RESEARCH ON NEW CHEMICAL INCAPACITATING AGENTS. PART I

PFIZER (CHAS) AND CO INC GROTON CT

30 JUN 1964

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Chas. Pfizer & Co., Inc.

Research on New Chemical Microorganism Agents

Army CRDL Contract #DA18-108-AMC-240(A)

ANNUAL REPORT NO. 1

June 26, 1963 - June 30, 1964

Part I

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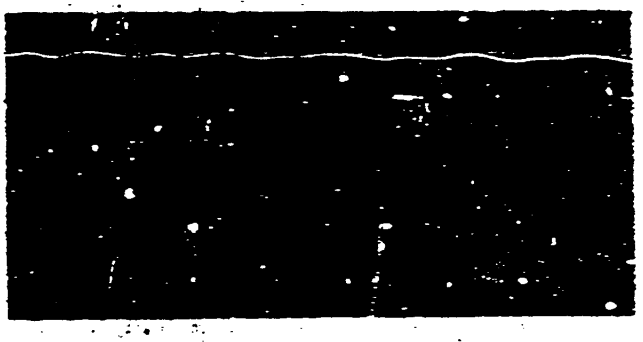

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TABLE OF CONTENTS

Pages

I. INTRODUCTION

The present report covers the work accomplished from June 28, 1963 to June 30, 1964, and represents the first year of the contract research on new chemical incapacitating agents.

Pfizer Research Laboratories began contract research on new chemical incapacitating agents on June 28, 1963. The research approach undertaken in this program is an integrated chemical-biological effort. The synthesis of agents for biological evaluation is guided by available leads and through correlation of chemical structure and biological activity. Empirical screening and the synthesis of compounds for empirical screening has not been a significant factor in this program. The overall strategy of this program consists of:

1. Selection of leads derived from the literature or from previous Pfizer research experience.

2. Chemical studies to exploit promising leads, and synthesis of rationally designed analogs of prototype compounds which possess potential or demonstrated incapacitating activity.

3. Preliminary screening of these analogs and other compounds for pharmacological activities and toxicity range.

4. Studying promising compounds more intensively to gather pertinent information on toxicity and mode of action, and

5. Development of specific test procedures appropriate to the type of incapacitating activity shown by the compound selected.

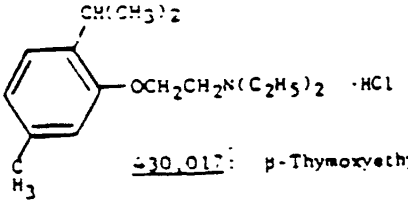
Data sheets for compounds screened during the fourth quarter of the contract are being submitted in a separately bound Part II of this report.

11. SUMMARY

During the first quarter of the contract period, it was necessary to devote a large part of our efforts to organizing the research program: Emphasis had been placed on (1) personnel assignment, orientation, briefing and training; (2) procurement and design of equipment and (3) initiation of the technical program. Since the end of the first quarter, the chemical synthetic program has been operating at full capacity, and the procedure for basic screening of new compounds has been standardized.

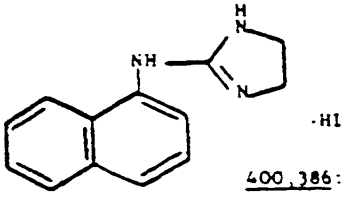
A variety of leads have been considered in our research program on incapacitating agents. Because of the size and budget of the contract, we have decided to limit the program to the selection of two prime groups of chemical structures for exploration. This is necessary in order not to spread out and dilute our research efforts. After a comprehensive review of the literature and also of leads originating from previous Pfizer research experience, our research program on new incapacitating agents has been directed toward two major groups of chemical structures for exploration. The prototype of each is represented below:

1. The thymol ethers and



430,017: p-Thymoxyethyl diethylamine hydrochloride

2. The substituted 2-aminoimidazolines



400,386: 2-(1-Naphthylamino)-2-imidazoline hydroiodide

In the thymol ether research program, primary efforts were directed toward the synthesis of analogs of the diethylaminoethyl ether, 430,017, and the development of specific biological tests in animals which would correlate with the desired incapacitating effects observed with this compound in man Because of the lack of pharmacologic test procedures capable of clearly characterizing the incapacitating effects observed in man with this type of compound, we are still not able to tell whether the many analogs prepared are potentially more potent than 430,017. Prime efforts will be continued to successfully develop biological methods that could be used to test new compounds in animals for the desired activities in man.

In the substituted aminoimidazoline program, 21 compounds were found in the MOTS to have an MED50 of less than 1 mg/kg, 13 of which had an MED50 of 0.5 mg/kg or less, and 6 of which had an MED50 of 0.1 mg/kg or less. After secondary screenings and further pharmacological investigations, 3 compounds (400,386; 400,483, and 400,487) were shown to be of exceptional interest.

Research on thymol ethers and substituted 2-aminoimidazolines is supplemented by the selection from the Pfizer research program of other compounds on the basis of potential incapacitating symptoms as additional sources of new leads to incapacitating agents. No new leads with incapacitating potential have been discovered.

Also included in our program is research on behavioral evaluation of amnestic drugs. The objective in this part of the program is to develop reliable and practicable animal screening methods for testing of compounds which may interfere with recent memory and disallow retention. Critical review of work to date on the retrograde amnesia research has recently been made. No satisfactory drug leads with retrograde amnesia activity have thus far been generated and certainly none approaches the potency required by the ACRDL objectives. We discussed with contract project officers Drs. Wills and Witten during their visit to our laboratories on May 25 to phase out this program and employ the freed potential in a new research program, that is, research on new agents derived from microbial sources. The assay procedure for retrograde amnesia will, however, continue to be available should agents having retrograde amnesia effect become of interest.

III. CHEMICAL SYNTHESIS PROGRAM

A. Selection of Leads

The overall research strategy adopted consists of the selection of specific leads derived from the literature or from prior Pfizer experience, followed by the development of these leads, through the coordinated synthesis and evaluation of analogs, into compounds possessing the properties sought in an incapacitating agent.

At the outset, the potential leads consisted of those described in the project proposal submitted by Pfizer to the Army CRDL. These had originated either from previous in-house programs or from investigations reported in the literature. The number of potential leads was then augmented by a survey of former Pfizer clinical candidates, searching for potentially incapacitating side-effects that had been observed in man. A thorough review of these prototypes from various sources and of their potential for development into effective incapacitating agents, followed by consultation with the Army CRDL, led to the selection of the two most promising ones for immediate investigation, and of several others as alternate possibilities.

1. Thymol Ether Analogs. The diethylaminoethyl ether of thymol, 430,017 was selected as the prime prototype on the basis of high potency, known activity in man, structural simplicity, and other criteria discussed below. It appears to represent a lead of exceptional interest which is entirely new to the research programs towards incapacitating agents carried out and coordinated by the Army CRDL.

The incapacitating activity of this compound in man was described by two independent reports (Staub, A. N., Ann. Inst. Pasteur., 63: 485, 1939 and Ashford, A., et al., Nature, 197: 969, 1963). In the report by Staub, an oral dose of 430,017 (1.5 mg) produces muscle fatigue and pain, difficulty in breathing and swallowing, hypersensitivity of the skin and hypotension. In the report by Ashford, et al., 10-12 mg of 430,017 produces severe muscle aching and tenderness throughout the body and tachycardia on exertion. According to Staub, a 20 mg dose produces violent muscle pains, violent headache, vomiting and prostration. More recently, difficulty in breathing and pronounced muscle stiffness were also observed in a human volunteer at the Army CRDL after an oral dose of 1.5 mg of 430,017, leaving little room for doubt that the compound adversely affects muscle function in man.

The experience of these investigators with 430,017 suggested that it fulfills sufficiently the biological and chemical requirements of the program to warrant detailed investigation. It is a well defined,

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(b)(3)

2. Aminoimidazoline (400,386) Analogs. Naphthylaminoimidazoline (400,386) was selected as a second prototype for synthetic development on the basis of previous Pfizer research experience. It is known to have CNS depressant and cardiovascular activity in man, is highly potent, produces effects that are clearly detectable in the mouse toxicity screen at very low doses, and has a structure that can be readily varied in a number of ways. In addition, it is free of the chemical instability problems encountered with some related types of compounds, e.g. aminooxazolines, which produce similar biological effects.

3. Miscellaneous Compounds. Several other prototypes were selected as alternate possibilities:

a. A number of quinazolones which produce tremors in various species, including man.

b. Dimethylhistamine and a number of other compounds which either release histamine or have histamine-like properties.

c. N-Nitrosopiperazine which produces pronounced hypotension.

d. Several trifluoromethyl- and halo-substituted amphetamines which produce CNS effects such as dysphoria, tremors and confusion in man.

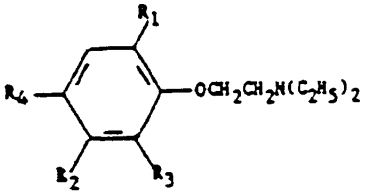
B. Synthesis of Thymol Ether Analogs

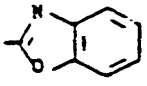
The first synthetic efforts in the program were devoted to preparing 430,017 and 3 other reference compounds to aid in the development of specific biological testing procedures. The tertiary butyl analog,

Table 1

Thymol Ether Analogs

Aromatic Variants



	R_1	R_2	R_3	R_4
430,017	$CH(CH_3)_2$	CH_3	H	H
430,021	$CH(CH_3)_2$	H	H	H
430,022	$CH(CH_3)_2$	H	$CH(CH_3)_2$	H
430,019	$C(CH_3)_3$	CH_3	H	H
430,027	$C(CH_3)_3$	H	CH_3	H
430,031	$C(CH_3)_3$	H	H	H
430,042	$C(CH_3)_3$	$C(CH_3)_3$	H	H
430,026	CH_3	CH_3	H	H
430,025	Cl	Cl	H	H
430,023	C_6H_5	"	H	H
430,024	$COCH_3$		H	H
430,040	$COCH_3$	[b e n z o]		H
430,034	COC_5H_{11}	CH_3	H	Cl
430,032	OCH_3	H	H	$CH_2CH=CH_2$
430,035	H	OC_6H_5	H	H
430,036	$CH=CHCOCH_3$	H	H	H
430,043	$CH_2CH=CH_2$	H	$CH_2C=CH_2$	H
430,076		H	H	H
430,077		H	H	H

