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MEDICAL RESEARCH LABORATORIES

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QUARTERLY REPORT NO. 3

from January 1 to March 31, 1964

Vol. 1

Research on New Chemical Incapacitating Agents

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

UNCLASSIFIED

Submitted

by

Medical Research Laboratories

Chas. Pfizer & Co., Inc.

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April 14, 1964

QUARTERLY REPORT

I. INTRODUCTION

The present report covers the work accomplished from January 1, 1964 to March 31, 1964 and represents the third quarter of the contract research on chemical incapacitating agents.

Chemical and other data sheets recording biological information on compounds tested during this quarter are submitted in a separate volume.

II. SUMMARY

Laboratory studies during the third quarter of the program continued to emphasize the thymol ether and the 2-aminoimidazoline related compounds.

In the chemical synthetic program, 24 compounds related to thymol ether 430,017 and 28 compounds related to 400,386 were submitted for biological evaluation. In addition, the files of Pfizer in-house therapeutic research programs were scanned and the punched card file was searched for new leads on compounds with potential incapacitating effects. A total of 27 compounds was selected this way for biological evaluation.

No new thymol ether of potential interest has been discovered, partly because no animal test procedure is yet available to clearly characterize the potential incapacitating effect of thymol ethers.

None of the thymol ethers tested during this quarter equalled the activity of 430,038, the most potent analog in the series, in the MOTS.

In the aminoimidazoline series, 2 compounds were found to be of particular interest. They are 400,487, 2-(2,5-Dichloroanilino)-2-imidazoline hydroiodide, and 400,483, 2-(2'4'-Dichloro-1'-naphthylamino)imidazoline hydrochloride. The pharmacological profile of these compounds was expanded.

Research and screening for drug-induced retrograde amnesia were continued. So far, no drug has been found to have a potent retrograde amnestic effect. It is of interest to note, however, that the

aminoimidazoline, 400,386, was the most potent of all. Other compounds in the aminoimidazoline series found to be potent by the MOTS will be tested for RA effect. A new one-reinforced-trial appetitive assay for RA seems promising.

III. CHEMICAL SYNTHESIS PROGRAM

A. Thymol Ether Analogs

A clinical grade sample of 430,017 was prepared and forwarded to the Army CRDL, where preliminary studies in volunteers will be undertaken to supplement the clinical data on this compound available from the literature.

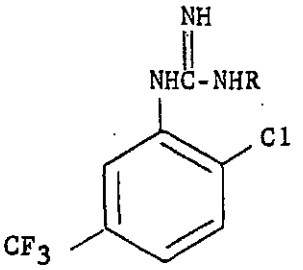
An additional 24 compounds related to 430,017 were synthesized during the quarter; the objective continues to be an agent that will produce the same type of incapacitation in man as 430,017, but at even lower dose levels. Most of the new compounds were patterned after analogs which showed higher activity than 430,017 in the mouse screen, such as 430,038 and 430,035 ("amine variants," Table 1). An added functional group was incorporated into some of them in the hope of thereby increasing interaction with receptor sites and, hence, potency. This same type of variation was combined with a lengthened side chain in the compounds listed under "Chain Variants" (Table 2).

B. Analogs of 400,386

A total of 28 compounds related to 400,386 was submitted for evaluation during the quarter. Developments in this area were highlighted by the finding that 400,487, 2-(2,5-Dichloroanilino)-2-imidazoline hydroiodide, is several times more potent than 400,386 in the mouse screen. Several other chlorinated anilinoimidazolines (Table 3) were subsequently submitted, and although these did not show the desired order of activity, high potency was found in a compound, 400,483 (Table 4), which is structurally a hybrid between 400,386 and 400,487.

Another type of variation that was investigated was alkylation on one of the ring nitrogens (N'). The N'-alkylated derivatives showed uniformly low potency in the mouse screen, paralleling their low potency as vasoconstrictors.

The following compounds were prepared with the objective of seeking higher potency through variations in the aminoimidazoline group:



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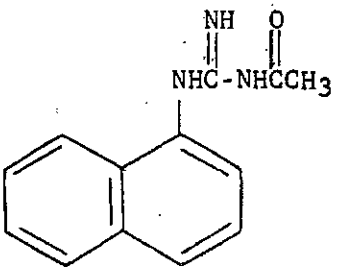
430,079: R = COϕ



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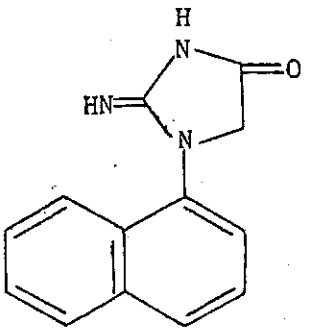


