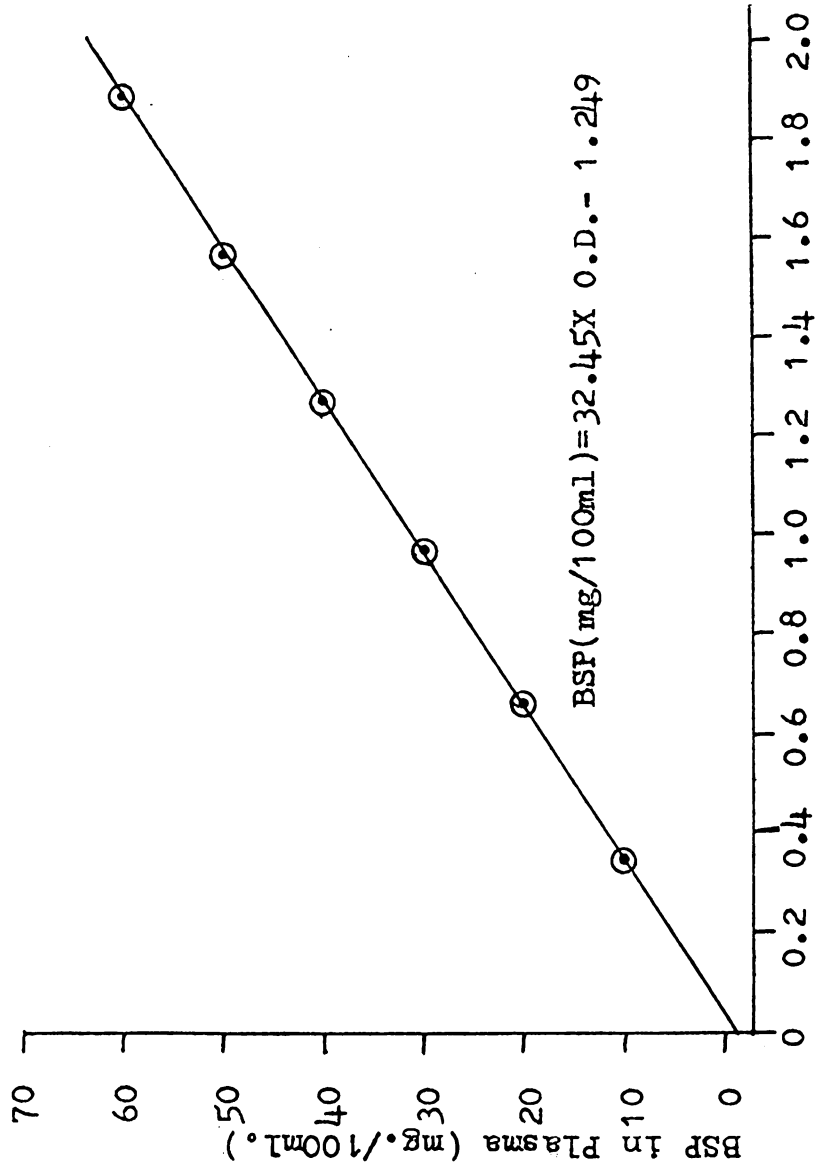


Figure 32. Calibration Curve for Plasma Levels of BSP (phenoltetrabromophthalein sodium sulphonate).

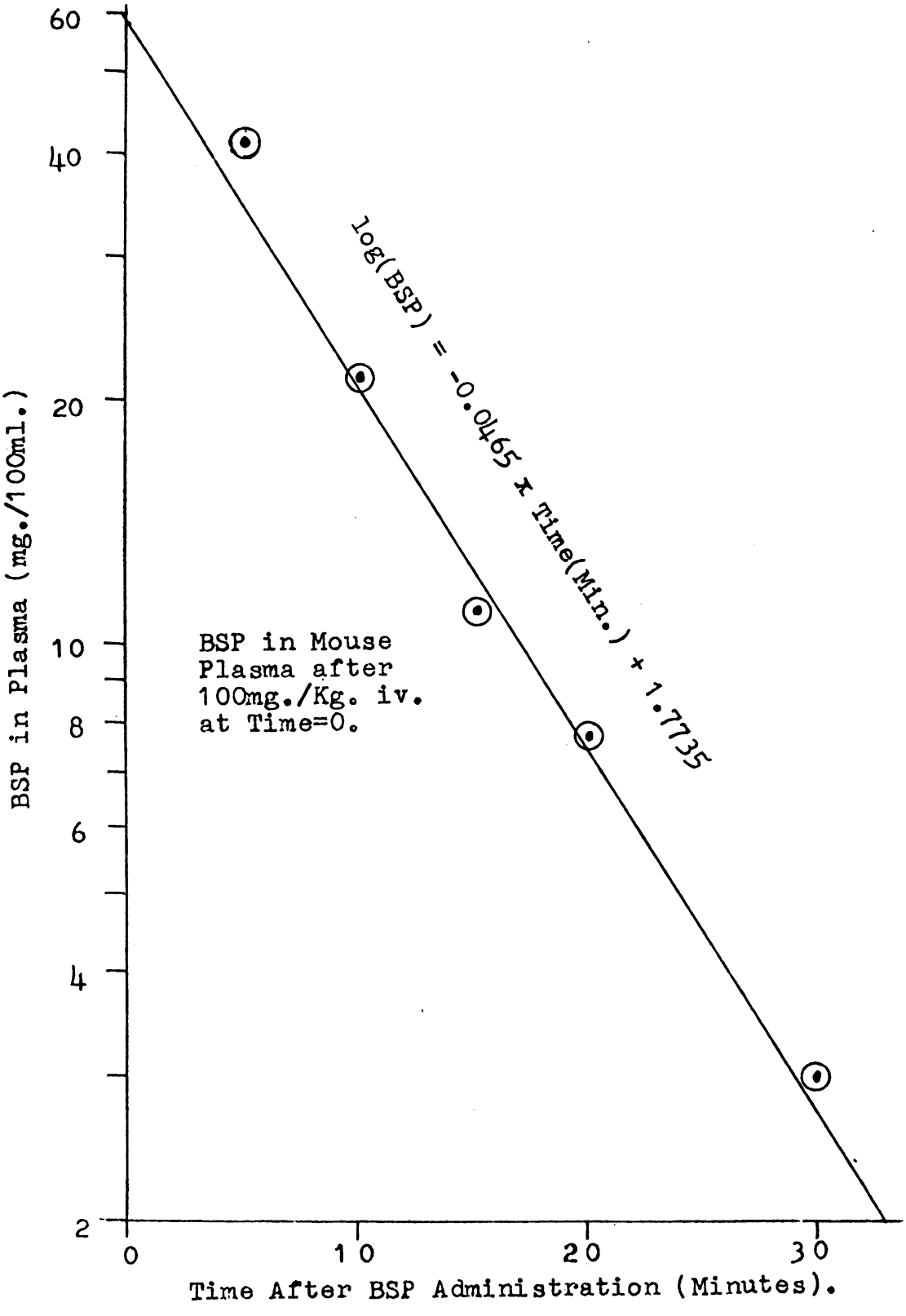


Figure 33. Disappearance of BSP from Mouse Plasma: Normal Control Group Subjects.

Only one time spot was identified in the testing of these animals. Thirty minutes (30 min.) was chosen as a good time to determine the BSP levels. These data are shown in Table 33 together with the control group values. The means are compared with a Student's t-test and found to be statistically identical. The mean for the treated animals was 3.1 whereas the control group was 3.0. A calculated t-value of $p = 0.054$ was much less than the theoretical value of 2.365, acceptable for significant difference. The raw absorptance (O.D., optical density) data for these samples are shown on this table as well. From these results it is apparent that the liver is not functionally impaired, at least as determined by this procedure.

Discussion of Toxicological Results

Acute lethality data for DPSD provided a calculated LD-50 (Lethal Dose--50th percentile) of 2,599 mg./kg. when administered i.p. to mice. The reported LD-50 from the Russian literature (Kelman et al., 1968) was 2,150 mg./kg. which is within the 95% confidence interval for this value (see Table 24) obtained here.

Acute lethality (LD-50) comparisons of DPSD with other anticonvulsant compounds appears in Table 34. Of these four compounds (diphenylsilanediol, phenobarbital, diphenylhydantoin, and diazepam), DPSD is definitely the least toxic in having the greatest LD-50 on single-dose administration. A further comparison of these four compounds on anticonvulsant and neurotoxic parameters will be covered below in the general discussion section.