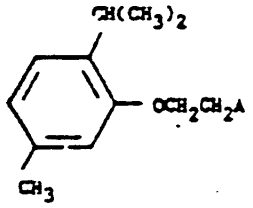
Commercial-Discreet

402,084	402,232	402,344	402,654
402,122	402,295	402,426	403,208
402,145	402,310	402,562	403,248
402,229		402,619	403,753

Table II

Thymol Ether Analogs

Amine Variants



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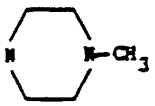
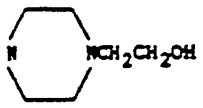
430,018	$N(CH_3)_2$
430,092:	$N[CH(CH_3)_2]_2$
430,028	
430,029	
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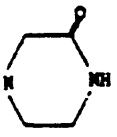
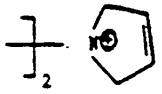
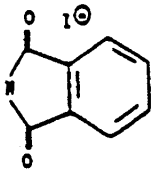
430,037	

	A
430,039	
430,046	$N(CH_2C_6H_5)_2$
430,056	
430,063	
430,038	

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	A
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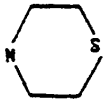
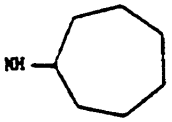
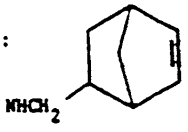
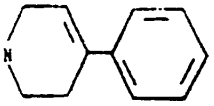
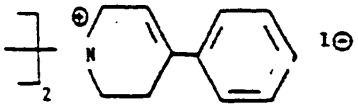
430,070:	
430,072:	
430,091:	
430,096:	
430,099:	$NHCH_3$
430,106:	$NH\ CH(CH_3)_2$
430,107:	$NH\ C(CH_3)_3$
430,109:	
430,108:	
430,122:	$NHCH_2C_6H_5$

	A
430,068:	
430,083:	
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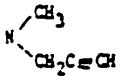
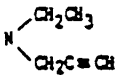
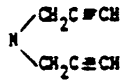
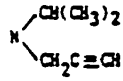
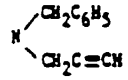
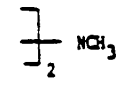
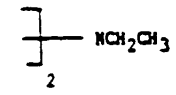
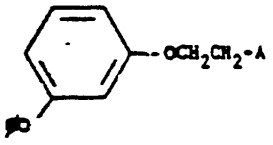
430,122:	
430,133:	
430,113:	
430,110:	
430,132:	
430,128:	
430,129:	

Table II (cont.)

Thymol Ether Analogs

Amine Variants



A

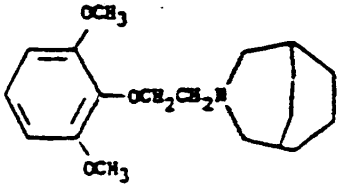
430,080: 

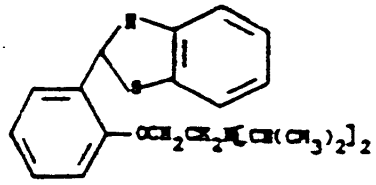
430,071: 

430,074: 

430,115: $NHCH_3$

430,114: $NHCH_2CH_3$

430,097: 

430,101: 

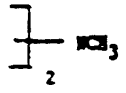
A

430,086: 

430,089: 

430,093: $N[CH(CH_3)_2]_2$

430,116: $NH\ CH(CH_3)_2$

430,127: 

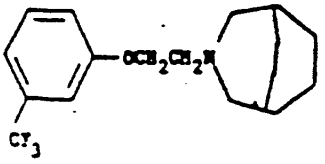
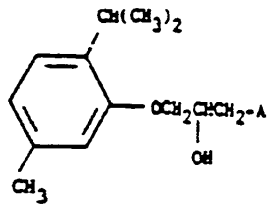
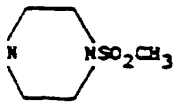
430,102: 

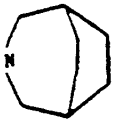
Table III

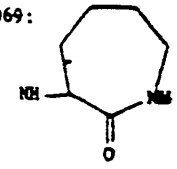
Thymol Ether Analogs

Chain Variants



430,066: 

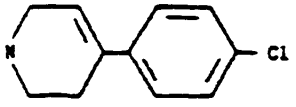
430,067: 

430,069: 

430,045 Cl

430,047 OH

430,054 

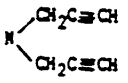
430,055 

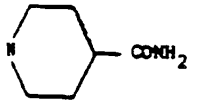
430,057 $N(C_2H_5)_2$

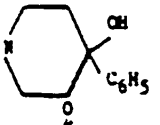
430,058 

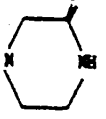
430,075: 

430,081: 

430,084: 

430,087: 

430,04- 

430,061 

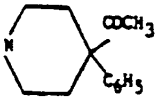
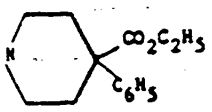
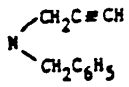
430,062 

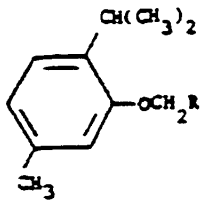
Table III (cont.)

430,064



430,065





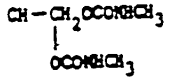
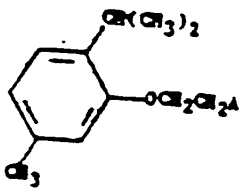
	R		R
430,044		430,048	

Table IV

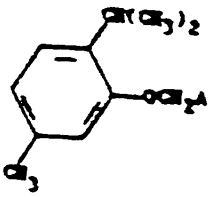
Thymol Ether Analogs

Hypothetical Metabolites

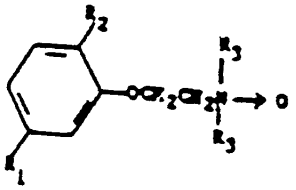


	A
430,121:	$O\rightarrow N(CH_2CH_3)_2$
430,131:	$N(CH_2CH_3)(CH_2CH_2OH)$
430,138:	CHO

	A
430,100:	$NHCH_2CH_3$
430,073:	NH_2



	A
405,106:	COOH

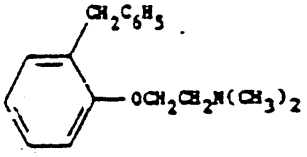


	R_1	R_2	R_3
430,134:	CH_3	$CH(CH_3)_2$	CH_3
430,135:	H	C_6H_5	CH_2CH_3
430,136:	CH_3	$CH(CH_3)_2$	
430,139:	CH_3	$C(CH_3)_3$	CH_2CH_3

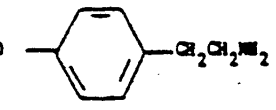
Table V

Thymol Ether Analogs

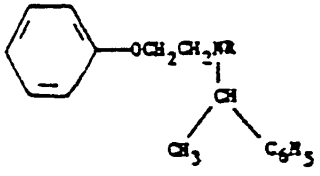
Miscellaneous



400,836 (Bristamine)

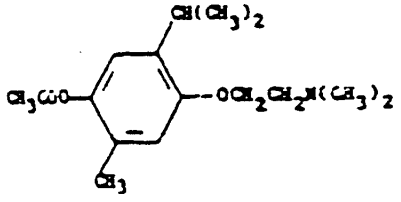


430,041



430,051 R = H

430,059 R = 



430,033 (Opilon)

430,019, is reported to be active in man; two other closely related compounds, 430,018 and 430,023, are reported not to show this activity in man, one of them, 430,023, even in doses as high as .00 mg. In addition, samples of two related commercially available drugs, Opilon (430,033) and Bristamine (400,836), which do not show incapacitating activity in man, have been procured for comparison.

A number of compounds related to 430,017 were then prepared with the aim of developing an agent that will produce the same type of incapacitation as 430,017 at even lower dose levels. These are arranged in Tables I through V according to structure. In the most general sense, the aryl moiety, the amino group, and the chain connecting the two were varied. Table I shows variations, such as changes in the size and nature of the substituents in the aromatic ring and changes in the positions of these substituents on the ring, that were meant to define what features of the aromatic moiety confer maximum activity.

Variations in the amino group (Table II) seemed particularly appropriate in view of the fact that a simple change from dimethylamino to diethylamino is involved in the transition from an inactive compound, 430,018, to an active one, 430,017. In many cases, another functional group -- amide, olefin, propargyl group, basic nitrogen -- was added in the hope of thereby increasing interaction with receptor sites and,hence, potency. One of these compounds, 430,038, was found to be about 7 times as active as 430,017 in the mouse screen, but failed to show any potency advantage in other species or any activity in man in doses up to 5 mg in testing conducted at the Army CRDL. A number of analogs with hindered amine groups were also prepared.

The length of the chain connecting the amino group and the aromatic group was lengthened by one carbon atom in some of the compounds (Table III), and a hydroxyl group was added so as to form a substituted 1-amino-2,3-propanediol pharmacophore. Various amino groups were used, many of them bearing additional functional groups as in the shorter chain series. These variants included a group that is related in structure to the potent neuroleptic haloperidol, which produces muscular incoordination at low doses.

The lack of correlation between the results observed with 430,017 and the related reference compounds in man, with those obtained to date in experimental animals, could possibly be due to a metabolic transformation of 430,017 that is unique to man. In particular, man might be relatively insensitive to 430,017 itself, but -- as suggested by the slow onset of action -- change it into an active metabolite. This possibility prompted the preparation of a number of hypothetical metabolites of 430,017 (Table IV). One logical possibility for such a metabolite would be the corresponding N-dealkylated amine, 430,100, which has

a structure suggestive of an adrenergic β-blocking agent and might block the activation of muscle phosphorylase. This compound was therefore prepared, together with several other related N-monoalkylated thymoxyethylamines (see also Table II). Results with 430,100 in the mouse screen were, however, disappointing. This bis-desalkylated compound, 430,073, was more active than 430,017 in the mouse screen, but not sufficiently so to be the active metabolite.

Another hypothetical metabolite of 430,017 is the N-oxide 430,121, which appeared to be an attractive candidate from several points of view: being more polar, it would be more apt to be concentrated in muscle than the highly lipophilic 430,017 free base; it might relate pharmacologically to the N-oxides which are physiological constituents of muscle in fish and, possibly, in mammals; and there are precedents among other quaternaries for some of the structural requirements for high activity -- diethyl rather than dimethyl substitution on N, a medium-sized ortho-substituent -- which seems so puzzling in the amines. The N-oxides of the other reference standards were prepared for comparison. The symptomatology of 430,121 in the mouse and the dog failed, however, to reveal any obvious potent incapacitating activity.