430,090

Besides the aminoimidazolines, a number of other imidazolines and guanidines from the Pfizer files were also submitted for evaluation:

400,805	404,405	405,505
402,005	404,746	405,506
404,404	405,504	452,559

C. Miscellaneous Compounds

The Pfizer punched card file was searched by machine for compounds which had shown indications of interfering with muscular function. From the resulting list, the following initial group (CD) was selected for submission to the mouse screen:

401,236	402,033
401,242	402,072
401,273	402,280
401,942	402,281

The results of current in-house therapeutic research programs are being scanned regularly for compounds that produced potentially incapacitating effects. Nineteen compounds (CD) were selected for submittal in this way during the quarter:

401,724	402,365	403,753	405,165
401,486	402,499	404,081	405,228
402,023	402,514	404,966	405,236
402,079	403,324	405,017	405,534
402,253	403,413	405,160	

IV. BIOLOGICAL PROGRAM

A. Mouse Toxicity Screen

A total of 101 compounds was screened in the mouse intravenous toxicity test. Among these, 43 were related to thymol ether, 42 were related to aminoimidazoline, and the remaining 16 were miscellaneous compounds selected from Pfizer in-house therapeutic research programs (Tables 5, 6 and 7). Eight thymol ether related and 12 aminoimidazoline related compounds showed an MED50 of 1 mg or less per kg body weight. To date, none of the new thymol ethers synthesized equalled the activity of 430,038 in the MOTS (Table 5). Noteworthy were 400,483 and 400,487, both of which were more potent than the reference standard 400,386 (Table 6). All compounds having a MED50 of 1 mg of less per kg body weight in mice will be submitted to a secondary screen in dogs and/or cats for pharmacologic activities and lethality.

B. Thymol Ether Analogs

As discussed in earlier reports, the lead in this program originated from a rather simple compound, the diethylaminoethyl ether of thymol (430,017). Two independent publications have reported that 430,017 produces incapacitating effects in man. The most prominent effect is a pronounced muscle fatigue after an oral dose of as low as 1.5 mg. The objective in this program is to develop a more potent agent that will produce a similar type of incapacitation.

Research on thymol ethers has been thwarted by the lack of specific pharmacological test procedures capable of clearly characterizing the incapacitating effects observed with this type of compound in man. To elucidate the mode of action of these compounds, the following experimental procedures had been pursued during the first and second quarters:

1. mouse toxicity test
2. dog symptomatology
3. rat swim test
4. cardiovascular effects
5. histamine like or antihistamine effects
6. electrolyte effects
7. behavioral effects

Practically all these procedures failed to differentiate compounds that produce incapacitation in man from those that do not (cf. 2nd Quarterly Report covering the period Oct. 1 to Dec. 31, 1963). No symptoms appeared in the mouse, rat, dog or monkey with any of the thymol ethers until doses well in excess of those at which they were known to produce incapacitation in man were reached. Furthermore, none of the symptoms produced by these compounds could be correlated with the known activity in man.

During the third quarter, the following additional experimental approaches were carried on:

a. Biochemical Studies

Similarities between the reported symptoms and signs produced by 430,017 and 430,019 in humans and those manifested by individuals affected with McArdle's Disease led us to speculate that the incapacitating thymol ethers act by a mechanism similar to that responsible for McArdle's Disease, namely, an absence of muscle phosphorylase. Patients with the disease are characterized by their inability to endure exercise due to the development of painful muscle cramps. Biochemically there is increased muscle glycogen; also, the blood lactic acid level fails to rise after ischemic exercise. It was thought, therefore, judicious to ascertain the effect of different thymol ethers on lactic acid levels after exercise.

Method: Rats, one at a time, were put in a water tank at zero time, allowed to swim until exhaustion, taken out, wiped dry and test compound injected, 10 mg/kg intravenously. One hour after zero time, the rats were put back into the water tank for 3 minutes (the 3 minute duration was found by previous experience to induce peak lactic acid levels), then sacrificed and blood samples collected for determination of blood lactic acid levels (by the method of Barker and Summerson, J. Biol. Chem., 138, 535, 1941). Groups of 13 to 14 rats each received the following prototype thymol ethers: 430,017; 430,018; 430,019; 430,023 and 430,038. The results are summarized in Table 9. It is rather disconcerting that no correlation could be established between

the depression of blood lactic acid elevation following exercise and compounds known to cause incapacitation. Compound 430,017 reduced the elvation of blood lactic acid following exercise, but 430,019 failed to have a similar effect on lactic acid levels. Compounds 430,018 and 430,023, which are not incapacitating in man, also reduced the lactic acid levels following exercise. Compound 430,038, the most potent of the thymol ethers by the MOTS, also produced an elevation of lactic acid similar to the controls.