Table 33

Plasma Levels of BSP in Treated vs. Control Mice

Control Mice# (Untreated diet for 90 days)		Treated Mice# (6.4 mg. DPSD/gm. Food for 90 days)	
O.D.	Calculated mg./100 ml.	O.D.	Calculated mg./100 ml.
0.16	4.0	0.054	0.63
0.11	2.4	0.12	2.7
0.12	2.7	0.16	4.0
		0.82	1.4
		1.44	6.7
		0.14	3.3
Mean:	3.0*		3.1*
S.E.:	0.49		0.87
%Dose Retained:	5.1		5.2

Note: #Blood plasma samples at 30 min. post dose.

*No significant difference (t = 0.054).

Table 34

Acute Lethality Comparisons for DPSD and Other Compounds

Compound	LD-50 in Mice	Reference
Diphenylsilanediol	2599 mg./kg., i.p.	(this investigation)
Phenobarbital	325 mg./kg., i.p.	(Reinhard et al., 1952)
Diphenylhydantoin	490 mg./kg., i.p.	(Fink and Swinyard, 1959)
Diazepam	620 mg./kg., i.p.	(Banziger, 1965)

Chronic toxicity comparisons between compounds are less easily made but a simple summary appears in Table 35. First, however, a brief summary of the observed toxicological responses to DPSD is required.

A re-examination of Table 28 provides a summary of the observed gross and microscopic differences between the treated and control groups that might be attributed to DPSD administration. Gross observations in treated animals compared with controls showed the body, adrenal, and spleen weights were slightly lower. Some large and hemorrhagic gall bladders were seen. On a microscopic level there were enlarged lymphatics in the small intestine, liver congestion with smooth endoplasmic reticulum proliferation, some mild kidney congestion, and hematological changes. The blood changes included an increase in the number of circulating eosinophils and reactive lymphocytes. The liver congestion and the gall bladder enlargements were examined further but there was no significant functional change utilizing BSP in a liver function test. The hematological changes could be serious toxicological effects and are discussed in some detail below.

Though eosinopenia is a common manifestation of drug toxicity, the occurrence of eosinophilia is not (Zbinden, 1963; Finch, 1972). The literature on eosinophilia shows that this state is a common response to most parasitic and allergic conditions (Donohugh, 1966). The physiological role of this blood cell is not well understood. It displays chemotaxis to histamine and has antihistaminic properties in its ability to render histamine inactive (Archer, 1963). These cells display only a small fraction of their total numbers in the circulating blood. The ratio of blood to tissue eosinophils has been estimated at

Table 35

Specific Toxicological Responses to the Chronic Administration of Anticonvulsant Compounds.

Compound	Observation	Reference
Diphenylsilanediol	eosinophilia	this investigation
	reactive lymphocytes	"
	swollen liver cells	"
	gall bladder enlargements	"
Phenobarbital	megaloblastic anemia	Mannheimer et al., 1952
	liver cell changes	Herdson et al., 1964
	allergy and skin rash	McGeachy et al., 1953
Diphenylhydantoin	megaloblastic anemia	Mannheimer et al., 1952
	encephalopathies	del Cerro et al., 1967
	connective tissue disorders	Hauck et al., 1972
Diazepam	no specific animal organ toxicities	Randall et al., 1960
	rare idiosyncratic effects in man	Wilcox, 1962; Gaul, 1961

1:200 (Rytomaa, 1960). From these basic data one can speculate on a variety of mechanisms for the observed eosinophilia associated with DPSD administration. These include such possibilities as DPSD being chemotactic for the eosinophils and drawing them from the tissue stores into the blood or selectively displacing these cells from the intestine or other tissues where they appear in large numbers (Vilpo et al., 1970). As the activation of complement components 5, 6, and 7 (Ward, 1969) is also chemotactic to eosinophils, these reactions might be mediated by an antigen-antibody reaction involving the DPSD. The possibility of some immunological-type mechanism is supported by the observed increase in reactive lymphocytes associated with DPSD administration. The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Schalm et al., 1975). The cytological changes seen in these cells have been associated with an increase in the production of immunoglobulins (Henry et al., 1972) which is considered the functional role of this increase in reactive lymphocyte numbers.

The other observed organ and tissue changes may be significant but appear to offer no serious threat when compared with the observed toxicities of known anticonvulsant compounds (see Table 35). Additional discussion of these comparisons appears in the GENERAL DISCUSSION section below. The observations summarized in this table were selected to represent what Zbinden (1963) calls "specific toxicity." These effects appear to be a direct result of a compound's activity and not an indirect effect through, for example, a reduction in food or water consumption or behavioral activity level.

ANTIEPILEPTIC STUDIES IN SEIZURE DISORDER ANIMALS

Introduction

The human epilepsies have been the source of much research and speculation from early times to the present (O'Leary and Goldring, 1976). In this section, the evaluation of DPSD as an antiepileptic agent is attempted, not in man, but in animals that exhibit spontaneous epileptiform-type seizures.

It is relatively easy to produce seizures in animals by chemical or electrical means. The section on anticonvulsant characterization above discusses some of these methods. In these situations one is forced to justify the experimental results by saying that the induced seizure has certain properties that make it appear to mimic an epileptic seizure state.

The existence of seizure phenomena in animals that is of a spontaneous nature, or triggered by phenomena that are relatively innocuous to non-epileptic animals, provides another opportunity to evaluate chemicals and procedures as antiepileptic agents.

A search of the scientific literature revealed only a few animals that have seizure states of an epileptic nature and that bear resemblance to the human maladies in being idiopathic and reasonably well-documented. Table 36 summarizes the seizure phenomena observed in several animal species. Three of these occur in rodents (the rat,

Table 36

Animals, In Addition to Man, Displaying Idiopathic Seizures

Species	Seizure Type(s)	Inducing Stimulus	Similarity to Man	Reference*
Mouse	Major with running often lethal	High intensity sound	Slight: Audiogenic rare, lethal rare in man	Hall, 1947
Gerbil	Major, minor	Emotional Stress: Territorial threat	Good: Stress not a common human factor	Kaplan and Miezejeski,
Rat	Major with running often lethal	High intensity sound	Slight: Audiogenic rare, lethal rare in man	Maier and Glaser, 1940
Chicken	Major, Psychomotor	Intermittent Photic Stimulation	Fair: EEG Slow Wave	Johnson et al, 1974
Dog	Major, Minor, Psychomotor	None: Stress may be a factor	Excellent: No minor to major, no maturation suppression	Cunningham, 1971
Baboon	Minor, myoclonus	Intermittent Photic Stimulation	Fair: Myoclonus rare in man	Killam et al, 1966

Notes: *Reference found in list of references.
#Dog seizure development is much like in man, but, unlike children, puppies do not outgrow minor seizures.

mouse, and gerbil). The rodent seizures are perhaps the best documented. The baboon and dog, as advanced mammals, have been moderately well investigated. The newest model to be examined is the chicken (Johnson et al., 1974), which is of course a non-mammalian species.

Audiogenic seizures in the mouse (Hall, 1947) and the rat (Maier and Glaser, 1940) have been used for the purposes of anticonvulsant drug characterization (Collins, 1972). The necessity for high intensity sound stimulation to evoke the seizures make them unlike seizures in man (Brazier, 1973). The patterning of the seizure (running followed by tonic-clonic seizures and then a major tonus followed by death) further make this animal model unlike the human disease.

The gerbil seizures are a more recent observation (Kaplan and Miezejeski, 1972) and have received little attention in anticonvulsant drug evaluation (Loskota et al., 1974). The seizure in these animals occurs spontaneously but can be triggered by stress and photic stimulation making these seizures similar to those in man (Harris and Mawdsley, 1974). The epileptic gerbil was one of the models chosen to be used for further anticonvulsant and antiepileptic characterization of DPSD.

The photomyoclonic baboon has received much attention as a model for human epilepsy due principally to the fact that this seizure (Killam et al., 1966) can be evoked by intermittent light stimulation. Though human seizures can often be evoked by photic stimulation (Brazier, 1973), myoclonic seizures are a rare phenomenon in human epilepsies (Schmidt and Wilder, 1968).

Canine epileptic animals are a common neurological observation to the veterinary practitioner (Hoerlein, 1965) and are managed by the same anticonvulsant medications used for humans (Siegmund et al., 1967). The dog shows a wide range of seizure types (Cunningham, 1971) and appears only rarely to require some external stimulation to evoke a seizure. This, then, was an additional model chosen for use in the evaluation of DPSD.