Other types of hypothetical metabolites include 430,131, which could arise by side-chain hydroxylation, and 430,138 and 405,106, by deamination.

The 96 analogs of 430,017 that were prepared specifically for the project were supplemented by 15 related compounds from the Pfizer files and 4 commercial drugs, so that altogether 115 compounds of this type are presently available for evaluation.

C. Synthesis of Aminoimidazoline Analogs

The primary objective of chemical modification in this series has also been the attainment of higher potency. The best results to date have been achieved by substitution of the aromatic ring with chlorine atoms (Tables VI and VII), which gave the most potent compound obtained so far, dichloronaphthylaminoimidazoline, 400,483, and 2 potent chloroanilinoimidazolines, 400,487 and 404,407. In the anilinoimidazoline series, 2 and 2,5-substitution by chlorine appeared to confer the highest activity; chlorine atoms located in the p-position and other types of substituents - alkoxy, trifluoromethyl, diethylsulfamyl — led to less active compounds. Alkyl substitution on the imidazoline ring generally decreased activity, while at the same time increasing lethality. Several aralkylaminodazolines (Table VIII) were less active than the arylaminodazolines.

A number of modifications of the aminoimidazoline function have also been tried, but so far none of these has yielded a compound that is more active than 400,386. They include a six-membered ring analog (400,432), guanidines, acylguanidines, glycocyamidines (Table IX), thioureas and isothiuronium salts (Table X). The 49 submittals listed in these tables include a considerable number of compounds that had been previously prepared at Pfizer, as well as the compounds prepared for the first time during the project. This list was supplemented by the following 31 additional related entries obtained from the Pfizer files:

400,463	402,714	404,746
400,466	402,715	404,833
400,559	402,720	405,066
400,648	402,722	405,247
400,806	403,439	405,249
401,236	403,684	405,504
401,273	403,496	405,505
401,427	404,013	405,506
402,005	404,404	406,040
402,026	404,405	452,559
		457,080

Many of these passed the mouse toxicity screen, although none was as active as 400,386.

D. Miscellaneous Compounds

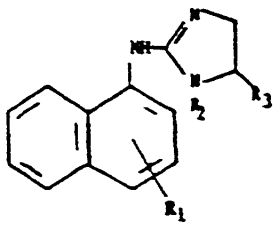
Continuing efforts are being made to uncover additional novel leads by perusal of the current literature, examination in depth of the published literature pertaining to certain types of compounds and use of the Pfizer files. The sections of the latter that are available on punched cards were searched by machine for compounds which had shown indications of interfering with muscular function. In addition, the results of current in-house therapeutic research programs were scanned regularly for compounds that had produced potentially incapacitating side-effects. These efforts resulted in the 101 compounds listed below being submitted to the mouse toxicity screen; however, none showed sufficient potency in the HOTS to merit further development.

400,945	402,016	402,307	402,755	405,236
401,242	402,023	402,316	402,951	405,263
401,249	402,033	402,317	403,215	405,480
401,320	402,034	402,320	403,315	405,534
401,356	402,072	402,326	403,316	405,700
401,460	402,073	402,329	403,324	405,774
401,500	402,079	402,334	403,340	405,773
401,523	402,146	402,337	403,413	406,006
401,539	402,185	402,364	403,436	406,007
401,641	402,253	402,365	404,081	456,729
401,724	402,269	402,367	404,245	456,730
401,727	402,278	402,381	404,346	456,731
401,846	402,280	402,473	404,466	456,732
401,908	402,281	402,492	404,580	456,733
401,942	402,282	402,499	404,825	456,734
401,977	402,284	402,514	404,966	456,735
401,991	402,302	402,528	405,017	456,736
401,993	402,303	402,537	405,160	456,737
401,994	402,304	402,563	405,165	456,738
401,995	402,305	402,747	405,228	456,739
				456,740

Table VI

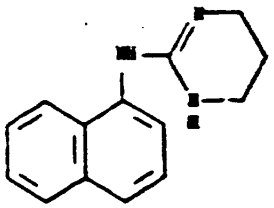
Analogs of 400,386

Naphthylaminoimidazolines



	R_1	R_2	R_3	Mocs Med_{50}*
400,386	H	H	H	0.032
400,483	Cl_2	H	H	0.01
400,433	H	H	Me	0.75
400,456	H	Me	H	> 1.0
400,628	H	$CH_2CH_2CH_3$	H	0.178
400,499	H	$COMC_6H_5$	H	> 1.0

400,432

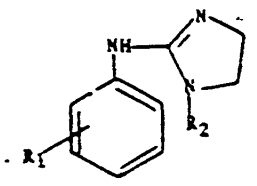


*mg/kg

Table VII

Analogs of 400,386

Substituted Anilinoimidazolines



	R1	R2	MOLS MED50*
400,777	H	Me	>1.0
400,735	H	Et	>1.0
400,611	M-EtO	H	>1.0
404,407	O-Cl	H	0.056
400,751	O-Cl	Me	>1.0
400,740	O-Cl	Et	>1.0
400,391	p-Cl	H	>1.0
400,679	p-Cl	Et	>1.0
400,465	2,4-Cl2	H	1.0
400,487	2,5-Cl2	H	0.01
400,725	2,5-Cl2	Et	>1.0
400,549	3,4-Cl2	H	0.316
400,772	3,4-Cl2	Me	>1.0
400,683	3,4-Cl2	Et	>1.0
400,759	3,5-Cl2	Me	>1.0
400,760	3,5-Cl2	Et	>1.0

*mg/kg

[redacted]

Table VII (cont.)

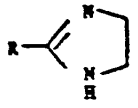
	R_1	R_2	MOT8 MED_{50}*
430,126	2-Cl-5-CF_3	H	0.1
430,103	2,5-Di-OCH_3	H	>1.0
430,105	2,4-Di-OCH_3-5-Cl	H	>1.0
430,120	2,5-Di-OCH_3-4-Cl	H	>1.0
430,130	2-OCH_3-5-$(CH_3CH_2)_2NSO_2$	H	>1.0

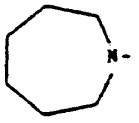
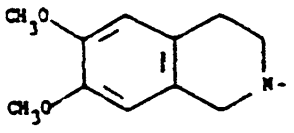
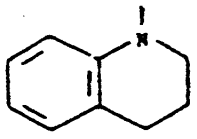
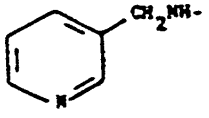
*mg/kg

Table VIII

Analogs of 400,386

Other Aminoimidazolines



		MOTS MED_{50}*
400,350		0.316
400,378		0.562
400,185		>1.0
400,769		>1.0

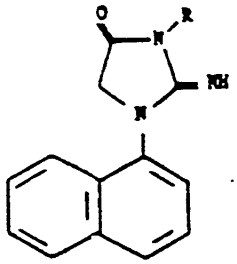
*mg/kg

Table IX

Analogs of 400,386

Guanidines and Glycocyamidines

	Ar	R	MDTS MED_{50}*
430,052	1-Naphthyl	H	>1.0
430,085	1-Naphthyl	$COCH_3$	>1.0
430,049	1-Naphthyl	COC_6H_5	>1.0
400,805	1-Naphthyl	$CH_2CH_2NHCH_3$	>1.0
430,053	2-Naphthyl	H	>1.0
430,050	2-Naphthyl	COC_6H_5	>1.0
430,082	2-Cl-5-$CF_3C_6H_3$	H	>1.0
430,079	2-Cl-5-$CF_3C_6H_3$	COC_6H_5	>1.0



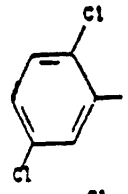
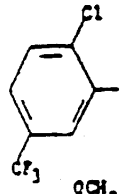
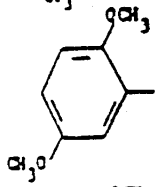
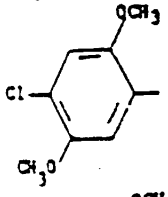
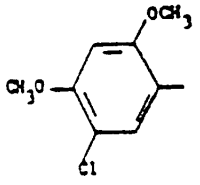
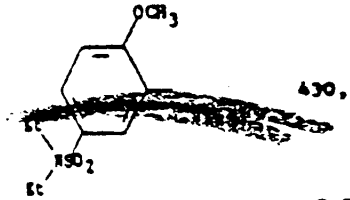
	R	
430,090	H	>1.0
430,111	CH_2CH_3	>1.0

*mg/kg

Table X

Analogs of 400,366

Thioureas and Isothiuronium Salts

	$NHCSNH_2$	SCH_3 NHC=NH	MSTS MED_{50}*
		430,094	>1.0
	430,125	430,124	>1.0 >1.0
		430,098	>1.0
	430,118	430,119	>1.0 >1.0
	430,095	430,104	>1.0 >1.0
	430,112	430,117	>1.0 >1.0

*mg/kg

IV. BIOLOGICAL PROGRAM

A. Screening Procedures

1. Primary Screening - Intravenous Mouse Toxicity Test (MOTS). The wide variety of measurable or observable drug effects that may result in incapacitation makes it a staggering undertaking to achieve new incapacitating agents. It is important to obtain in the earliest phase of screening the information essential to decide whether a compound is potentially incapacitating, so that compounds of little or no apparent value can be discarded quickly.

Observational screening of biological activities and toxicity in the mouse has been adopted as the standard primary screening procedure. The program is designed to screen or distinguish compounds having incapacitating potential from those that do not, as rapidly, comprehensively and inexpensively as possible.

The following procedures have been adopted in the program for screening and evaluating new agents.

1. Unless justified for a specific reason, intravenous injection is used throughout the entire program as a standardized procedure. Diluents used are, in order of preference: saline or water, dilute acid or alkaline, 10% aqueous ethanol or polyethylene glycols. Compounds which are insoluble in these solvents are injected as fine suspensions in 0.5% methylcellulose.

2. Primary screening is done in a minimal number of white male Swiss mice of approximately 20-30 gms. Observations of gross signs are made -- activity, mydriasis, ataxia, muscular signs, etc. Because of small numbers of animals per dose level, the statistical method of Weil and Thompson is used for the estimation of the LD_{50} and the MED50.

[redacted] (b)(3)

Other factors considered (besides MED50)

[redacted]

During the year, 290 compounds were tested in the primary mouse screening. Fifteen thymol ether related, 21 aminoimidazoline related and [redacted] miscellaneous compounds produced biologic activities at intravenous doses of less than 1 mg/kg body weight (Table XI).