Since physical exertion is required to produce the increase in blood lactic acid levels, any drug which decreases physical exertion would also tend to diminish the rise in lactic acid. Consequently, the results obtained with 430,017; 430,018 and 430,023 in the rat after swimming do not necessarily rule out the possibility that interference with muscle phosphorylase plays an important role in the production of incapacitation in man by the thymol ethers.

The Army CRDL has tested the activity of 430,017, in vitro, and found that it inhibits the enzyme ATP-creatine transphosphorylase, which plays a key role in muscle function.

Despite these findings, it is felt, nevertheless, that the similarity in effects produced by thymol ethers and McArdle's Disease represents a significant clue. Our immediate plan is to attempt to determine the effects of the different thymol ethers on the muscle glycogen and phosphorylase levels.

b. Guinea Pigs

In further attempts to characterize the potential incapacitating properties of thymol ethers, the prototype ethers, 430,017; 430,018; 430,019; 430,023 and 430,073 were screened for activities in guinea pigs. Compounds 430,017 and 430,019 had been reported to produce incapacitating symptoms in man whereas 430,018 and 430,023 did not. Compound 430,073, which is a possible metabolite of 430,017, was included also in this study.

i. Dermal Wheals

Each test compound (0.1 cc of 1% solution) was injected intracutaneously to a group of 2 guinea pigs. All compounds produced a dermal wheal of approximately 1 cm in diameter immediately after injection. No differences in the wheals produced by the different thymol ethers were evident. The wheals usually turned necrotic after 24 hours. An Intracutaneous injection of 0.1 cc of 0.1% solution of the histamine releaser 48/80 also produced a dermal wheal. A saline injection produced no such effect.

ii. Systemic Effects

Each of the 5 thymol ethers was given intravenously and also orally, 1 mg/kg body weight, to groups of 2 guinea pigs. No apparent symptoms were noted in these animals with any of these compounds.

c. Dogs - Secondary Screen of Thymol Ethers

Compounds 430,021, β-ortho isopropyl phenoxyethyl diethylamine hydrochloride; 430,022, 2-(2,6-diisopropylphenoxy)triethylamine hydrochloride; 430,027, β-(2-methyl-6-t-butylphenoxy)triethylamine hydrochloride; 430,038, N-(2-thymoxyethyl)-1,2,5,6-tetrahydropyridine hydrochloride; 430,039, 1-(2-thymoxyethyl)-3-pyrroline hydrochloride, and 430,073, β-thymoxyethylamine hydrochloride, which had previously been found to have a MED50 of 1.0, 1.0, 0.75, 0.24, 0.32 and 0.56 mg/kg body weight, respectively, in the MOTS, were screened in dogs for symptoms and signs (Table 10). No activities of specific interest were noted.

d. Behavioral Effects of Compound 430,038

In the last Quarterly Report, compound 430,038 was described as being seven times more potent than 430,017 in the mouse toxicity screen. This analog was subsequently submitted to the Army CRDL for evaluation in the conditioned avoidance, sustained physical exercise, and visual discrimination tests, which failed to confirm the potency advantage over 430,017 indicated by the mouse screen. It was also found (Dr. Wills) that 430,038 was without effect in a volunteer at 1.5 mg orally, a dose at which 430,017 is reported to have produced muscle pain and other symptoms in man.

C. Substituted Aminoimidazolines

The substituted aminoimidazolines were selected from prior Pfizer research files as an interesting lead. The potency of one of these compounds, 400,386, in the intravenous mouse toxicity test, is considered commensurate with the requirements for new incapacitating agents.

During the third quarter of contract research, work was continued on biological screening of the aminoimidazoline analogs. Two compounds in the series, 400,487 and 400,483, particularly the latter, were found to be of interest because of high potency in the MOTS. The pharmacological profile of these compounds was expanded.

a. 400,487

i. Mouse and Rat Toxicity Tests.

The MED50 and LD50 of compound 400,487 in mice were 0.01 and 31.6 mg/kg body weight, respectively (Table 6). In rats, the corresponding values were 0.018 and 56.2 mg/kg body weight, respectively (Table 8).

ii. Dogs

Although 400,487 was more potent than 400,386 in the mouse and rat toxicity tests, it was less potent in dogs. The principal pharmacological effects of the aminoimidazolines are those related to the central nervous and cardiovascular systems. The comparative symptomatology in dogs with compounds 400,487 and 400,386 is given in Table 11.