Epileptic Gerbil Model

The mongolian gerbil, Meriones unguiculatus, as found in laboratories in the United States, displays convulsive seizure phenomena in 2% to 20% of the adult population (Kaplan and Miezejeski, 1972). The seizure displayed is idiopathic and of a grand mal (major motor) type displaying generalized symmetrical electroencephalographic signs (Loskota et al., 1972). A feature that makes these seizures both interesting and convenient for experimental study is that they can be evoked in susceptible animals by stressing them. Given the highly territorial nature of these rodents, an adequate stress is placement into a new environment (Arrington et al., 1973; Loskota et al., 1974). Typical animals can be induced to have seizures every two to four days. Loskota and Lomax (1974) have shown that these seizures are controlled by treatment with antiepileptic drugs. As an epilepsy model, this seizure type was used to examine the effects of DPSD.

Methods

Gerbils were obtained from various laboratories on the M.S.U. campus, principally physiology and zoology, and screened over a four-week period for seizure susceptibility. Testing was done by placing the gerbil in a large rat cage lined with paper towels. Seizures were elicited in susceptible animals within 30 minutes. All animals were tested daily. The animals showing seizures were marked for identification and their seizures recorded.

Table 37 lists the seizure histories for gerbils selected as having seizure susceptibility. Gerbils were selected for this experiment as those with short seizure latencies and seizure rates of at least one every three days. Twenty-four animals were randomly assigned to one of four treatment groups of four animals each. Group I received 2 ml./kg. body weight of 0.5% methyl cellulose solution; Group II, 50 mg./kg. of a methyl cellulose suspension of diphenylhydantoin; Group III, 20 mg./kg. of phenobarbital in methyl cellulose; and Groups IV, V, and VI received DPSD at 20, 50, and 100 mg./kg. as methyl cellulose suspensions.

Each animal received daily injections of the drug and was observed for seizures for two hours (one hour before and one hour after dosing). The last hour was accompanied by the animal being placed in a rat box as noted above. The seizures of each animal over a four-day period were noted and recorded.

Table 37
Seizure Characteristics for Gerbils in Colony
Summary from Four Week Observation Period

Total Number of Seizures	Number of Gerbils*	Shortest Time Between Seizures	Longest Time Between Seizures
0	36	28 days	28 days
1	10	28 days	28 days
2	16	1 day	22 days
3	9	2 days	15 days
4	9	1 day	17 days
5	6	1 day	12 days
6	7	1 day	8 days
7	8	1 day	6 days
8	6	1 day	6 days
9	9	1 day	5 days
10	5	1 day	4 days
11	1	2 days	5 days
12	2	1 day	5 days
13	1	2 days	5 days
14	3	1 day	4 days
15	3	1 day	3 days
16	1	1 day	4 days
17	5	1 day	3 days
18	6	1 day	3 days
19	8	1 day	3 days
20	2	1 day	3 days

Notes: *Number of animals having the seizure totals listed in Column 1 (Total "epileptic" gerbils - 117).

Results

Table 38 summarizes the incidence of seizure for the epileptic gerbils in each of the treatment groups. At the doses tested, all compounds reduced seizure frequency over the four-day test period. Each animal had had a seizure on day zero. And each would have been expected to have another seizure before day four. The protection by diphenylhydantoin and phenobarbital corresponds with the data obtained by Loskota and Lomax (1974) on their inbred strain of epileptic gerbils (WJL/UC). The DPSD data suggests and antiepileptic activity for this compound. As apparent ED-50 was calculated to be 32 mg./kg. from these data using Finney's probit method (Finney, 1952).

Epileptic Dog Model

The domestic dog, Canis familiaris, displays a wide variety of neurological disorders (Hoerlein, 1965) many of which have direct correspondence to human heurological disorders (Foster, 1973). Some of the more common canine neurological diseases include seizure disorders, intervertebral disc abnormalities, brain tumors, meningitis, and hydrocephalus. Observations from the Veterinary Medical Teaching Hospital of the University of California, Davis (Holliday et al., 1970), revealed that epilepsy-like disorders in dogs comprise about 1% of the total canine diseases diagnosed at that clinic. Furthermore, convulsive seizure is the most commonly seen neurologic symptom for the dog requiring veterinary medical treatment (Cunningham, 1971a).

Table 38

Effects of Anticonvulsant Drugs on the Idiopathic Seizures of Mongolian Gerbils

	Control Treatments, i.p.			Experimental Treatments, i.p.		
	Drug Vehicle 2 ml./kg.	Diphenyl-hydantoin 50 mg./kg.	Pheno-barbital 20 mg./kg.	Diphenylsilanediol (DPSD) 20 mg./kg.	50 mg./kg.	100 mg./kg.
Observed Seizures Day 1	0	0	0	1	1	0
Observed Seizures Day 2	1	0	0	0	0	0
Observed Seizures Day 3	3	1	1	1	0	0
Observed Seizures Day 4	1	1	0	1	0	0
Total Seizures	5	2	1	3	1	0
Expected Totals	4	4	4	4	4	4
Protection Index	0%	50%	75%	25%	75%	100%

Notes: *ED-50 for DPSD = 32 mg./kg. (4.1-59.9).

Dogs, like man, show symptomatic as well as idiopathic seizures (Cunningham, 1971b; Hoerlein, 1965). The canine idiopathic seizure shows genetically determined trends in offspring seizure frequency (Cunningham, 1971a), as is known for the human disorder (Schmidt and Wilder, 1968). Dogs generally have one of three types of seizure displays: generalized, partial, or psychomotor (Cunningham, 1971a; Siegmun et al., 1967). The most common generalize seizure is a major motor (grand mal) type which has motor components, shows loss of consciousness, autonomic signs, and complex behavior signs developing immediately before or after a generalized seizure (Cunningham, 1971a). Partial seizures tend to be asymmetric in appearance; lifting one paw, facial twitching, etc., and frequently display a localizing sign suggesting an intracranial lesion. Psychomotor seizures have infrequently been carefully diagnosed (Hoerlein, 1965; Siegmund et al., 1967) but can be suspected in dogs displaying episodes of sudden and inappropriate fright, disorientation, with barking and stereotyped motor patterns.

The diagnostic criterion for idiopathic generalized seizures is usually one of exclusion of probable causes that would identify the seizure as being symptomatic. In the methods section below, the role of most of the diagnostic tests in the selection of dogs to receive DPSD was to eliminate animals showing symptomatic seizures. This was done principally to eliminate dogs from the study that might have disease states showing poor prognosis (i.e., brain tumor) and to avoid confounding the results.

Methods

The three dogs used in this study were clinical research patients of the neurology teaching unit of the Michigan State University Small Animal Veterinary Medicine Clinic. Each animal was recommended for therapy after having failed to be controlled by conventional anticonvulsant medication regimens (Hoerlein, 1965; Schmidt and Wilder, 1968). It was decided to begin treatment of each dog with DPSD doses of 20 mg./kg. two times per day orally as powdered DPSD-filled gelatin capsules. DPSD was used as supplied by Aldrich Chemical Company (Lot 102717) and pulverized in a mortar and pestle before being packed into empty gelatin capsules (Eli Lilly and Company, Indianapolis, Indiana). The dose and dosage sequence was estimated from the mouse data obtained against maximal electroshock seizures (see Maximal Electroshock Generalized Seizures, page 21, above). The plan for treatment was to increase the dose if an animal had a seizure close in time after an individual dose was given and to shorten the dosing period if an animal had a seizure in the middle of a dosing period (12 hours between doses). The initial dose of DPSD was to be added to the current medications, which were then sequentially withdrawn by dividing the dose by two each day and starting with the compound having the least potential for physical dependence liability (see Tolerance and Physical Dependence Studies, page 45, above) and progressing to that compound with the most potential for physical dependence. Thus, as an example, with patient A.G., Table 39, on diphenylhydantoin, primidone, and phenobarbital, the diphenylhydantoin would be withdrawn first, followed by the primidone, and

Table 39

Case Summary on Epileptic Dog A.G. Treated with DPSD

General and Diagnostic Summary:

Breed: German Shepherd, Age: 1.5 yrs., Sex: Male, Wt.: 25 kg.
Primary Diagnosis: Idiopathic Epilepsy-six month duration.
Control Period Medication: 500 mg. Diphenylhydantoin tid.
250 mg. Primidone tid.
32.4 mg. Phenobarbital tid.
Control Period Observations: 6 seizures in 1 month (1/wk.)
Control Period Laboratory Results:
EEG: Occasional spikes, bilaterally symmetrical.
Spinal Tap: 135 mm. CSF pressure, 6 mg. % protein.
Chemistries: BUN = 13 mg. %, SGPT = 20 IU., Fast. Glu. = 94 mg. %, BSP = 1% retention.
Hematology: WBC = 7100, Diff.: Neut. 67 (Seg. 60), Lymph. 22, Mono. 7, Eos. 4, Baso. 0. Hb. 15.5 gm. %, TP. 6.7 gm. %, PCV. 54%

Treatment Summaries on DPSD:

Week 1

Dose: 500 mg. bid. (20mg./kg.)
Seizure: none
Clinical Laboratory Results:
Chemistries: BUN= 18mg. %, SGPT = 62 IU.
Hematology: WBC = 15800, Diff.: Neut. 68 (Seg.67), Lymph. 24, Mono. 5, Eos.3, Baso. 0. Hb. 15 gm. %. TP. 6.7 gm %, PCV. 46 %.