~~CONFIDENTIAL~~

Table XI

MOTS Data

[redacted] (b)(3)

Type of Compound	Compound No.	MED50 mg/kg	LD50 mg/kg	LD50/MED50
Thymol Ether Related	430,038	0.24	31.6	133.0
	403,208	0.316	28.2	89.2
	430,039	0.316	31.6	100.0
	430,033	0.562	44.7	79.5
	430,035	0.562	39.8	70.8
	430,043	0.562	28.2	50.2
	430,048	0.562	10.0	17.8
	430,064	0.562	79.4	141.0
	430,073	0.562	44.7	79.5
	430,075	0.562	44.7	79.5
	430,076	0.562	35.5	63.2
	430,027	0.750	25.1	33.5
	430,051	0.750	20.0	26.7
	430,054	0.750	35.5	47.3
	430,056	0.750	12.6	16.8
Aminoimidazoline Related	400,483	0.01	22.4	2240.0
	400,487	0.01	31.6	3160.0
	400,386	0.032	20.0	625.0
	400,648	0.056	89.1	1591.0
	404,407	0.056	44.7	795.0
	430,126	0.10	39.8	398.0
	400,559	0.178	15.8	88.8
	400,628	0.178	2.51	14.1
	404,404	0.178	22.4	126.0
	400,348	0.316	79.4	251.0
	400,350	0.316	17.8	57.6
	400,466	0.316	28.2	88.1
	400,549	0.316	20.0	63.3
	400,378	0.562	31.6	56.2
	402,714	0.562	79.4	141.0
	404,405	0.562	31.6	56.2
	404,746	0.562	7.08	12.6
	404,833	0.562	15.8	28.1
	405,247	0.562	70.8	126.0
	405,504	0.562	35.5	63.2
	400,433	0.750	28.2	37.6
Miscellaneous	402,514	0.178	12.6	70.8
	456,736	0.237	14.1	59.5
	402,278	0.316	2.24	7.09
	402,316	0.316	7.94	25.0
	403,315	0.316	7.08	5.76
	402,747	0.562	15.8	28.1
	404,825	0.562	5.01	8.91

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2. Secondary Screening. Dogs (rats, guinea pigs, rabbits and/or cats) are used in secondary screening for pharmacological activity. Lethality is checked for rough limits only. All new compounds having an MED50 of [redacted] less per kg body weight in the MOTS were subjected to secondary screening. In secondary screenings, no thymol ether related compound produced biological activity at less than 1 mg/kg (Table XII). Eight of the aminoimidazoline related compounds produced biological activities at less than 0.1 mg/kg in the dog. The most potent among these are 400,483 and 400,368. The pharmacological activities of these compounds will be discussed in section IV.C. of this report.

(b)(3)

3. Pharmacologic Studies of Thymol Ethers

Given the fact that 430,017 is active in man, the first problem that presented itself was to find an assay that could be used to screen new compounds in animals for the desired activity in man. The selection of such a screen is rendered more meaningful by the fact that human data are available on three other very closely related compounds. One of them, 430,019, has the same effect in man as 430,017, while the other two, 430,018 and 430,023, are innocuous in man at much higher doses. One of the obvious requirements of an appropriate animal screen is, then, that it should clearly differentiate between the two compounds that are incapacitating in man and the two that are not.

The four reference compounds were first injected into various species of animals to see whether any symptoms relatable to the effects in man could be observed. These studies led to two main conclusions. First of all, no symptoms or signs appeared in the mouse, dog, or monkey with any of the compounds until doses which were well in excess of those at which 430,017 produced incapacitation in man were reached. Secondly, none of the symptoms produced could be correlated with the known activity of the four compounds in man. For instance, there were no marked differences among the rather high MED50 the four compounds in the mouse symptomatology screen.

One of the thymol ether related compounds, 430 ., which was shown to have greater biologic activities than 430,017 in the mouse and rat toxicity tests, was later found by the ACRDL to have no incapacitating activity in man at a dose of up to 5 mg whereas 430,017 produced symptoms in man at a dose of [redacted] similar to those reported in the literature. It would appear, therefore, that neither the mouse nor the rat toxicity test is able to distinguish between the incapacitating and the non-incapacitating thymol ethers.

(b)(3)

XII

Secondary Biologica. Screening Data

Type of Compound	Compound #	Species	MED50 mg/kg	LD50 mg/kg
Thymol ether related	430,038	Rat	1.33	17.8
	430,038	Dog	>1, <5	>5.0
	430,038	Monkey	>10 (i.p.)	
	403,208	Dog	>1.0, <5.0	>5.0
	430,039	Dog	>1.0, <5.0	
	430,035	Dog	>1.0, <5.0	
	430,043	Dog	~1	
	430,048	Dog	>1	
	430,064	Dog	>1	
	430,073	Dog	>1.0, <5.0	
	430,073	Rabbit	>3.16	
	430,073	Guinea Pig	>3.16	
	430,075	Dog	>1	
	430,076	Dog	>1	
	430,027	Dog	>5	
	430,051	Dog	>1	
	430,054	Dog	>1	
	430,056	Dog	<1	
	430,021	Dog	<5	
	430,021	Rabbit	>3.16	
	430,022	Rabbit	>1, <5	
Aminoimidazoline related	400,483	Rat	0.002	3.2
	400,483	Dog	0.001	1.5
	400,487	Dog	~0.1	>5.0
	400,386	Rat	0.006	22.4
	400,386	Dog	0.01	1.5
	404,407	Dog	<0.1	
	430,126	Dog	<1.0	
	400,559	Dog	>1.0	
	400,628	Dog	>1.0	
	404,404	Dog	<0.1	
	400,348	Dog	<0.1	
	400,350	Dog	>1.0	
	400,466	Dog	<0.03	
	400,549	Dog	<0.1	
	400,378	Dog	compd. not avail.	
	402,714	Dog	>1.0	
	404,405	Dog	<0.1	
	404,746	Dog	>1.0	
	404,833	Dog	>1.0	
	405,247	Dog	not done	
	405,504	Dog	1.0	
	400,433	Dog	compd. not avail.	
	405,505	Dog	1.0 or less	

A lack of correlation was also obtained when other criteria, such as various specific symptoms or the LD50, were applied. Similarly, the dog required relatively high doses to elicit symptoms, and of these, ataxia did not correlate with the known activity in man at all, while emesis did show some correlation when a certain dosing regimen was followed.

The compounds were also injected into dogs and monkeys that were subjected to the various performance tests at the Army CRDL. All four compounds were again found to be alike in potency and effects in these animals.

Pharmacological studies made on diethylamino thymoxy ether (430,017) and its 3 closely related derivatives (430,018; 430,019 and 430,023) are summarized below.

1. Primary and Secondary Screening

a) Mice and Rats. Symptoms produced by the four thymol ethers were compared to see if there were any common to the compounds active in man and absent in the others. It seemed from these studies that no correlation existed.

Recently the ACRDL had requested that the Hazelton Laboratories compare the intravenous MED50 of 430,017 and 430,038. Their tests suggested that tremors were produced by 430,017 and not by 430,038. Re-examination of our earlier data showed that 430,038 also produced tremors. The LD50 and MED50 of 430,017 and 430,038 obtained by Pfizer Laboratories and Hazelton Laboratories were very similar.

Table XIII

LD50 and MED50 of 430,017 and 430,038 In Mice

Compound	Species	MED50 mg/kg	LD50 mg/kg
430,017	mouse	1.78 (1.07-2.96)	35.5 (28.2-44.7)
430,038	mouse	0.237 (0.133-0.421)	31.6 (14.1-70.8)
430,017	rat	0.215 (0.065-0.715)	22.4 (17.8-28.2)
430,038	rat	1.33 (0.750-2.37)	17.8 (12.6-25.1)

b) Guinea Pigs. In guinea pigs, no significant symptoms or signs were noted either after oral or intravenous administration of 1 to 5 mg of the following thymol ethers: 430,017; 430,018; 430,019 and 430,023. It is to be recalled that compounds 430,017 and 430,019 have been reported to produce incapacitating effects in man, while 430,018 and 430,023 did not.

c) Rabbits. To see whether using other species would enable us to differentiate among the thymol ethers, 430,017; 430,018; 430,019 and 430,023 were administered intravenously and orally at 1 mg/kg to rabbits. All four compounds produced the same minor symptomatology after intravenous administration. The only unusual sign was increased muscle response when the haunches were squeezed, but this response was elicited by all compounds. After oral administration, this muscle response was the only observable sign.

d) Dogs. The outstanding symptoms produced by some thymol ethers are emesis and bloody urine. A positive correlation may exist between incapacitation in man and emesis in dogs after large doses, e.g. 20 mg/kg (Table XIV). However, if retching is also considered as a positive response, this correlation disappears. Further investigation concerning this phenomenon is in progress.

2. Advanced Pharmacologic Studies

a) Effects on Blood Pressure. Since the thymol ether, 430,017, has been reported to lower the blood pressure in man, each of the 4 thymol ethers was given intravenously, 2 and 8 mg/kg, to anesthetized rats. All caused a substantial drop in blood pressure. There was no apparent difference between the two groups (Table XV).

b) Effects on Electrolyte Metabolism. A mechanism which might explain the observed muscle pain is the loss of intracellular potassium. Accordingly, studies were made in rats to investigate the effects of the thymol ethers on electrolyte excretion and tissue electrolyte concentrations. Rats dosed orally with 430,017, 20 and 60 mg/kg, exhibited a significant increase in urine volume, output of potassium ion and uric acid, but no change in sodium or chloride ion excretion. However, six structurally related compounds including 430,018; 430,019 and 430,023 were also tested at 20 mg/kg, and none had a pronounced effect on potassium excretion. It is concluded that there is no correlation between effects on potassium excretion in the rat and incapacitating symptoms in man.