Because of the observation by the duPont Laboratories that the central nervous system depressant effects of tetrahydronaphthyloxazoline is potentiated by the simultaneous administration of atropine or scopolamine, we have explored the effect of atropine on the central nervous system depressant effects of 400,386 and 400,487. A slight potentiation of the central nervous system depressant effects was noted, but was not as pronounced as that reported by the duPont group for the oxazoline (Table 12).

b. 400,483

i. Mouse and Rat Toxicity Tests

The MED50 and LD50 of compound 400,483 in mice were 0.01 and 22.4 mg/kg body weight, respectively (Table 6). In rats, the corresponding values were 0.0017 and 0.316 mg/kg body weight, respectively (Table 8). The unusually high lethality of this compound is worth noting.

ii. Dogs

In dogs, 400,483 was more potent than both 400,386 and 400,487. Like 400,386 and 400,487, the principal pharmacological effects were those related to the central nervous and cardiovascular systems (Table 13). Bradycardia was noted after the intravenous injection of 0.001 mg/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 of up to 1 mg/kg, the overt symptoms consisted of: blanched gums and ears (indicating vasoconstrictor effects), ataxia, exophthalmos, mydriasis, sub-convulsive jerking, recurrent clonic spasms, clonic-tonic convulsions, salivation, nasal discharge, piloerection and emesis. Compound 400,483 seemed to be from 5 to 10 times

more potent than 400,386 and considerably more potent than 400,487 in dogs (cf. Table 11). The lethal dose of this compound in dogs has not yet been determined. The behavioral effects of 400,386, 400,487 and 400,483 will be examined.

D. Retrograde Amnesia Research

a. Screening

In a continued effort to uncover drugs with significant retrograde amnesia effects, 15 compounds were examined in six separate experiments during the last quarter. Tables 14, 15 and 16 summarize the data obtained. Several of the compounds exhibited suggestive activity. To facilitate quantitative comparison of these drugs across the various experiments, a relative index was devised. As previously described, the "RA scores" in the tables are median ratios of the post-foot shock to pre-foot shock number of bar pressing in a standard five minute period (cf. Quarterly Report). Since RA scores of the saline control groups vary from experiment to experiment, it was decided to express results from each drug in relation to data from its own saline control group. Thus for each experiment, the RA score of each drug was converted into the following relative RA index:

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

When the RA index is negative, it indicates that there is a retrograde amnesia effect, when zero, no effect at all, and when positive, it might loosely be regarded as "improved memory" for the aversive foot shock. Although some of the assumptions of this index are subject to question, it is a convenient index that makes comparisons clearer.

Using this index, Figure 1 compares the relative effects of the cholinergic and anticholinergic agents tested in several experiments. Although none of these effects is individually statistically significant, and none approaches in magnitude the usual RA effect of ECS, there is a general tendency for anticholinergic drugs to produce retrograde amnesia (with the exception of one of the atropine sulfate experiments) and for the cholinergic drugs to produce "improved memory."

Figure 2 shows results of experiments employing hallucinogens, LSD and mescaline, and selected CNS stimulants. Like the anticholinergics, both hallucinogens resulted in an RA effect. The CNS stimulants (Fig. 2) and the anesthetics (Fig. 3) did not produce any consistent effects.

Electroconvulsive shock, which produces reliable retrograde amnesia, has cerebral vasoconstriction effects. Since it is possible that the RA effect might be the result of interferences with cell metabolism through the reduced blood supply, the effects of 2 vasoconstrictors, ergotamine and 400,386, were used. Both of these (Figure 3) resulted in a RA effect but so did ATP, which has vasodilator actions. This area of drug selection will be explored further.

To date, no drug has produced as potent an RA effect as ECS. However, the qualitatively consistent effects of the anticholinergics and related hallucinogens and the relatively high RA potency of 400,386, suggest that these classes of drugs should be further explored.

b. Development of New Techniques

For the past several months attempts have been made to develop a simple appetitive task to further examine potential amnestic drugs. The ongoing assay examines drugs that might produce retrograde amnesia for an aversive event (foot shock). Since drugs might be selectively effective in producing retrograde amnesia, we have sought to develop a technique for examining amnestic effects of amnestic drugs on appetitively reinforced response.

Rapid, preferably "one-trial," learning is greatly to be desired in retrograde amnesia experiments, since only by such techniques can the actual time of acquisition be stated with assurance. Knowing the time of acquisition, one can then administer potential amnestic treatments a constant time thereafter.