Week 2

Dose: 500 mg. bid. (20mg./kg.)
Seizures: none
Clinical Laboratory Results:
Chemistries: BUN= 12mg. %, SGPT = 51 IU
Hematology: WBC = 9700, Diff.: Neut. 66 (Seg.66), Lymph. 20, Mono. 9, Eos. 5, Baso. 0. Hb. 15.5 gm. %, TP. 6.7 gm. %, PCV. 45%

Table 39 (Continued)

Case Summary on Epileptic Dog A.G. Treated with DPSD

Week 3

Dose: 250 mg. bid. (10 mg./kg.)
Seizures: none
Clinical Laboratory Results:

Chemistries:	BUN = 11 mg. %, SGPT = 34 IU.
Hematology:	WBC = 7400, Diff. : Neut. 67 (Seg. 67) Lymph. 17, Mono. 10, Eos. 6, Baso. 9. Hb. 17 gm. %, TP. 6.9 gm. %, PCV. 54%
Necropsy:	Patient euthanized, no gross nor microscopic evidence of drug toxicity.

finally the phenobarbital. If seizures were exhibited during this withdrawal period, the plan was to stop the next scheduled reduction and to increase the dose of DPSD before starting up the withdrawal sequence again. The criterion for DPSD efficacy was to be a comparison of seizure frequency before DPSD treatment with the seizure frequency while the patient was on DPSD treatment. A reduction of seizure incidence associated with the DPSD therapy would be indicative of antiepileptic efficacy.

Patients were selected having a diagnosis of idiopathic epilepsy based on history, physical and neurological examination, clinical laboratory data, and baseline electroencephalogram results (Hoerlein, 1965; Cunningham, 1971a). Patient A.G. was a male, German Shepherd, age 1.5 years, with a seizure history of six months duration. This animal was donated to the clinic to be euthanized if not needed and was the first dog to be treated with DPSD. The diagnostic and history data are summarized for this animal in Table 39. Physical and neurological examination showed no focal deficit evidence nor symptoms of other disease states which might display seizures as part of a complex syndrome. This patient was started on 20 mg./kg. DPSD twice a day with capsules given at 8:00 a.m. and 8:00 p.m.

Patient R.D. was a male, Great Dane, age 2.5 years, with a seizure history of one year duration. This animal was being considered for euthanasia by its owners when introduced into this test program. Table 40 summarizes the diagnostic and history data for this animal. Therapy was begun with 10 mg./kg. of DPSD twice a day at 8:00 a.m. and

Table 40

Case Summary on Epileptic Dog R.D. Treated with DPSD

General and Diagnostic Summary:

Breed: Great Dane, Age: 2.5 years, Sex: Male, Wt.: 60 kg
Primary Diagnosis: Idiopathic Epilepsy- 1 year duration.
Control Period Medication: 500 mg. Diphenylhydantion qid
750 mg. Primidone qid
10 mg. Diazepam qid
Control Period Observations: 15 seizures in 6 months (1/2 weeks)
Control Period Laboratory Results:

EEG: Occasional spikes, multiple spikes, symmetrical
Spinal Tap: 160 mm. CSF pressure, 5 mg. % Protein
Chemistries: BUN 11 mg. %, SGPT 4 IU., Fast Glu. 107 mg. %, Alk. Phos. 100 IU., BSP. 0% retention
Hematology: WBC 5400, Diff.: Neut. 76 (Seg. 70), Lymph. 9, Mono. 11, Eos. 4, Baso. 0. Hb. 17.5 gm. %, TP. 6.0 gm. %, PCV. 54%

Treatment Summaries on DSPD:

Two Week Period 1

Dose: 600 mg. bid (10 mg. /kg.) + 750 mg. Primidone bid
Seizures: one episode with status
Clinical Laboratory Results:
Chemistries: BUN 15 mg. %, SGPT 8 IU, Alk. Phos. 86 IU., BSP 0% retention.
Hematology: WBC 8300, Diff.: Neut. 76 (Seg. 67), Lymph. 11, Mono. 3, Eos. 10, Baso. 0. Hb. 16.0 gm. %, TP 7.0, PCV 48%.

Two Week Period 2

Dose: 1200 mg. bid (20 mg./kg.) + 750 mg. Primidone bid
Seizures: none
Clinical Laboratory Results:
EEG: Occasional spikes, spike wave complexes, symmetrical.
Chemistries: BUN 11 mg. %, SGPT 8 IU.
Hematology: WBC 8000, Diff.: Neut. 75 (seg. 65), Lymph. 13, Mono. 0. Eos. 12, Baso.0 Hb. 16.3 gm. %, TP 5.9 gm. %, PCV. 45%, RBC 4.6 M.

Table 40 (Continued)

Case Summary on Epileptic Dog R.D. Treated with DPSD

Two Week Period 3

Dose: 1200 mg. bid (20 mg./kg.) + 750 mg. Primidone bid.
Seizures: One episode, 21 days since last.
Laboratory Results:
Chemistries: BUN 13 mg. %, SGPT 19 IU.
Alk. Phos. 66 IU, BSP. 0% retention.
Hematology: WBC 7000, Diff. : Neut. 66 (Seg. 55), Lymph. 20, Mono. 0, Eos. 14, Baso 0.
Hb. 16.4 gm. %, TP 6.4 gm. %,
PCV. 48%, RBC 6.56M.

Two Week Period 4

Dose: 2400 mg. bid (40mg./kg.) + 750 mg. Primidone bid
Seizures: none
Clinical Laboratory Results:
Chemistries: BUN 16 mg. %, SGPT 26 IU,
BSP 4 % retention
Hematology: WBC 10,500 Diff.: Neut. 62 (Seg. 61), Lymph. 14, Mono. 4, Eos. 20, Baso. 0.,
Hb.14.3 gm. %, TP 6.1 gm. %, PCV. 43%,
RBC 5.74M.

Two Week Period 5

Dose 6000 mg. bid (100 mg./kg.) + mg. Primidone bid
Seizures: One episode, 16 days since last.
Clinical Laboratory Results:
Chemistries: BUN 14 mg. %, SGPT na., BSP 3% retention
Hematology: WBC 6000, Diff. : Neut. 59 (Seg. 58), Lymph. 18, Mono. 5, Eos. 17, Baso. 1,
Hb. 15.0 gm. %, TP 4.6 gm. %, PCV 45%,
RBC 6.54M.

Two Week Period 6

Dose: 6000 mg. bid (100 mg./kg.) + 750 mg. Primidone bid
Seizures: none
Clinical Laboratory Results:
Chemistries: BUN 24 mg. %, SGPT 10 IU.,
Alk. Phos. 970 IU.
Hematology: WBC 9400, Diff.: Neut. 64 (Seg. 62), Lymph 15, Mono 5, Eos. 16, Baso).
Hb.17.2 gm. %, TP 6.2 gm. %, PCV 51%,
RBC 7.08M.

Table 40 (Continued)

Case Summary on Epileptic Dog R.D. Treated with DPSD

Two Week Period 7

Dose: 6000 mg. bid (100 mg./kg.) + 750 mg. Primidone bid.
Seizure: none
Clinical Laboratory Results:
Chemistries: BUN na., SGPT 34 IU., Alk Phos. 1840 IU., BSP 0% retention.
Hematology: WBC 11400, Diff : Neut. 73 (Seg. 69), Lymph.11, Mono.2, Eos. 14, Baso. 0, RBC 6.44M, Hb 16.7 gm. %, TP 6.0 gm. %, PCV 50%.