To test the effect on tissue electrolytes after repeated doses, 430,017 was given orally to ten rats, 60 mg/kg/day, for 15 days. At the end of this period, sodium and potassium ion concentrations were

Taole XIV

Emesis Following Intravenous Injection of Thymol Ethers

Dog #	Compound # 430,017	430,018	430,019	430,023	Emesis*	Retching*
8588	E (1)		O-R (3)	O (2)	1/3	1/3
D-8	E (2)	O-R (1)		O (3)	1/3	1/3
8283	O (3)	O-R (2)	E (1)		1/3	1/3
D-106		O (3)	E (2)	E (1)	2/3	0/3
Emesis	2/3	0/3	2/3	1/3		
Retching	0/3	2/3	1/3	0/3		
Total Response	2/3	2/3	3/3	1/3		

( ) = day compound was injected
E = emesis; O-R = retching; O = no response
*No. of animals showing responses/no. of animals tested

Table XV

Effects of Prototype Thymol Ethers
on
Blood Pressure in Anesthetized Rats

Compound	DOSE, i.v. 2 mg/kg	8 mg/kg
430,017	slight transient fall	↓75 mm. (>30 min)
430,018		↓80 mm. (>30 min)
430,019		↓65-90 mm., ↑ 10 mm. (<20 min)
430,023		↓20-25 mm., ↑ 10-25 mm. (<20 min)

measured in skeletal muscle, aorta, and plasma (Table XVI). Although there appeared to be some changes in aorta and plasma electrolytes, there were no alterations in either sodium or potassium content of the skeletal muscle.

Table XVI

Effects of 430,017 on Tissue Electrolytes in Rats

	MUSCLE[a]		AORTA[a]		PLASMA	
	Na^+ mEq/g	K^+ mEq/g	Na^+ mEq/g	K^+ mEq/g	Na^+ mEq/l	K^+ mEq/l
Control	103.2±2.9	400.3±5.1	400.4±13.3	169.5±11.6	127.7±5.9	6.72±0.5
430,017	100.0±1.6	395.1±3.4	367.3±13.6	149.5±4.5	136.7±1.6	4.98±0.3

[a]The values of Na^+ and K^+ in muscle and aorta are expressed mEq/g of dry tissue.

c) Histaminic and Antihistaminic Effects

1. Bronchoconstriction. Since the literature suggests that 430,017 has histamine like action in addition to being a potent antihistaminic, the four prototype thymol ethers were compared for their bronchoconstrictor effects in the guinea pig (Konzett preparations). After intravenous injection, 10 mg/kg, all four compounds have bronchoconstrictor effects. Quantitative differences seemed to exist between these four compounds. The order of activity was as follows: 430,017 > 430,019 > 430,018 > 430,023 (Table XVII).

Almost all of the four compounds are antagonists to the bronchoconstrictor action of histamine, serotonin or acetylcholine, but no differentiating pattern could be established to distinguish the incapacitating from the non-incapacitating thymol ethers.

Table XVII

Comparison of Prototype Thymol Ethers

Bronchoconstriction*

Compound	Dose mg/kg i.v.	No. Animals	% Increase in Baseline	% Inhibition to 5-HT	Hist.	Ach.
430,017	1	3	3	7	74	31
	5	2	25	74	97	29
	10	1	304	--	--	--
	20	2	254	--	--	--
430,018	1	3	0	56	96	17
	10	1	121	100	100	89
	20	2	294	100	100	96
430,019	1	3	0	0	15	4
	5	1	91	21	93	0
	10	3	205	63	100	3
430,023	1	3	7	6	16	0
	10	3	87	81	100	52
	20	2	386	--	--	--

*Konzett Preparation, male guinea pigs anesthetized with sodium pentobarbital, 40 mg/kg i.p.

ii. Dermal Wheal. Although 430,017 was originally reported in the literature as an antihistaminic agent, it was also demonstrated to possess histamine-releasing properties. We have further explored whether a correlation might be found between histamine release and the incapacitating action of thymol ethers in man. The four key thymol ethers, 430,017; 430,018; 430,019 and 430,023, were injected intradermally to groups of guinea pigs using 0.1 ml of 1.0, 0.1 and 0.01% solutions. All compounds evoked equivalent wheal responses.

In guinea pigs pretreated by an antihistamine (Benadryl, 20 mg/kg i.p.), dermal wheals were produced by a 0.01% solution of both 430,018 and 430,019; wheal formation did not occur or was much reduced for 430,017 and 430,023. Comparable wheal formation was seen for all compounds at the 0.1 and 1% concentrations. There is, therefore, no correlation between histamine or histamine releasing activity and incapacitating action in humans.

d) Behavioral Effects

i. Army CRDL Evaluation. The four prototype thymol ethers, 430,017; 430,018; 430,019 and 430,023 were tested at the ACRDL in the conditioned avoidance response, sustained physical exercise and visual discrimination tests. The results, as provided by Dr. Wills, are shown in Table XVIII. The high dose levels required and the absence of correlation between the effects of 430,017 and 430,019, as compared with 430,018 and 430,023, indicate that these tests are not discriminatory for the incapacitation seen with the former pair in man.

Retching, usually slight to moderate and not dose consistent, was reported to occur in the test animals with all compounds at the two higher dose levels. Additional symptoms seen in these tests included decreased activity, hyperpnea, ataxia and convulsions.

ii. Pfizer Evaluation. 430,017 was tested for its effects on conditioned avoidance behavior in a standard rat assay procedure. No disruptive effect on conditioned avoidance was observed at 3.2 or 10 mg/kg i.p. in the rat.

This compound was also injected intraperitoneally into six pigeons, three at 1 mg/kg and three at 3.2 mg/kg. At 1 mg/kg, there was no consistent effect, although two animals exhibited ptosis, and one appeared bloated. At 3.2 mg/kg, ptosis and a crouching posture were noted. The experiments in pigeons are to be expanded. In addition, studies are planned for the investigation of the behavioral effects of the thymol ethers in squirrel monkeys.

e) Rat Swim Test. In the rat swim test, which in effect is a test of muscle performance, all four compounds significantly decreased swimming time. Preliminary trials with 430,017 did not demonstrate a significant effect on swimming time after a single dose. However, a pronounced reduction was seen if the compound was given during recuperation from a preliminary swim, and then the animals were put for a second time in the tank. Additional evidence suggested that this effect was greatest when the drug was injected shortly after completion of the first swim.

The method employed was essentially that reported by Laborit, et al. (Comp. rend. soc. Biol., 151: 1383, 1957). The bath temperature was maintained at 20 ± 1°C in order to shorten the swimming time. The tank, approximately 2 feet in diameter, was filled with 18 inches of water leaving approximately 7 inches to the top edge of the tank so that the rats could not climb out. The water was agitated by a stream of air from the bottom of the tank. Rats, in groups of six, were left to swim in the water until exhausted (staying below the surface for at least 20 seconds and having apparent difficulty returning to the surface).

Table XVIII

Comparison of Behavioral Effects of Prototype Thymol Ethers

CAR, SPE, VDT[a]

Compound and Test	3.16 mg/kg i.v.		5.8 mg/kg i.v.		10.0 mg/kg i.v.	
	Physical Effects[b]	Behavioral Effects	Physical Effects	Behavioral Effects	Physical Effects	Behavioral Effects
430,017						
CAR	+,+	0,0	+,+	0,0	++,	+,
SPE	0,0	0,0	+,+	0,++	++,	0,
VDT	0,+	0,+	+,+	+,+	+,	0,
430,018						
CAR	+,+	0,0	+,++	0,0	+,	0,
SPE	0,0	0,0	+,++	0,0	+++,	0,
VDT	0,+	0,+	+,+	++,++	+,	+++,
430,019						
CAR	0,0	0,0	+,+++	0,0	+,	0,
SPE	0,0	0,0	,+++	0,+++	++,	++
VDT	0,0	0,0	0,0	0,+++	0,	+,
430,023						
CAR	0,0	0,0	+,++	0,+	+++,	+++,
SPE	0,0	0,0	+,++	0,++	+++	0
VDT	0,0	0,0	0,+	0,+	+++c	+++c

(a) CAR = Conditioned Avoidance Response; SPE = Sustained Physical Exercise; VDT = Visual Discrimination Test. Data provided by ACRDL.

(b) 0 = No Effect; + = Slight Effect; ++ = Moderate Effect; +++ = Severe Effect

(c) Scrub Monkey - at 10 mg/kg - convulsed 1 to 5 min post drug.
VDT monkey at 9 mg/kg convulsed 1 to 10 min post drug. Marked VDT effects during 1st hr only.

They were then removed from the tank, wiped dry, and the elapsed swimming time recorded for each rat. The animals were returned to the tank 60 minutes after the beginning of the first swim.

The four prototypes were injected (10 mg/kg i.v.) 5 minutes after the first swim along with a saline control and swimming times in the second swim were compared. The data (Table XIX) indicate that all four compounds significantly diminished swimming time as compared to the control in the second swim. In order of potency they were: 430,018 > 430,023 > 430,017 > 430,019. It was of interest that the greatest effects were seen in the second swim, and appeared to be related to interference with recuperation from muscle work. We have to conclude, however, that the test was not discriminating for the type of activity of present interest.

Table XIX

Comparison of Prototype Thymol Ethers

Rat Swim Test

	SWIM TIME, MINUTES									
	Controls		430,017		430,018		430,019		430,023	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	9.42	13.32	9.32	7.93	10.00	3.45	10.83	11.82	8.92	5.68
	9.66	13.95	10.35	-	10.92	5.50	11.28	8.03	9.83	6.75
	11.35	11.38	10.70	7.97	12.17	3.18	12.23	9.62	11.67	3.53
	12.90	10.40	12.58	6.25	12.75	1.88	12.50	8.15	12.43	8.03
	14.53	9.03	12.63	5.95	13.13	5.67	12.92	7.60	14.27	6.53
	14.88	15.12	13.38	7.80	18.62	5.53	18.62	4.78	18.62	5.68
Median	-	12.35	-	7.80	-	4.47	-	8.09	-	6.10
Mean	-	12.20	-	7.18	-	4.20	-	8.67	-	6.03

f) Blood Lactic Acid Levels after Exercise. A comparison of the similarities of symptoms and signs in individuals affected by McArdle's disease (Rowland, et al., Arch. Neurol., 9: 325, 1963; J.A.M.A., 185: 860, 1963), and the clinical manifestations in man receiving an oral dose of 430,017, have provided a clue to the possible mechanism of action of 430,017 in man. McArdle's disease is attributed to a rare hereditary absence of muscle phosphorylase. Its characteristic symptoms include pigmenturia and the inability to sustain exercise due to the development of painful cramps. Biochemically, in McArdle's disease, there is a lack of utilization of muscle glycogen so that the usual elevation of lactic acid after exercise, especially ischemic exercises,

does not occur. The marked similarities between the symptoms of the disease and those noted after the administration of 430,017 suggest the possibility that the latter may act by interfering with muscle phosphorylase. Accordingly, animal experiments to measure the effect of 430,017 and its congeners on lactic acid levels after exercise were made.