Previous pilot studies have shown simple rapid appetitive learning to be technically quite difficult. However, one of the procedures investigated this quarter appears to be encouraging. This pilot study suggests that rats, when placed into an enclosure that has a hole in the wall, will explore the area and hole readily. After a few exposures to the area their exploration diminishes considerably. If, however, the water-deprived rat is given brief access to water at the hole, he will increase his exploration of this hole when tested subsequently (as compared with the control non-reinforced rats). The dependent variables measured were frequency of exploration of the hole in a standard length of time, total time spent at the hole, and latency to first explore the hole.

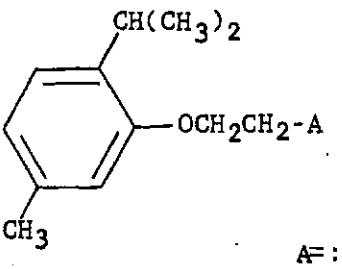
A second pilot study replicated those findings and further showed that if electroconvulsive shock was given immediately following this single reinforcement, the frequency of head explorations of the hole does not significantly increase and is almost equal to the non-reinforced group. This suggests that the technique is sensitive to retrograde amnesia agents.

/gac

Table 1

Thymol Ether Analogs

Amine Variants



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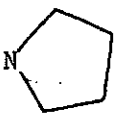
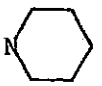
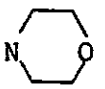
430,078: 

430,070: 

430,072: 

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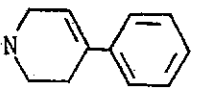
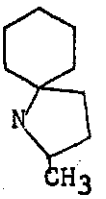
430,068: 

430,088: 

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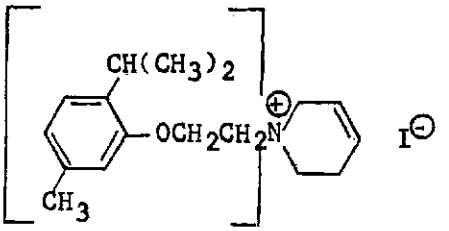
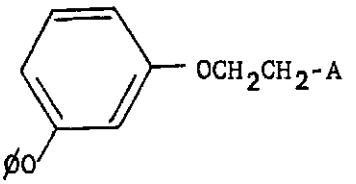
430,083: 

Table 1 (cont.)

Thymol Ether Analogs

Amine Variants



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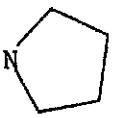
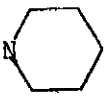
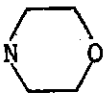
430,080: 

430,071: 

430,074: 

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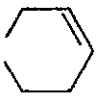
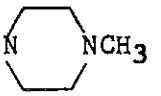
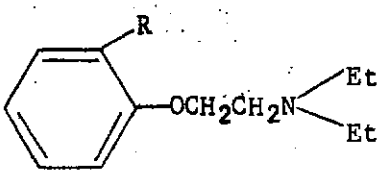
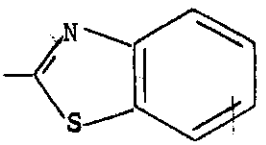
430,086: 

430,089: 

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

Aromatic Variants



430,076: 

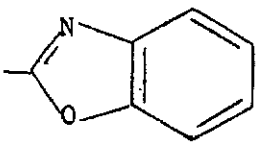
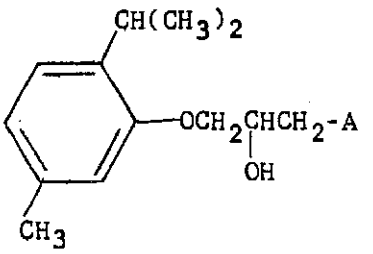
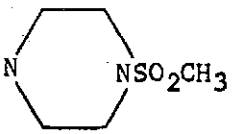
430,077: 

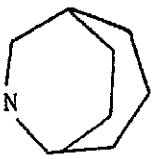
Table 2

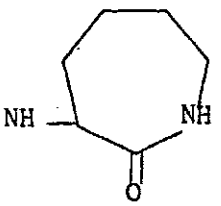
Thymol Ether Analogs

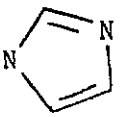
Chain Variants

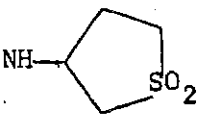


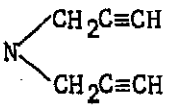
430,066: 

430,067: 

430,069: 

430,075: 