Two Week Period 8

Dose: 4200 mg. bid (70 mg./kg.) + 750 mg. Primidone bid
Seizures: One episode, 56 days since last.
Clinical Laboratory Results:
Chemistries: BUN 12 mg. %, SGPT 8 IU., Alk. Phos. 679 IU.
Hematology: WBC 11400, Diff.: Neut. 80 (Seg. 73), Lymph. 10, Mono. 2. Eos. 8, Baso. 0. Hb. 17.1 gm. %, TP 5.5 gm %, PCV 50 %, RBC 5.65M.
Necropsy: Patient euthanized, no gross or microscopic evidence of drug toxicity. Brain showed signs of anoxic damage.

8:00 p.m. The lower dose was based on the results seen with patient A.C. (see Results and Discussion sections below).

Patient B.S. was a female, Laborador Retriever, age 4.5 years, with a seizure history of eight months duration. The diagnostic and history data for this animal are summarized in Table 41. Note also that this animal has a second diagnosis of hepatitis possibly of viral origin. The decision to include this animal in the DPSD study was prompted, in part, by the hepatitis diagnosis. An assumption was made, that if DPSD showed any liver toxicity directly, it would be likely to show up in an animal with an already impaired liver (Becker, 1974). This patient was donated to the clinic for experimental purposes and maintained as a research animal. Therapy was begun with 20 mg./kg. DPSD twice a day at 8:00 a.m. and 8:00 p.m.

Results

Table 42 summarizes the treatment results for these three canine patients. In each case the addition of diphenylsilanediol to the therapy for these animals either eliminated seizures completely or reduced the seizure frequency. The reduction in seizure frequency for these three patients as a group was both statistically and clinically significant. Each of these animals will be discussed with regard to seizure data, observations relating to potential toxicity, and incidental findings that could become important or could be of little consequence.

Table 41

Case Summary on Epileptic Dog B.S. Treated with DPSD

General and Diagnostic Summary:
Breed: Labrador Retriever, Age: 4.5 years, Sex: Female
Wt.: 30 kg.
Primary Diagnosis: Idiopathic Epilepsy- 8 month duration.
Control Period Medication: 600 mg. Diphenylhydantion tid
32.4 mg. Phenobarbital tid
5 mg. Diazepam tid
Control Period Observations: 11 seizures in 4 months. (1 seizure / two weeks).
Control Period Laboratory Results:
EEG: Occasional Spikes, Symmetrical
Spinal Tap: 135 mm CSF pressure, 25 mg. % protein.
Chemistries: BUN 11 mg. %, SGPT 17 IU., Fast Glu. 96 mg. %, BSP 0% retention, NH_3 54 gm. %.
Hematology: WBC 7800, Diff.: Neut. 72 (Seg. 68), Lymph. 20, Mono. 1, Eos. 7, Baso. 0. Hb. 17.3 gm. %, TP 6.1 gm. %, PCV 48%.
Secondary Diagnosis: Hepatitis, Chronic, relapsing.
Laboratory Results on Secondary Diagnosis:
Chemistries: SGPT 148 IU., Alk. Phos. 158 IU., BSP 19% retention, T Bili. 0.4 mg. %, NH_3 117 g. %.
Hematology: WBC 20,000, Diff.: Neut. 84 (seg. 71) Lymph. 10, Mono 6, Eos. 0, Baso. 0.

Treatment Summaries on DPSD:

Month 1

Dose: 600 mg. bid (20 mg./kg.) + 32.4 mg. phenobarbital
Seizure: None
Clinical Laboratory Results:
Chemistries: SGPT 66 IU., Alk. Phos. 949 IU., BSP. 6% retention.
Hematology: WBC 7600, Diff.: Neut. 77 (seg. 74), Lymph 20, Mono 1, Eos. 2, Baso. 0. Hb. 17.1 gm. %, TP 7.0 gm. %, PCV 50%

Table 41 (Continued)

Month 2

Dose: 600 mg. bid (20 mg./kg.) + 32.4 mg. phenobarbital
Seizures: None
Clinical Laboratory Results:
Chemistries: SGPT 140 IU. Alk. Phos. 1790 IU., BSP 0% retention.
Hematology: WBC 9400, Diff.: Neut 75 (Seg.73), Lymph. 19, Mono. 4, Eos. 2, Baso. 0. Hb. 16.3 gm. %, TP 6.8 gm. %, PCV 46%.

Month 3

Dose: 600 mg. bid (20 mg./kg.) + 32.4 mg. phenobarbital
Seizures: None
Clinical Laboratory Results:
Chemistries: SGPT 700 IU., Alk. Phos. 3070 IU., BSP 22.5% retention.
Hematology: WBC 8300, Diff.: Neut. 71 (Seg. 66), Lymph. 23, Mono. 3, Eos. 3, Baso.0. Hb. 16.4 gm. %, TP 5.76 gm. %, PCV 47%

Month 4

Dose: 600 mg. bid (20 mg./kg.) + 32.4 mg. phenobarbital
Seizures: None
Clinical Laboratory Results:
Chemistries: SGPT 400 IU., Alk. Phos. 2620, BSP 17%
Hematology: WBC 6000, Diff.: Neut. 66 (seg. 66), Lymph. 31, Mono. 0, Eos. 3, Baso. 0. Hb. 16.1 gm. %, TP 5.8 gm. %, PCV 48%

Month 5

Dose: 600 mg. bid (20 mg./kg.) + 32.4 mg. phenobarbital
Seizures: None
Clinical Laboratory Results:
Chemistries: SGPT 90 IU., Alk. Phos. 420 IU., BSP 17% retention.
Hematology: WBC 6000, Diff. : Neut. 73 (Seg. 70), Lymph. 22, Mono. 2, Eos. 3, Baso. 0. Hb. 15.9 gm. %, TP 6.3 gm. %, PCV 45%

Table 42

Summary Of Treatment Results With Epileptic Dogs On DPSD

Patient Initials	Dose Of DPSD	Seizure Rate Before DPSD	Longest:Seizure-Free On DPSD	Duration Of Treatment	Number of Seizures Expected	Seizures Observed**
A.G.	20 mg./kg. bid.	1 per week	3 weeks	3 weeks[1]	3	0
R.D.	100 mg./kg. bid.	1 per 2 weeks	6 weeks	16 weeks[2]	8	4
B.S.	20 mg./kg. bid.	1 per 2 weeks	21 weeks	21 weeks[3]	10	0

Notes: 1. Slight eosinophilia observed.
2. Marked eosinophila and elevated alkaline phosphatase observed.
3. Elevated alkaline phosphatase observed. Patient still on DPSD.
** Significant treatment difference (.05 level) for group (x^2=15, df.=2).

Table 42 and Table 39 show the treatment results with DPSD given to canine patient A.G. While being treated with 500 mg. of diphenylhydantoin, 250 mg. of primidone, and 32.4 mg. of phenobarbital three times per day, this animal had at least one seizure per week. During the first week on DPSD, this animal had no seizures and the other three drugs were completely withdrawn during the same period. The second week showed no seizures either, so that the dose was reduced to 10 mg./kg. for the third week. Still no seizures occurred. From the previous history and the controlled observation period, this dog was expected to have had at least three seizure episodes during the same three week period. At the end of the third week the DPSD was withdrawn and the animal had a seizure 26 hours after the last dose and then began having multiple daily seizure episodes. The dog A.G. was euthanized with an intravenous dose of pentobarbital and examined at necropsy using both gross and microscopic techniques. The histopathology exam revealed no abnormalities. Routine clinical laboratory results obtained during the control and DPSD observation periods revealed no large significant changes. Table 42 has a note indicating a slight eosinophilia associated with the DPSD dosing period. The slight rise in eosinophil (eos.) white blood cell count is noted in the light of the marked eosinophilia noted in patient R.D. below.