Table XX

The Effect of Thymol Ethers on
Blood Lactic Acid Levels in Rats after Exercise (Swim)

Compound	Dose mg/kg i.v.	(N)	Mean Lactic Acid µg/ml, Blood	% of Control	p+
430,017	10	(14)	387.43	62.1	<0.001
Control	-	(14)	624.07	100	
430,018	10	(14)	405.78	64.8	<0.01
Control	-	(14)	625.86	100	
430,019	10	(13)	635.54	95.3	>0.10
Control	-	(14)	666.71	100	
430,019	10	(14)	556.9	95.2	>0.10
Control	-	(14)	584.4	100	
430,023	10	(14)	303.00	67.6	<0.01
Control	-	(14)	447.86	100	
430,038	6.3	(14)	497.79	91.3	>0.10
Control	-	(14)	544.86	100	
*430,017	10	(14)	164.00*	95.3	>0.10
*Control	-	(14)	172.00*	100	

*Samples drawn from unexercised rats (no swim)
+Non-parametric (Rank Sum) test
(N) = number of rats per treatment group.

Compound 430,017 depressed elevation of blood lactic acid after exercise (swim), but 430,019 and 430,038 both failed to have a similar effect. Compounds 430,018 and 430,023, which were reported not to produce incapacitation, also showed a reduced lactic acid level (Table XX).

g) Effects on ATP-Creatin[ine] Trans-phosphorylase. This in vitro test conducted by the ACRDL d[e]termines the ability of a compound to inhibit the enzyme ATP-creati[ne] trans-phosphorylase, which plays a very important role in muscle [fu]nction. A recent report from the pharmacology branch of the AC[RDL] (Quarterly Progress Report, U.S. ACRDL, Jan.-March, 1964)indicated th[at] a concantration of 1 x 10-5M of the five thymol ethers, 430,017; 430,0[18]; 430,019; 430,023 and 430,038, has no significant inhibitory effect [on] muscle ATP-creatinine trans-phosphorylase.

h) Adrenergic Blocking Ef[fects]. Since the symptoms produced by 430,017 in man seem to resemb[le] those noted in patients with McArdle's disease, which is characteriz[ed] by the lack of phosphorylase a, and since epinephrine is known to [be] able to activate phosphorylase from form b to form a, it was post[ulat]ed that the thymol ethers might produce the incapacitating sympt[oms] by interfering with epinephrine activation of phosphorylase. Sev[eral] experiments were made attempting to demonstrate this point.

i) Effects on Free Fatty Acid Release by Norepinephrine. When segments of rat epididymal adi[pos]e tissue are incubated with norepinephrine, free fatty acids (FFA) [a]re released. Each of the four thymol ethers, 430,017; 430,018; 430,[019] and 430,023, was added to this system and the per cent inhibition of [FF]A release produced by norepinephrine was measured (Table XXI). This [t]est also failed to differentiate the thymol ethers.

Table XXI

Inhibition of Norepi[nep]hrine-Induced Free Fatty Acid Release from Rat Epid[idy]mal Adipose Tissue in vitro

Compound	Concentration	% Inhibition
430,017	10^{-3}M	42
	10^{-4}M	0
430,018	10^{-3}M	30
	10^{-4}M	22
430,019	10^{-3}M	59
	10^{-4}M	36
430,023	10^{-3}M	41
	10^{-4}M	0

ii. Isolated Cat Papillary Muscle. In isolated cat papillary muscle preparations, isoproterenol induces an increase in contractile force of the heart muscle. A β-adrenergic blocking agent blocks this phenomenon. We have tested only compound 430,017 at a concentration of 1 x 10^{-5} g/ml. It did not antagonize the isoproterenol induced increase in contractile force of isolated papillary muscle. DCI and Nethalide both have a blocking effect at this concentration.

i) Effects on Muscle Work and Muscle Glycogen

i. Cats. In further pursuing the analogy of effects produced by 430,017 to symptoms manifested in individuals affected with McArdle's disease, it was considered worthwhile to examine the response of muscle to work as well as the fate of muscle glycogen after the administration of the different thymol ethers. The tendon of each gastrocnemius in spinal cats was attached to an isometric lever. Both sciatic nerves were cut high in the thigh and the peripheral ends placed on shielded electrodes for stimulation. Repetitive stimuli of 1-5 v., 1.0 msec. duration, 2 per second, were applied from a Grass S4C stimulator, while 125 gm. weights were placed on the tendons. There was no significant difference in muscle performance during this forced work between thymol ether dosed or undosed animals.

Small samples of the gastrocnemius were taken during these experiments for glycogen determinations. A sample taken before stimulation was used as the control. Samples were taken immediately after a 20-minute stimulation and then after appropriate periods of rest. The glycogen values were expressed as per cent of the control. To date, no clear-cut pattern of muscle glycogen response is discernible. Further work will be undertaken to clarify the effects of thymol ethers on muscle glycogen.

ii. Guinea Pigs. Groups of 5 guinea pigs each were treated with 430,017 and 430,038, 1 mg/kg i.v., on three successive days. One hour after the last dose, muscle samples were taken for glycogen determination. A decrease in the muscle glycogen level was noted in animals receiving 430,017 (Table XXII). The large standard deviation found with 430,017 arose from a marked decrease of muscle glycogen in some animals in each group, while the muscle glycogen levels of the remainder were similar to that of control animals; it is difficult to interpret these results at the moment. Further experiments will be undertaken to explore the effects of the different thymol ethers on muscle glycogen.

Table XXII

Effect of Thymol Ethers on Muscle Glycogen in Guinea Pigs

Compound	Dose* mg/kg	Route	Muscle Glycogen mg%
430,017	1	i.v.	596 ± 720
430,017	1	p.o.	658 ± 369
430,038	1	i.v.	912 ± 62
430,038	1	p.o.	980 ± 165
Control	-	-	1,000 ± 103

*For 3 successive days

iii. Mice. In preliminary experiments, attempts were also made to test the effect of thymol ethers on muscle glycogen after exercise in mice. Normal undosed mice were forced to exercise for ten minutes on the rotorod. Gastrocnemii and diaphragms were analyzed for glycogen content immediately after exercise and after rest periods of 1/2, 1 and 2 hours. The data (Table XXIII) showed a decrease in glycogen content of borderline significance only in diaphragms immediately after exercise. The glycogen content of both muscles was significantly elevated after a rest of 1 hour.

Table XXIII

Normal Mice
Muscle Glycogen after Exercise

	Control	Rest (Hours) 0	1/2	1	2
Gastrocnemius	181 ± 78	180 ± 51	213 ± 62	273 ± 39	236 ± 61
"p"		0.9	0.5	<0.05	0.3
Diaphragm "p"	167 ± 68	96 ± 36	218 ± 77	297 ± 99	201 ± 49
		0.1	0.4	<0.05	0.4

C. Substituted Aminoimidazolines

During the course of the research program, a total of 88 2-aminoimidazoline related compounds were screened in primary screening and 21 in secondary screening. Of those tested, compounds 400,386; 400,483 and 400,487 were found to have unusually high biological activity, and compound 400,483 is the most potent of all.

The pharmacological profile of the 3 compounds are summarized below:

1. Primary and Secondary Screening Data for 400,386, 400,483 and 400,487

a) Mouse and Rat Toxicity. The MED50 and LD50 of the 3 aminoimidazoline derivatives 400,386; 400,483 and 400,487 in the mice and rats, are shown in Table XXIV.

In the mouse, 400,483 and 400,487 both have an MED50 of 0.01 mg/kg. In the rat, 400,483 is unusually potent and also highly toxic (MED50 = 0.0018 and LD50 = 3.16 mg/kg).

b) Dog

i. General Symptomatology. In dogs, 400,483 is more potent than both 400,386 and 400,487. Like 400,386 and 400,487, its principal pharmacological effects were those related to the central nervous and cardiovascular systems (Table XXV). Bradycardia was noted after the intravenous injection of 0.001 mg of 400,483 per kg body weight. The heart rate was reduced from 100 beats per minute to 60 beats per minute after this dose. A slight decrease of motor activity was also evident. With increasing doses up to 1 mg/kg, the overt symptoms consisted of: blanched gums and ears (indicating vasoconstrictor effects), ataxia, exophthalmos, mydriasis, recurrent clonic spasms, clonic-tonic convulsions, salivation, nasal discharge, piloerection and emesis. Compound 400,483 seemed to be 5 to 10 times more potent than 400,386 and considerably more potent than 400,487. The lethal dose of both 400,483 and 400,386 in dogs was approximately 1.5 mg/kg i.v. The lethal dose of 400,487 was greater than 5 mg/kg.

ii. Potentiation of Effects by Atropine in Dogs. The duPont Laboratories reported that the central nervous system depressant effects of 119902, a substituted oxazoline, is potentiated by the simultaneous administration of atropine or scopolamine, we have explored the effects of atropine on the central nervous system depressant effects of 400,386; 400,483 and 400,487. A slight potentiation of the central nervous system depressant effects was noted with 400,386 and 400,487 (Table XXVI). A definite potentiation of the CNS depressant effects was noted when 400,483 and atropine were given together. Atropine also antagonized the bradycardia effect of all 3 compounds. Instead of bradycardia, tachycardia was usually observed in dogs receiving one of the substituted 2-aminoimidazoline and atropine.

Table XXIV

House and Rat Toxicity Screen

400,386-Related Compounds

MOTS

Compound	MED50 mg/kg	LD50 mg/kg	LD_{50}/MED_{50}	mg/kg	Symptoms and Signs
A. MOUSE					
400,483	0.01	22.4	2240.0	(0.01)	exophthalmos blank stare phonation-induced piloerection
400,487	0.01	31.6	3160.0	(0.01)	muscle tone inc. sensitivity to touch inc. aggressive phonation-induced dec. activity piloerection
400,386	0.032	20.0	625.0	(0.032)	inc. respiratory depth + rate
				(0.1)	exophthalmos piloerection dec. activity ataxia phonation-induced
B. RAT					
400,483	0.0018	3.16	1859.0	(.003)	dec. act., vasoconstr.
400,487	0.018	56.2	3160.0	(.1)	exoph., pilo.,vasoconstr.
400,386	0.0056	22.4	4000.0	(.01)	vasoconstr., respir., rate dec.