430,081: 

430,084: 

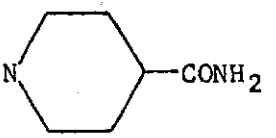
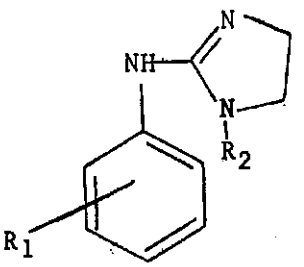
430,087: 

Table 3

Analogs of 400,386

Substituted Anilinoimidazolines

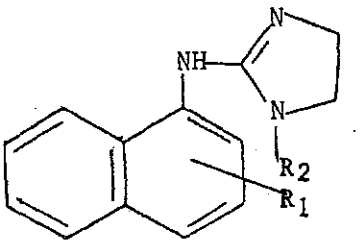


Code Number	R_1	R_2
400,777	H	Me
400,735	H	Et
404,407	O-Cl	H
400,751	O-Cl	Me
400,740	O-Cl	Et
400,391	p-Cl	H
400,679	p-Cl	Et
400,725	2,5-Cl_2	Et
400,549	3,4-Cl_2	H
400,772	3,4-Cl_2	Me
400,683	3,4-Cl_2	Et
400,759	3,5-Cl_2	Me
400,760	3,5-Cl_2	Et

Table 4

Analogs of 400,386

Naphthylaminoimidazolines



Code Number	R1	R2
400,483	Cl_2	H
400,499	H	CONHØ

Table 5

MOTS Data

Thymol Ether Related Compounds

Compound	MED_{50} mg/kg	LD_{50} mg/kg	LD_{50}/MED_{50} Ratio	Biological Activities
430,048	0.56	10.0	17.8	(1) motor deficit, inclined n. strip
430,064	0.56	79.4	141	(1) decreased muscle tone
430,073	0.56	44.7	79.5	(1) dec. act., mot. def., hypersensitivity
430,075	0.56	44.7	79.5	(1) ptosis, dec. act.
430,076	0.56	35.5	63.2	(1) ptosis, dec. act.
430,054	0.75	35.5	47.3	(1) pilo., mot. def.; (3) hypersensitive
430,051	0.75	20.0	26.7	(1) pilo. dec. act.; (3) hypersensitive
430,056	0.75	12.6	16.8	(1) ataxia, motor deficit
430,017*	1.78	35.5	19.9	
430,060		20.0		
430,077		22.4		
402,084		> 31.6		
402,122		< 31.6		
402,145		> 31.6		
402,344		31.6		
402,426		> 31.6		
402,562		> 31.6		
402,619		> 31.6		
430,070		31.6		
430,055		35.5		
430,057		35.5		
430,080		44.7		
430,058		50.1		
430,066		50.1		
430,067		50.1		
430,078		50.1		
430,089		50.1		
430,047		56.2		
430,062		56.2		
430,088		56.2		
430,065		63.1		
430,069		63.1		
430,071		63.1		
430,083		63.1		
430,086		63.1		
430,072		70.8		
430,061		79.4		
430,068		89.1		
402,310		< 100.		
430,063		> 100.		
430,074		100.		
430,087		> 100.		
430,081		112.		
430,084		112.		

*Reference Standard

MOTS Data

Aminoimidazoline (400,386) Related Compound

Compound	MED_{50} mg/kg	LD_{50} mg/kg	LD_{50}/MED_{50} Ratio	Biological Activities
400,483	0.01	22.4	2240.0	(.01) exophthalmos, piloerection (.03) vasoconstriction
400,386*	0.032	20.	625.0	
404,407	0.056	44.7	795.0	(0.1) decreased activity
400,559	0.178	15.8	88.0	(0.3) dec. act., (1) tremors, deficit
400,628	0.178	2.51	14.1	(0.3) ptosis, (1) pilo., tremors
404,404	0.178	22.4	126.0	(0.3) mydriasis, dec. activity
400,348	0.316	79.4	251.0	(1) exoph., piloerection, vasoconstriction
400,549	0.316	20.0	63.3	(0.3) piloerection., exophthalmos, dec. act.
402,714	0.562	79.4	141.0	(1) hypersens., motor deficit
404,405	0.562	31.6	56.2	(1) mydriasis, dec. act.
404,746	0.562	7.08	12.6	(1) ptosis
405,504	0.562	35.5	63.2	(1) ptosis, dec. act., flaccid
405,505	1.0	4.47	625.0	(1) hematuria, (3) ataxia, tremors
400,772		11.2		
400,805		12.6		
403,439		14.1		
400,679		17.8		
400,725		20.0		
400,759		25.1		
400,391		28.2		
400,683		31.6		
400,740		31.6		
401,320		> 31.6		
405,506		35.5		
430,052		35.5		
430,053		35.5		
430,085		35.5		
400,735		39.8		
402,005		39.8		
400,777		44.7		
400,751		56.2		
400,760		70.8		
430,079		79.4		
430,050		89.1		
430,059		89.1		
400,499		> 100.		
401,724		> 100.		
402,528		> 100.		
404,013		> 100.		
430,082		100.		
430,090		> 100.		
452,559		> 100.		
430,049		158.		