Tables 40 and 42 summarize the results obtained with patient R.D. This patient was being treated with 500 mg. of diphenylhydantoin, 750 mg. of primidone, and 10 mg. of diazepam four times per day. Even though this patient was drowsy and mildly ataxic, he still managed to

have one seizure episode, usually status epilepticus, every two weeks. By the end of the first two weeks of dosing with DPSD at 10 mg./kg., the diphenylhydantoin and the diazepam had been completely removed and the primidone had been reduced to 750 mg. twice a day, but the dog had a seizure episode. For the second two-week period the dose of DPSD was increased to 20 mg./kg. and no seizures were seen. During the third two-week period there was another seizure episode so the dose of DPSD was increased to 40 mg./kg. At the beginning of two-week period five the dog had another seizure episode, so the dose was increased to 100 mg./kg. of DPSD twice a day. On this dose there were no seizures during the next two (6 and 7) two-week periods. This prompted a reduction of the DPSD dose to 70 mg./kg. during two-week period eight. The dose reduction was followed shortly by another episode of seizures which occurred 35 days after the last seizure episode. The animal was transferred to another experimental program and, as he was having repeated episodes of status epilepticus during attempts to withdraw him from the DPSD, was euthanized with an intravenous dose of pentobarbital three days later. At necropsy most tissues and organs appeared normal but the brain showed areas of damage in the cerebral hemispheres that appeared to be similar to those seen after anoxic episodes (Sandritter and Wartman, 1973). These areas of encephalomalacia were confined to cerebral grey matter and at the junctions of white and grey matter. The clinical laboratory data associated with this therapeutic program identified two areas for concern. The serum enzyme alkaline phosphatase was found to be markedly elevated during

DPSD therapy. The blood-formed element, eosinophil, was found to have increased in number. The resulting eosinophilia showed a maximum of 20%, compared with 4% for the control period, during two-week period number four. The serum alkaline phosphatase level increased from a baseline level during the control period of 100 IU. to 1,840 IU. during two-week period number seven. The implications of these two elevated values is not clear but some hypothetical considerations are covered in the discussion below.

Table 41 and 42 summarize the treatment results for patient B.S. This dog is still under treatment and has not experienced a seizure since beginning therapy with DPSD five months ago. Before therapy with DPSD was begun this patient was being treated with 600 mg. diphenylhydantoin, 32.4 mg. phenobarbital, and 5 mg. diazepam three times per day. On this treatment regimen the animal was having a seizure episode every two weeks. As with the previous patient, this dog showed a marked elevation in serum alkaline phosphatase levels during DPSD treatment. The maximum value was recorded during the third month of treatment at a level of 3,070 IU. compared with 158 IU. recorded earlier and which was elevated due to the associated hepatitis. There was no evidence of an eosinophilia seen here. All other drugs except the phenobarbital had been withdrawn by the end of the second week of DPSD therapy. The phenobarbital was left at 32.4 mg. twice a day to provide enzymatic stimulation of the liver and some mild sedation of the animal.

Discussion of the Antiepileptic Studies

It is apparent from these investigations with the epileptic gerbil and dog that DPSD also controls seizures in epileptic animals. The results with the dog, even though the number of animals is small, provides some evidence that this compound may be albe to antagonize convulsions which are not well-controlled by the conventional anti-convulsant drugs. Some speculation on this possibility appears below (GENERAL DISCUSSION).

The dog also showed some signs of eosinophilia, with a large increase in one animal (R.D.), a small increase in another (A.G.), and no increase in the third (B.S.). This serves as additional support for the observed increase in these cells in the circulation in the mouse. Refer to the Toxicological Studies section (pages 87ff.) of this report for a discussion of the potential implications of this eosinophilia. Two dogs showed a very large increase in serum alkaline phosphatase levels (B.S. and R.D.). The third dog (A.G.) did not have this parameter determined as it was the first subject to be studied. With the introduction of B.S. and R.D. to the study, an attempt was made to monitor more clinical parameters due to the planned longer period of administration.

This huge increase in alkaline phosphatase deserves some discussion as to possible implications of its significance. Alkaline phosphatase enzyme activity in the blood serum has three major sources: bone, liver, and intestine (Fishman, 1974). In females there is also

an important contribution from the placenta. Normally the elevation of alkaline phosphatase levels is interpreted as indicating liver damage (Hoe, 1969). For the dogs discussed here, other parameters of liver function (BSP excretion, SGPT levels, serum bilirubin levels, and plasma protein concentration) appear normal. This tends to direct attention to other sources for this enzyme elevation. Despite the extreme values for alkaline phosphatase observed, there was no evidence that any of the dogs experienced any debilitation that might be expected to generate this enzyme from bone to intestinal damage. Until a detailed examination of the possible isoenzymes for the origin of this enzyme elevation is accomplished, its source will remain unknown. It may evern be that the enzyme concentration has not been increased but that its activity is somehow potentiated by DPSD.

GENERAL DISCUSSION

In this section, an attempt will be made to consider the characterization program for DPSD. First, an overall summary of the effects of DPSD on the test methods conducted in this investigation will be presented to provide a convenient reference for comparison. Secondly, discussions of this compound as an anticonvulsant will center around speculations about mechanism of action and structure-activity relations compared to conventional anticonvulsant compounds. Finally, DPSD as a potential antiepileptic drug will be addressed.

Results of the Characterization Program

Table 43 summarizes each of the test procedures on which DPSD was evaluated. Table 43 has five major divisions: Anticonvulsant Testing, Behavioral Characterization, Toxicological Studies, Antiepileptic Studies, and General Pharmacology. The general pharmacology of DPSD was not systematically pursued in these studies and only represents findings incidental to the other four major investigational areas. Table 43 lists the test method used, the species of the animal used, what measurement was made, and the dose level at which the observations were made. A single dose indicates an ED-50 value calculated from the data. A range of values indicates those doses which were actually tested. Doses that were administered in the food

Table 43

Summary of Anticonvulsant Program for DPSD

Test Method	Species	Measurements and Results	Dose(s) Tested or ED-50
I. Anticonvulsant Testing			
electroshock	mouse	inhibit tonic extension	20 mg./kg. i.p.
	mouse	inhibit tonic extension	2.9 mg./kg. p.o.
	mouse	inhibit tonic extension	0.43 mg./g. food (68.3 mg./kg./day p.o.)
	rat	inhibit tonic extension	30 mg./kg. i.p.
	gerbil	inhibit tonic extension	42 mg./kg. i.p.
pentylenetetrazol	mouse	inhibit tonic extension	48 mg./kg. i.p.
	rat	inhibit clonic seizures	58 mg./kg. i.p.
	mouse	elevate seizure threshold	0.4-6.4 mg./g. food (66.6-1066 mg./kg./day p.o.)
strychnine	rat	inhibit clonic seizures	60 mg./kg. i.p.
picrotoxin	rat	inhibit clonic seizures	150 mg./kg. i.p.
topical penicillin	rat	inhibit clonic seizures	30-100 mg./kg. i.p.
after-discharge in neocortex	rat	increase threshold and decrease duration	30 mg./kg. i.p.
post-tetanic potentiation	cat	reduce amplitude and duration of tetanization	25-50 mg./kg. i.p.

Table 43 (Cont'd)

II. Behavioral Characterization			
rotating rod apparatus	mouse	inhibition of performance	200 mg./kg. i.p.
	mouse	inhibition of performance	6.62 mg./g. food (1050 mg./kg./day p.o.)
	rat	inhibition of performance	170 mg./kg. i.p.
	gerbil	inhibition of performance	80 mg./kg. i.p.
operant FR-40 for food	rat	increase rate of performance	10 mg./kg. i.p.
		decrease rate of performance	300 mg./kg. i.p.
operant CES model	rat	threshold facilitation	10 mg./kg. i.p.
		peak facilitation	100 mg./kg. i.p.
III. Toxicity Studies			
acute lethality	mouse	death within 8 hours	2599 mg./kg. i.p.
sub-acute lethality	mouse	deaths during 5 days	32.4 mg./g. food (5144 mg./kg./day p.o.)
chronic toxicity	mouse	reduction in body weight, reduction in adrenal weight, reduction in spleen weight, liver congestion, eosinophilia, reactive lymphocytes	6.4 mg./g. food (1016 mg./kg./day)
	dog	eosinophilia, serum alkaline phosphatase elevation	20-100 mg./kg. bid. (40-200 mg./Kg./day p.o.)

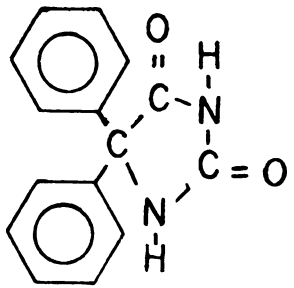
Table 43 (Cont'd)

IV. Antiepileptic Studies			
idiopathic seizure animals	gerbil	suppression of seizures	32 mg./kg./day i.p.
	dog	suppression of seizures	10-100 mg./kg. bid. (20-200 mg./kg./day p.o.)
V. General Pharmacology			
time course-anticonvulsant	mouse	peak time at 30 min. half time about 4 hrs.	25 mg./kg. i.p.
tolerance development	mouse	no decrease in seizure threshold with chronic (14-90 day) dosing	0.4-6.4 mg./g. food (63-1016 mg./kg./day p.o.)
physical dependence	mouse	no decrease in seizure threshold after withdrawal from chronic dosing	0.4-6.4 mg./g. food (66.6-1066 mg./kg./day p.o.)
antianxiety activity	rat	operant behavioral model of anxiety state	10-100 mg./kg. i.p.