Table XXV

General Symptomatology in Dogs
of Compounds
400,483; 400,487 and 400,386

Dose mg/kg i.v.	400,483	400,487	400,386
0.001	Blanching ++ Dec. activity + Bradycard. 100-60 b/min.		
0.003	Blanching Dec. activity ++ Ataxia + Bradycard. 104-76 b/min		
0.01	Blanching + Dec. activity + Ataxia + Bradycard. 104-80 b/min.	No effects	Dec. activity Ataxia
0.032	Blanching +++ Dec. activity ++ Ataxia ++ Exophthalmos + Bradycard. 104-44 b/min.	No effects	Blanching ++ Dec. activity ++ Ataxia + Emesis Bradycard. 80-40 b/min.
0.1	Blanching +++ Dec. activity +++ Ataxia +++ Exophthalmos + Convul. of H. qtrs. Muscle spasticity Fasciculation of H. qtrs. Bradycard. 76-40 b/min.	Dec. activity +	Blanching +++ Dec. activity ++ Ataxia +++ Emesis Bradycard. 80-36 b/min.
1.0	Blanching ++++ Mydriasis Sub-convul. jerking Clonic-tonic convul. Exophthalmos Nasal discharge Piloerection Recurrent clonic spasms Emesis Bradycard. 76-24 b/min.	Blanching ++ Dec. activity +++ Ataxia ++ Exophthalmos Tremors Convulsion Piloerection Bradycard. 80-34 b/min.	Mydriasis Exophthalmos Irreg. respiration Emesis Lacrimation, salivation Inability to walk Tremors & convulsions Catatonia Heart rate not taken Death -- 4 1/2 hr.
1.5	Death -- 1 hr.	No death up to 5.0 mg/kg	Death -- 20 hr.

Table XXVI

Symptomatology in Dogs

Effects of Atropine on 400,386, 400,487 and 400,483

Compound	Route	Dose mg/kg	Symptoms & Signs
400,386 + Atropine	i.v. i.v.	0.1 } 0.2	Dec. activity and ataxia ++ Slight tachycardia Emesis (at 2 min. & 5 hrs.) Piloerection Exophthalmos Mydriasis Pupillary light reflex loss Fasciculation of h. quarters
400,487 + Atropine	i.v. i.v.	0.1 } 0.2	Dec. activity and ataxia +++ Slight tachycardia Emesis (at 35 min., 105 min. & 12 hrs.) Mydriasis Pupillary light reflex loss Fasciculation of h. quarters
400,483 + Atropine	i.v. i.v.	.03 .2	Dec. activity and ataxia Tachycardia Pupillary light reflex loss Mydriasis Deep sleep. Can be aroused Muscle weakness

2. Advanced Pharmacologic Studies of 400,386, 400,487 and 400,483

a) Effects on Blood Pressure. The effects of the 3 aminoimidazolines on blood pressure of anesthetized rats were determined (Table XXVII).

Compounds 400,386 and 400,483 both have very potent pressor effects.

[redacted]

Table XXVII

The Effect of Aminoimidazoline Related Compounds on Blood Pressure in Anesthetized Rats

Compound	Dose µg/kg i.v.	No. Test	Blood Pressure mm Hg Control	ΔBP
400,386	0.63	5	96 ± 7.3	+13 ± 5.8
	2.5	5	92 ± 9.5	+44 ± 4.8
	10	5	98 ± 7.8	+59 ± 7.5
	40	5	93 ± 5.1	+77 ± 3.4
400,487	10	4	103 ± 2.4	+13 ± 6.1
	40	4	110 ± 6.4	+34 ± 11.9
	160	4	109 ± 7.4	+69 ± 9.2
400,483	0.63	3	102	+16.6
	2.5	5	95 ± 6.3	+38 ± 5.9
	10	5	98 ± 8.4	+66 ± 6.4
	40	5	113 ± 5.0	+66 ± 4.2
	100	5	117 ± 4.8	+55 ± 4.8
	1000	2	95	+70

b) Behavioral Effects. The three most active imidazolines, 400,386, 400,483 and 400,487, were tested in rats on conditioned avoidance behavior by a standard assay procedure. Compounds 400,386 and 400,487 have no disruptive effects at 1 and 3.2 mg/kg i.p. Disruption of avoidance behavior was observed in 1 of 5 rats with compound 400,483 at 1 mg/kg i.p. At 3.2 mg/kg i.p., 2 rats died within 1 hour, 1 within 2 hours and 2 within 24 hours.

c) General Pharmacologic Profile. As noted earlier in this report, the principal pharmacological effects of 400,483, like 400,386 and 400,487, are those relating to the central nervous and cardiovascular systems. The outstanding effects are: bradycardia, rise in blood pressure and central nervous system depression. The comparative pharmacological profiles of 400,483, 400,487 and 400,386 are summarized in Table XXVIII. It is apparent that 400,483 is the most potent of all the aminoimidazoline derivatives tested so far.

We are planning to compare the pharmacological profile and toxicity of 400,483 with the duPont compound, 119902. If 400,483 should prove to be as potent as the duPont compound, it may merit consideration for limited study in man.

[redacted]

Table XXVIII

Pharmacological Profile of 400,386
400,487 and 400,483

Test or Symptom	Animal Species	Dose (mg/kg) 400,386	400,487	400,483
MED-50	Mouse	0.032	0.01	0.01
	Rat	.0.0056	0.018	0.0018
	Dog	0.01	0.1	0.001
LD-50	Mouse	20	31.6	22.4
	Rat	22.4	56.2	3.16
	Dog	ca. 1.5	>5	ca. 1.5
Bradycardia	Dog	0.032	0.316	0.001
Dec. activity	Dog	0.01	0.1	0.001
Ataxia	Dog	0.01	1.0	0.003
Convulsion	Dog	0.316	1.0	0.1
Emesis	Dog	0.032	>5	1.0
Atropine potentiation	Dog	+	+	+
Pressor Effect	Rat	++++ (0.00063)	+ (0.01)	++++ (0.00063)
Conditioned avoidance	Rat	>3.2	>3.2	1.0 (1/5)

D. Retrograde Amnesia Research

Retrograde amnesia has been considered as a novel type of incapacitation that a drug may produce and was, therefore, included as a part of our research program.

Retrograde amnesia is the disruption of acquired information by a subsequent stimulus, usually traumatic. It may be produced in man by concussion, brain damage, acute cerebral anoxia, severe hypothermia and electroconvulsive shock.

The following considerations indicate that a chemical agent capable of inducing retrograde amnesia might be developed. [redacted]

(b)(3)

[redacted]

[redacted] (b)(3)

During the past year, the retrograde amnesia research has been directed to:

1. The development of appropriate assays.
2. Exploitation of leads in the literature with the eventual goal of developing potent amnestic agents.

1. Assay Method

To screen compounds for retrograde amnesia in rats, we have adopted with modifications a method originated by Pearlman, Sharpless, and Jarvik (J. comp. physiol. Psychol. 54: 109-112, 1961). The ongoing procedure is described below.

Retrograde amnesia is produced when certain stimuli or drugs negate a behavioral suppressant influence of prior foot shock on ongoing stable conditioned behavior. The assay procedure is as follows:

a) Establishment of stable conditioned behavior.
b) Suppression of conditioned behavior by foot shock.
c) Administration of the amnestic agent
d) Determination of suppression.

a) Establishment of Stable Conditioned Behavior. Naive male Sprague-Dawley rats are first acclimated to the laboratory for four days. All rats are then trained during the course of a week to press a lever for water reinforcement after 48 hours of water deprivation. Initial training takes place in automated Skinner boxes (Lehigh Valley Rotor Wheels). When each rat completes initial training, it is kept on ad lib water and food until the entire batch of rats for the assay is trained.

Beginning the next Monday, each rat is exposed, after 24-hour water deprivation, to a 10-minute lever pressing session for three consecutive days in a Foringer-type Skinner box. During the sessions, each animal remains on a simple continuous reinforcement (CRF) schedule; that is after every lever press, a 3-second access to a dipper of water is permitted. Following a session, the rat is removed to an individual home cage and allotted 10 minutes of access to water. Food pellets are allowed ad lib. During the three daily CRF sessions, lever pressing behavior in the individual rat becomes fairly stable, although considerable variability exists among rats.

b) Suppression of Conditioned Behavior by Foot Shock. The fourth CRF session begins just as did the preceding three, with water reinforcement following each lever press. This session, however, is abbreviated as follows: When 5 minutes have elapsed the first subsequent lever press is punished with 1-2 mA foot-shock of 5 seconds duration. The animal is then rapidly removed from the Skinner box. The foot shock delivered to the rat has the effect of suppressing subsequent CRF behavior (see below).

c) Administration of the Amnestic Agent. Within five minutes following foot shock, the rats (in groups of 15) are given their treatments. The negative control treatment consists of the same volume of the vehicle. The positive control treatment consists of supramaximal electroconvulsive shock. The parameters of ECS are 150 mA, 60 cycles AC, 0.2 seconds, passed through ear clip electrodes. The ECS source is a Hans Electroshock Seizure apparatus in which internal resistance and voltage are extremely high in order to counteract the variability of external (cranial) resistance. The "amnestic" drugs chosen for investigation thus far have been derived mostly from the literature where they have been reported under certain conditions to induce amnesia or interfere with memory.

Dosage administered depends on the acute toxicity of the compound. Ordinarily based on these data, a dose-multiple of 1 or 3.2 mg/kg is chosen which is between 1/3.2 and 1/10 of the estimated LD50.

Following treatment, the rats are returned to home cages, but no water is allowed. This procedure will equate the various drug-treated groups, which would otherwise drink different amounts.

d) Determination of Suppression. About 24 hours after treatment (on Friday) each rat is exposed to a final 10-minute CRF session. In general, failure to respond with the usual response rates during this session indicates that lever pressing has been suppressed by the foot shock on Thursday. Relative stability of response rates indicates that the usual suppressing effects of foot shock have been negated.

In order to quantitate the suppression exhibited by individual animals or groups, a retrograde amnesia score has been adopted (Pearlman, Sharpless and Jarvik, J. comp. physiol. Psychol., 54: 109, 1961). This consists of the following percentage:

$$\frac{\text{Responses during first 5 minutes on test day}}{\text{Responses during first 5 minutes on pretreatment day}} \times 100$$

This score is determined for each animal and the median retrograde amnesia score of the group serves as an index of the amount of retrograde amnesia caused by the proposed amnestic agent. When the score is low, suppression is in evidence, and retrograde amnesia is not. On the contrary, retrograde amnesia is indicated by a high score. A score of 100 presumably would indicate that retrograde amnesia is "complete."