*Reference Standard

Table 7

MOTS Data

Miscellaneous Compounds

Code #	LD_{50} mg/kg
402,337	>31.6
402,563	50.1
405,534	50.1
402,365	56.2
402,079	70.8
401,991	100
401,993	100
401,846	>100
402,023	>100
402,253	>100
402,324	>100
405,017	>100
405,160	>100
405,228	>100
405,165	>100
401,994	>100

Table 8

Rat Toxicity Screen

400,386-Related Compounds

Compound	MED-50** mg/kg	LD-50 mg/kg	Ratio	$\frac{Dc^*}{Dt}$	$\frac{Rt^*}{Rc}$	(mg/kg) Symptoms & Signs
400,483	0.0017	0.316**	186	3.29	0.046	(.003) dec. act., vasoconstr.
400,487	0.018	56.2	3160	0.31	0.79	(.1) exoph., pilo., vasoconstr.
400,386***	0.0056	22.4	4000	1.0	1.0	(.01) vasoconstr., respir. rate dec.

*Note: D_c = MED-50 of reference standard
D_t = MED-50 of test compound
R_c = Safety ratio of reference standard
R_t = Safety ratio of test compound

**Approximate

***Reference Standard

Table 9

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

Table 10

General Symptomatology in Dogs
of Thymol Ethers

Compound	Dose mg/kg i.v.	Symptoms & Signs
430,021 (1.0)*	1.0	No Effect
	5.0	Salivation, ataxia +++, dec. activity ++, fine tremors, muscle spasticity, gen. muscle weakness, groaning, moves in crouched position
430,022 (1.0)	5.0	Retching, salivation (foaming), blanched gums, H.l. lateral exten., ataxia +, dec. activity, diarrhea, hematuria +++.
430,073 (0.56)	1.0	No Effect
	5.0	Sl. blanched gums, ataxia +, dec. activity +, tremors of h. quarters.
430,039 (0.32)	5.0	Ataxia +, dec. activity +, hematuria +++.
430,027 (0.75)	5.0	No Effect
430,038 (0.24)	1.0	No Effect
	5.0	Dec. respiration, irreg. respiration, dec. activity +, ptosis, peripheral vasoconstriction, hematuria for 2 days.

*Fig. in parentheses = MED, mg/kg, of the compound in MOTS.

Table 11

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

Dose mg/kg I.V.	400,487	400,386
0.01	No effects	Decreased activity; Ataxia
0.0316	No effects	Blanching; Emesis; Decreased activity; Ataxia; Slight rise in body temp. 1°C; Bradycardia (80→40 in 7 min.) duration 2-3 hrs.
0.1	Decreased activity	Blanching; Emesis; Decreased activity; Ataxia; Slight rise in body temp. 1°C; Bradycardia (80→36 in 25 min.) duration 4-5 hrs.
0.316	Decreased activity; Ataxia; Slight rise in body temp. 1-2°C; Bradycardia (84→48 in 10 min.) duration about 1 hr. Exophthalmos	Blanching; Emesis; Decreased Ataxia; Slight rise in Body temp. 1°C; Bradycardia (88→32 in 4 min.) duration 6 hrs; Exophthalmos; Depressed respir.; Piloerection; Tremors of head and trunk; Lying down
0.6	Blanching; Decreased activity; Ataxia; Bradycardia (100→40 in 5 min.) duration 4 hrs.	Not done
1.0	Blanching; Decreased activity; Ataxia; Slight rise in body temp. 1°C; Bradycardia (80→34 in 28 min.) duration 6 hrs; exophthalmos; Piloerection; Tremors and convulsion, slight.	Pupil dilation; Emesis; exophthalmos Irreg. respiration; Tremors and convulsion; Lacrimation and salivation; Inability to walk; Catatonia; Death 4 1/2 hrs.; Heart rate not taken