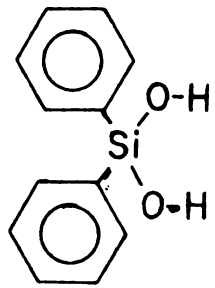
are shown together with a calculated estimate of the total daily dose received by an animal or group of animals.

Some Considerations of DPSD as an Anticonvulsant Compound

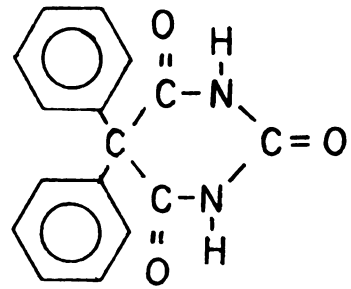
With the demonstration by Putnam and Merritt (1937) that diphenylhydantoin elevated the electroconvulsive shock threshold to produce tonic seizures in cats, the potential for developing new anticonvulsive drugs in the laboratory became apparent. Supported by their clinical data, Merritt and Putnam (1938) demonstrated the effectiveness of diphenylhydantoin in man. In a review paper eight years later (Merritt and Putnam, 1945), the screening of over 1,000 compounds resulted in approximately 75 that showed good seizure protection. The majority of these compounds contained phenyl groups or were otherwise aromatic. This tended to support an earlier (1937) postulate that phenyl-containing compounds appeared to have the best potential as anticonvulsant drugs. Toman and Goodman (1948), after a consideration of structure-activity relationships for clinically effective anticonvulsant drugs, concluded that phenyl-substitution was essential for drugs used to treat grand mal seizures or psychomotor displays, but was either non-essential or detrimental to drugs used in the treatment of petit mal seizure types. This position has tended to be supported throughout many reviews of anticonvulsant structure-activity data (Close and Spielman, 1961; Spinks and Waring, 1963; Delgado and Isaacson, 1970; Mercier, 1973). Some of these phenyl-containing compounds are shown in Figure 34 together with DPSD for comparison



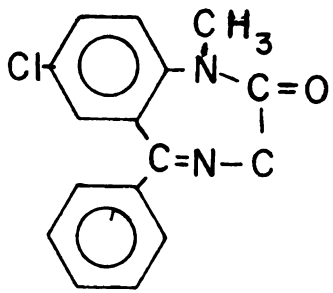
diphenylhydantoin



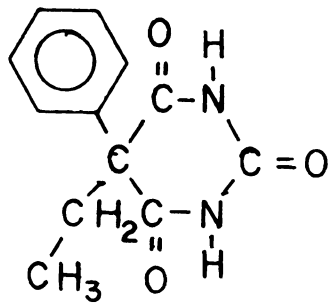
diphenylsilanediol



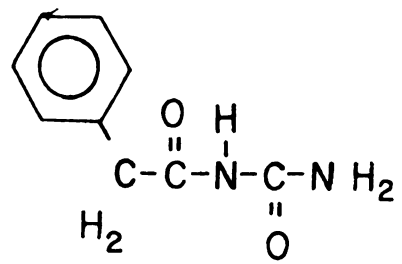
diphenylbarbituric acid



diazepam



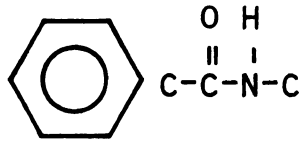
phenobarbital



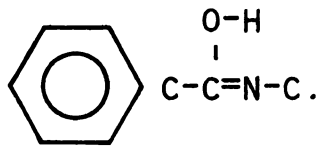
phenurone

Figure 34. Structural Forms of Phenyl-Containing Anticonvulsant Drugs

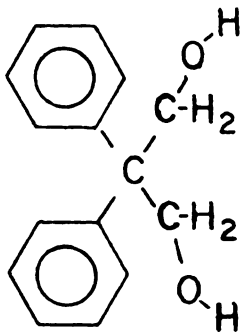
purposes. If one attempts to make direct structural comparisons between DPSD and these other compounds, the phenyl groups become obvious. On the other hand, what might be said about the remaining structures? Most of the organic anticonvulsants have the common feature of



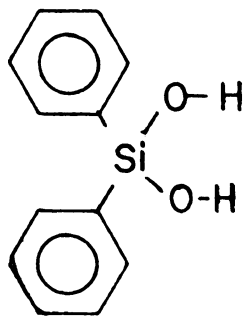
and if one considers keto-enol tautomerism, an alternate version of this common feature becomes



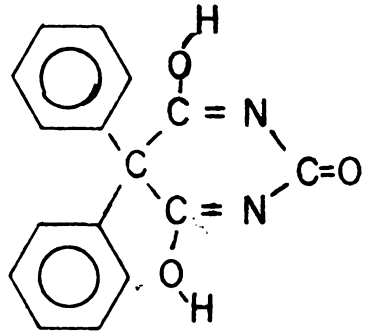
With the success of diphenylhydantoin therapy, there has been some renewed interest in other diphenyl-containing compounds. Diphenylbarbituric acid was originally introduced as a sedative-hypnotic and dismissed because of its low potency (McElvain, 1935). A current examination of this compound as an anticonvulsant (Raines, 1973; Raines et al., 1973) shows it to be potentially a broad spectrum anticonvulsant compound having a better safety margin, in terms of therapeutic indices, than phenobarbital. Many years ago Berger (1951) showed that the propanediol, 2,2-diphenyl-1,3-propanediol, antagonized electroshock seizures in mice. This compound was ignored due to its short duration of action. Attempts to prolong the action of the propanediols ultimately produced the carbamate esters of these compounds. Of these, meprobamate became the major one of interest (Berger, 1952). Figure 35 shows a collection of anticonvulsant compounds compared with DPSD. The classical keto forms are redrawn as



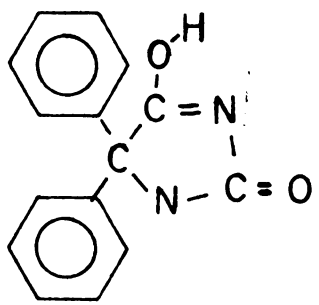
2,2-diphenyl-1,3-propanediol



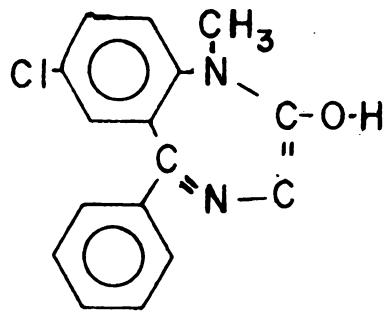
diphenylsilanediol



diphenylbarbituric acid



diphenylhydantoin



diazepam

Figure 35. Comparisons of Alcoholic Forms of Selected Anticonvulsant Compounds.

enol forms showing a direct comparison with DPSD. The larger size of the silicon atom appears to place the phenyl and alcohol groups in a position making it similar to each of these compounds.

As is currently true for nearly all central nervous system acting drugs, the mechanism of action for any anticonvulsant drug has not been well defined. Most attempts to deal with mechanisms for these compounds have to contend with a wealth of conflicting electrophysiological and biochemical studies. Woodbury (1969) suggested that there may be three general types of anticonvulsant effects. These include (1) effects of non-neural systems to prevent changes which may precipitate or facilitate seizure activity, (2) effects confined to the pathologically altered neurons of the seizure focus to prevent their seizure discharge, and (3) effects on normal neurons to prevent their excessive discharge by the seizure focus. Anticonvulsant drugs probably have their most significant action attributable to the latter general mechanism. There are probably some mechanistic subcategories based on how the various anticonvulsant drugs accomplish this suppression of excessive discharge in normal neurons by so-called "epileptic neurons." Such subcategories can include a general depression of neural activity through neurotransmitter or electrogenic means or a facilitation of physiologically active inhibitory mechanisms. Circumstantial evidence obtained from the results of various anticonvulsant test methods permits inferences about possible mechanisms of action for each drug tested.

Investigations into the mechanisms of action of these drugs is coupled with investigation into the mechanisms of the seizures themselves. Phenobarbital has been investigated most extensively though diphenylhydantoin appears to have been best defined in terms of its action. Investigations on diazepam have tended to center around its behavioral effects and not its anticonvulsant properties.