Other scoring systems are feasible. For example, one can deal directly with the latency of the first lever pressing response on the test-day (Friday) session; latencies approximately equal to pre-treatment latencies would indicate the presence of retrograde amnesia; abnormally prolonged latencies could indicate suppression.

During the past year, two major procedural changes were made. The first was an increase in the number of pre-treatment sessions from 4 to 9 in order to provide a more stable baseline with which to make comparison. The second was an increase from 1 to 3 days in the interval between treatment with a potential amnestic agent and the following test session. This was done to make certain that the animals were no longer under the direct depressant effects of the drug while being tested.

2. Results

During the year 51 compounds were tested in 27 separate assays (the time required per assay is 2 weeks). Fifteen compounds were either retested several times or tested at more than one dose level. Each assay had its own positive control, consisting of electroconvulsive shock treatment (a reliable retrograde amnestic stimulus) and negative control, consisting of saline treatment. The data from these experiments are summarized in Table XXIX. The last column of this table gives the "relative RA index" of the retrograde amnesia score for each drug. This index is a crude attempt to compare retrograde amnesia scores among experiments by making them relate to their own saline controls. If the index

$$\text{Relative RA Index} = \frac{\text{Saline RA Score} - \text{Drug RA Score}}{\text{Saline RA Score}} \times 100$$

is negative, it indicates that there is a retrograde amnesia effect. If zero, no effect is indicated, and if positive, the score might signify either a non-specific prolonged depressant effect, or improved "memory" for the aversive foot shock. The index should be viewed with caution since spuriously large negative relative indexes can result

Table XXIX

DRUGS GROUPED BY PHARMACOLOGICAL CLASS

Drug	Dose (I.P.) mg/kg	R. I.
Adrenolytic		
Ethoxybutamoxane (405,476)	.5	+95
	.5	+57
	.4	
Anesthetic		
Ethyl Ether (405,481)	1 1/2 min. expos. to 4.4 cc/l	+28
Hexobarbital (405,884)	125	- 6
Pentobarbital Sodium (405,334)	40	- 6
Secobarbital (405,885)	50	+30
G-29505 (405,887)	150	+55
Anticholinergic		
Atropine Sulfate (405,342)	30	+38
	30	-22
Cogentin Methanesulfonate (Benzotropine) (406,066)	10	-27
	10	-13
Dibutoline Sulfate (405,938)	10	-25
	1	0
Ditran (406,065)	10	+91
EA 3443	10	-24
Methantheline Bromide (405,343)	5	- 3
N-Methyl-3-Piperidyl Benzilate	50	- 8
Propantheline Bromide (405,347)	5	-50

Table XXIX (cont.)

Drug	Dose (I.P.) mg/kg	R. I.
Scopolamine HBr (430,020)	100	+67
	100	
	100	51
	100	-1
	100	-35
430,006	10	-90
	10	+37
	10	-31
	10	-11
Anticholinesterase		
Neostigmine (405,886)	0.200	+15
	0.200	+ 6
Physostigmine Salicylate (405,389)	0.75	+21
Anticholinergic and Hallucinogen		
Mescaline HCl (406,075) and Scopolamine HBr (430,020)	30 (Mes.) 100 (Scop.)	0
Central Amine Depletor		
Tetrabenazine (402,937)	32	-144
	32	+25
Cholinergic		
Carbochol (456,597)	0.200	±49
Pilocarpine HCl (405,693)	7.5	+10

Table XXIX (cont.)

Drug	Dose (I.P.) mg/kg	R. I.
CNS Stimulant		
Aminophylline (400,768)	30	0
Caffeine (405,304)	30	+87
d Amphetamine Sulfate (403,329)	3	-19
Nicotine (405,634)	1.8	+41
	6	+33
Strychnine SO_4 (430,015)	0.3	-52
405,304	30	+24
430,015	0.3	+45
430,016	10	+50
Estrogen		
430,012	30	-189
Hallucinogen		
A.4	25	+89
LSD-25 (430,008 Lot P-5291)	1	-39
	1	- 7
Mescaline HCl (406,075)	30	-14
Mescaline SO_4 (405,290)	30	-152
	30	+61
Psilocin (406,152)	20	-29
405,290	30	+44
430,008	1	+50

Table XXIX (cont.)

Drug	Dose (I.P.) mg/kg	R. I.
Hypothermic		
2,4-Dichlorophenoxy Acetic Acid (402,831)	30	+78
	30	
	30	+37
MAO Inhibitor		
Nialamide (401,133)	100	+42
	100	
	100	+14
430,011	30	+ 9
Norepinephrine Precursor		
d,l-DOPA (401,047	32	+28
Parasympathomimetic		
405,337	10	-53
RNA Inhibitor		
8-Azaguanine (400,226)	30	+52
	30	
	30	+43
Serotonin Precursor		
5-HTP (405,386)	32	+89
	32	+28
Tranquilizer		
Chloropromazine HCl (400,225)	30	-78
400,225	30	+56

Table XXIX (cont.)

Drug	Dose (I.P.) mg/kg	R. I.
Vasoconstrictor		
Ergotamine Tartrate (405,352)	.75	-49
400,386	2	- 1
400,483	1	-33
Vasodilator		
ATP (405,696)	.75	-49

from unusually low control retrograde amnesia scores. This appears to be the case, for example, with tetrabenazine in one of the 2 experiments.

In each of the experiments, the positive control, electroconvulsive shock, produced retrograde amnesia. On the average, the negative control responded at 34% of their preshock response rate level, signifying retention of the aversive foot shock, whereas rats treated with electroconvulsive shock following the aversive foot shock responded at 75% of the preshock level, signifying impaired retention. This difference was statistically significant ($p < 0.001$). No drug tested reached the retrograde amnestic effect of its own ECS control. Although in many instances the retrograde amnesia score of a drug was higher than the score of the corresponding saline control, this difference was never statistically significant. Perhaps the most suggestive results were produced by the anticholinergic drugs which almost consistently caused scores indicating some retrograde amnesia. Exceptions were Ditran and one test of Atropine. Mescaline gave initial evidence of a retrograde amnesia effect, but the results obtained were not subsequently corroborated. Drugs with vascular activity also yielded scores suggesting mild retrograde amnesia effects.

3. New Technique Development

In addition to testing of drugs for retrograde amnesia by the technique described, several attempts were made during the past year to develop a simple appetitive task with which to examine drugs for their amnestic effect on positive reinforcement. The ongoing assay, which tests drugs for retrograde amnesia of an aversive event (foot shock), might not have selected potential amnestic drugs working on such positively reinforced responses.

To evaluate drugs that might produce an amnestic effect for positively reinforced responding, it was necessary to develop a task which could be reliably and very rapidly conditioned. After a series of unsuccessful pilot studies, the following technique was shown to work satisfactorily: water deprived rats were placed into a chamber with a hole in one wall. The chamber is a 12.5 cm. high plastic box with a 15 x 23 cm. grid floor. Centered on the 23 cm. wall, 3.5 cm. above the floor is a 3.8 x 5 cm. rectangular opening to a tapering tunnel. A photoelectric cell and opposing light bulb were placed on the tunnel wall 2 cm. from the opening. Interruption of the light beam would operate a counter and timers. Initially the rats repeatedly explored this hole but after a few such sessions, the frequency of hole exploration diminished markedly. The rats were then given very brief access to water at the hole (positive reinforcement) and removed. During subsequent testing, the frequency of hole exploration with these

reinforced animals was shown to be significantly higher than that of the controls. This procedure, when standardized, was used in conjunction with electroconvulsive shock to see if it were sensitive to a retrograde amnesia agent. Animals were given 4 familiarization (pre-reinforcement) sessions of 4 minutes each; afterwards, water reinforcement was given for ten seconds. The relevant findings were: the frequency of hole exploration of the reinforced group was significantly higher than that of the non-reinforced group, demonstrating learning and retention. The reinforced group scored also significantly higher in hole explorations than a group given electroconvulsive shock immediately after reinforcement, demonstrating that the technique is sensitive to an amnestic stimulus. Furthermore, in order to determine if an aversive event following reinforcement disrupts retention, one group received a foot shock immediately following the reinforcement. Since this foot shock group exhibited retrograde amnesia scores about as high as the reinforced group and significantly higher than the non-reinforced control group, it was concluded that this technique is sensitive to agents inducing retrograde amnesia, but not to agents acting as negative reinforcements. No potential retrograde amnesia drugs have, however, been studied using this technique.

Future Plan

After a careful review of work to date on the retrograde amnesia research program, no satisfactory drug leads with retrograde amnesia activity have thus far been generated and certainly none approaches the potency required by the ACRDL objectives. [redacted] (b)(5) The ongoing procedure will, nevertheless, continue to be available should agents suspected of producing retrograde amnestic effects come to our attention.

E. New Incapacitating Agents from Microbial Sources

Since May 1964, we have supplemented our research on new agents by the incorporation of a program to derive compounds from microbial sources. This addition was made possible by an agreement with ACRDL project officer, Dr. Wills, to terminate the research program on retrograde amnesia.

The rationale for the use of microbiologically derived substances in these investigations is founded on the large varieties of pharmacologically active compounds found in inoculated broths. An intensive literature search has provided support for these contentions. The

general pharmacological effects (malaise, abdominal cramps, nausea, vomiting, diarrhea, neurotoxicities, etc.) of toxins and antibiotics have been well documented (4,5,6). Of special significance are the extremely high potencies of some toxins, notably, botulin and tetanus toxin (7). Other exo- and endotoxins, e.g., enterotoxins, are well known for their incapacitating effects. With respect to the antibiotics, there are some, e.g. Aureolic Acid, Carzinophilin A, Xanthomycins, etc., which manifest exceptionally potent toxicities (6). In addition to the foregoing substances, one can cite the pharmacodynamically active compounds derived from mushrooms and other fungi, e.g., psilocybin, psilocin and d-lysergic acid diethylamide (1,2,3), which act to temporarily derange humans. It is emphasized that these compounds act at exceedingly low doses.

It is evident from the examples specified above that it is feasible to discover new incapacitating agents from microbial sources. In this regard, Chas. Pfizer & Co., Inc., is in a particularly advantageous position because of its vast background and experience in fermentation research.

The program will proceed initially along three lines. Firstly, some of the toxic compounds of microbiological origin, which are already available from the J. L. Smith Memorial for Cancer Research at Maywood, New Jersey, will be screened. Secondly, a number of substances of microbiological origin selected from the literature will be produced in our laboratories for similar evaluation. Lastly, those microbial cultures from Maywood known to produce especially toxic broths will undergo further scrutiny.

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