Early studies on phenobarbital implicated it as an inhibitor of brain respiration (Jowett and Quastel, 1937). Brody and Bain (1951) were able to show that the barbiturates uncouple oxidative phosphorylation lending support to the effects of these compounds on brain metabolism. Nachmanson and Wilson (1951) suggested that the barbiturates inhibit acetylcholine synthesis and produce its seizure-inhibiting effects through this mechanism. The net effect of the barbiturates in general, and phenobarbital in particular, is the stabilization of the neuron in reference to the generation of transmitted impulses (Seeman, 1972). This property is evidenced in the effectiveness of phenobarbital in antagonizing seizures generated by a variety of means (see discussion in anticonvulsant section above). The observation that levels of brain serotonin in animals are increased by 50% or more after treatment with phenobarbital (Bonnycastle et al., 1957) adds another inference that altered transmitter functions are involved in the mechanism of action of phenobarbital. In support of this idea, the pretreatment of animals with iproniazid has been found to increase the anticonvulsant activity of phenobarbital (Yen et al., 1960).

Unlike phenobarbital, diphenylhydantoin does not have a wide spectrum of activity in anticonvulsant test methods. Esplin (1957)

was able to demonstrate that diphenylhydantoin inhibited post-tetanic potentiation in the spinal cord of cats.

More refined mechanistic studies for these actions of diphenylhydantoin (Woodbury, 1969) have shown a decrease in the concentration of intracellular sodium and a lowered ratio of intracellular to extracellular sodium in brain neurons even after maximal electroshock seizures.

Diazepam shows the same wide spectrum of anticonvulsant activity as does phenobarbital but electrophysiological studies show a number of regional differences for these two compounds. Though both inhibit after-discharges in the neocortex (Schallek et al., 1969), the benzodiazepines appear to actually lower the threshold for after-discharges evoked from the amygdala. Effects on the electrocorticogram are also different for these two compounds. Phenobarbital tends to produce a synchronization effect or lowering of the observed frequencies, while diazepam produces an "arousal" (desynchronization) effect with a shift to higher frequencies.

In terms of its anticonvulsant action, DPSD appears to share similarities with each of these three compounds.

Some Considerations of DPSD as an Antiepileptic Drug

Looking once again at Toman's definition of an ideal anti-epileptic drug (Toman, 1970) as summarized by Table 2, we can re-examine DPSD in the light of these criteria: (1) suppresses all seizure types, (2) is effective orally, (3) is long acting, (4) shows

no tolerance to its action, (5) shows no withdrawal effects, (6) has no sedation or CNS toxic action, (7) shows no systemic toxicity, (8) shows no idiosyncratic toxicity, (9) has a wide margin of safety, and (10) is inexpensive to buy.

Looking at these in reverse order, consider first the cost of DPSD. We can estimate from the dog data a therapeutic dose of 20 mg./kg./twice a day or 6.8 grams per day for an average man (weight of 70 kg.). Based on the Aldrich Chemical Company price of $10.00 per 100 grams, this would cost the patient 68 cents per day or about $248 per year.

Table 44 illustrates margin of safety considerations where therapeutic indices are compared for DPSD, diazepam, phenobarbital, and diphenylhydantoin. Three indices are calculated based on the maximal electroshock antagonism ED-50, the rotating rod depression TD-50 and the lethal dose LD-50. When the neurotoxic dose is compared with the anticonvulsant dose, both DPSD and diphenylhydantoin appear similar and are safer than either diazepam or phenobarbital. Comparing the neurotoxic dose with the lethal dose (LD-50/TD-50), one sees an improved safety margin of DPSD over diphenylhydantoin. The seemingly large safety margin for diazepam is counter balanced by the poor separation of anticonvulsant from neurotoxic doses for this compound.

Idiosyncratic toxicity probably cannot be evaluated except in an extensive clinical trial.

Systemic toxicity for DPSD is not well-defined at this point but the observations made with the rat and the dog appear to require some cautious additional study.

Table 44

Acute Dose Safety Comparisons of DPSD with Other Anticonvulsant Compounds

	Mouse Data mg./kg. i.p.			Calculated Therapeutic Indices		
Compound Name	ED-50*	TD-50#	LD-50+	TD-50/ED-50	LD-50/ED-50	LD-50/TD-50
Diphenylsilanediol	20	200	2599	10	130	13
Diazepam	3.4	3.9	620	1.1	182	159
Phenobarbital	50	126	325	2.5	6.5	2.6
Diphenylhydantoin	12.8	133	490	10.4	38.3	3.7

Notes: * Effective Dose 50% from Maximal Electroshock Testing.
Toxic Dose 50% from Rotating Rod Testing.
+ Lethal Dose 50% After Acute Dosing

The sedation and CNS toxicity issues were already covered in brief discussion of margin of safety aspects. It appears that DPSD is at least as free of neurotoxicity as diphenylhydantoin which appears to be the standard anticonvulsant in this regard.

The lack of withdrawal effects of any significant magnitude with DPSD is perhaps one of the more interesting observations made on this compound.

Tolerance development appears to be mild and, over the longest time tested (90 days), appears to be insignificant.

An estimated elimination half-time of four hours is not really impressive, but due to the large therapeutic indices for this compound it is possible to elevate the dose to obtain whatever dosing regimen is required. Experience with the dog suggests that a twice a day dosing regimen is adequate. This animal normally requires the administration of diphenylhydantoin three or four times per day for effective treatment due to its rapid excretion in this animal.

Both the mouse and dog data attest to DPSD being effective orally, and well tolerated by this route.

The therapeutic indication for DPSD at this point is for the major motor seizures, i.e., grand mal and psychomotor types. The possibility for adjunctive use in minor seizures (petit mal or absence types) should be considered based on the wide spectrum of action for this compound.

An overall comparison of DPSD with diphenylhydantoin, phenobarbital and diazepam is given in Table 45.

Table 45.

Spectrum of Action Comparisons of DPSD with Other Anticonvulsant Compounds.

Anticonvulsant:	diphenylhydantoin	phenobarbital	diazepam	diphenylsilanediol
Prevents Tonic Seizures	yes	yes	yes	yes
Prevents Clonic Seizures	no	yes	yes	yes
Raises Seizure Threshold	no	yes	yes	yes
Prevents Seizure Spread	yes	some	yes	yes
Behavioral:				
Neurotoxicity Important	no	yes	yes	no
Antianxiety Effect	no	some	yes	yes
Toxicity:				
Margin of Safety Good	yes	no	yes	yes
Reported Systemic Toxicity	yes	yes	no	yes

RECOMMENDATIONS

In this section an attempt will be made to discuss casual observations about the activity of DPSD that are not well substantiated. In addition, some suggestions will be made as to where future investigations on this organosilicon compound should be directed.

The most pressing problems are an explanation of the observed toxicological responses to DPSD. The hematological changes of reactive lymphocytes and eosinophilia suggest some immunological effects for this compound. A systematic examination of DPSD on immune mechanisms should be undertaken. Note that silicosis can be considered an immune complex disease and DPSD has both the silicon and the silanols common to silica. An exploration of the increases in alkaline phosphatase is also required. An initial approach could be the identification of the isoenzyme fraction which is selectively increased for a hint as to which tissue is involved here.

There is an additional observation concerning the toxicity of DPSD that deserves mention, though it is not well documented. This compound may have some centrally-acting emetic properties. Three dogs dosed orally with this compound exhibited emesis an hour or so after initial dosing. An additional dog that was dosed with DPSD intravenously vomited shortly after the dose was administered. If this is an important property it appears to tolerate out relatively rapidly. However, emesis may become a problem with human administration.

The structure-activity comparisons of DPSD with other anticonvulsant drugs suggests that a direct comparison of this compound with diphenylbarbituric acid and with 2,2-diphenyl-1,3-propanediol is in order.

Based on the strong anti-anxiety effect observed for DPSD, the behavioral properties of this compound could become more therapeutically useful than its broad anticonvulsant activity spectrum of action. This pharmacological activity has been much examined for the benzodiazepines (Garattini and Shore, 1967; Zbinden and Randall, 1967) for which the role of endogenous inhibitory mechanisms seems to be of paramount importance. It may be that the anticonvulsant as well as the anti-anxiety effects of DPSD could ultimately be attributable to the effects of this compound on normal inhibitory mechanisms.

Based on the general observation that most anticonvulsant compounds are also antiarrhythmic agents (Morgan and Mathison, 1976), it would be beneficial to examine the antiarrhythmic activity for DPSD. Of course, the general cardiovascular pharmacology for this compound needs examination in depth to further characterize this compound.

Further characterization of other areas of pharmacology is definitely required. Pharmacokinetics and metabolism studies are needed to unravel some of the implications of the tendency of DPSD to polymerize. There is always the possibility that the polymeric forms of this compound are responsible for its spectrum of action or account for aspects of its toxicity.

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