Table 12

Symptomatology in Dogs
400,386 + Atropine and 400,487 + Atropine

Compound	Route	Dose mg/kg	Symptoms & Signs
400,386 + Atropine	I.V. I.V.	0.1 0.2	Immed. blanched gums and ears +++ Dec. activity ++ Ataxia ++ Emesis (at 2 min. & 5 hrs.) Piloerection Exophthalmos Mydriasis +++ Pupillary light reflex loss Appears blind Fasciculation of h. quarters
400,487 + Atropine	I.V. I.V.	0.1 0.2	Immed. blanched gums and ears +++ Dec. activity +++ Ataxia ++ Emesis (at 35 min., 105 min. & 12 hrs.) Mydriasis Pupillary light reflex loss Appears blind Fasciculation of h. quarters

Table 13

General Symptomatology in Dogs
of Compound 400,483

Dose	Route	Symptoms & Signs
0.001	I.V.	Immed. blanched gums and ears ++ Dec. activity + Bradycardia 100 → 60 beats/min.
0.00316	I.V.	Immed. blanched gums and ears ++ Dec. activity ++ Ataxia + Diarrhea (?) Bradycardia 104 → 76 beats/min.
0.01	I.V.	Immed. blanched gums and ears +++ Sl. ataxia Dec. activity + Bradycardia 104 → 80 beats/min.
0.0316	I.V.	Immed. blanched gums and ears +++ Ataxia ++ Dec. activity ++ Exophthalmos + Bradycardia 104 → 44 beats/min.
0.1	I.V.	Immed. blanched gums and ears +++ Ataxia +++ Dec. activity +++ Exophthalmos + Convul. of H. quarters Muscle spasticity Fasciculation of H. quarters Bradycardia 76 → 40 beats/min.
1.0	I.V.	Immed. blanched gums and ears ++++ Mydriasis Sub-convulsive jerking Clonic-tonic convulsions; exophthalmos Nasal discharge; salivation; piloerection Recurrent clonic spasms; H.l. altern. extensor spasms Bradycardia 76 → 24 beats/min.; emesis

Table 14

Median Retrograde Amnesia Scores after Various Drugs and Electroconvulsive Shock
(During January 1 to January 31, 1964)

EXPERIMENT XVII

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	5 cc/kg	12	35
Positive Control	Electroconvulsive shock	150 ma., 0.2 sec.	12	74
Vasodilator	ATP (405,696)	.75 mg/kg	12	52
Vasoconstrictor	Ergotamine Tartrate (405,352)	.75 mg/kg	12	52
Anesthetic	Pentobarbital Sodium (405,334)	40 mg/kg	12	37

EXPERIMENT XVIII

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	5 cc/kg	12	34
Positive Control	Electroconvulsive shock	150 ma., 0.2 sec.	12	82
Anti-cholinesterase	Physostigmine Salicylate (405,389)	.75 mg/kg	12	27
Anticholinergic	Methaneline Bromide (405,343)	5 mg/kg	12	35
Anticholinergic	Propantheline Bromide (405,347)	5 mg/kg	12	51

Table 15

Median Retrograde Amnesia Scores after Various Drugs and Electroconvulsive Shock
(During February 1 to February 29, 1964)

EXPERIMENT XVIII

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	1 cc/kg	12	53
Positive Control	Electroconvulsive shock	150 ma., 0.2 sec.	12	86
Anesthetic	Hexobarbital (405,884)	125 mg/kg	12	56
Anesthetic	Secobarbital (405,885)	50 mg/kg	12	37
Anti-cholinesterase	Neostigmine (405,886)	200 micro g./kg	12	45

EXPERIMENT XIX

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	1 cc/kg	12	33
Positive Control	Electroconvulsive shock	150 ma., 0.2 sec.	12	54
Anesthetic	G-29505 (405,887)	150 mg/kg	12	15
Cholinergic	Carbachol (456,597)	200 micro g./kg	12	17
Anti-cholinesterase	Neostigmine (405,886)	200 micro g./kg	12	31

Table 16

Median Retrograde Amnesia Scores after Various Drugs and Electroconvulsive Shock
(During March 1 to March 30, 1964)

EXPERIMENT XX

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	1 cc/kg	12	24
Positive Control	Electroconvulsive shock	150 ma., 0.2 sec.	12	66
Anticholinergic	Atropine Sulfate (405,342)	30 mg/kg	12	15
Anticholinergic	Dibutoline Sulfate (405,938)	1 mg/kg	12	24
Anticholinergic	Dibutoline Sulfate (405,938)	10 mg/kg	12	30

EXPERIMENT XXI

Class	Condition	Dose (I.P.)	N	Score
Negative Control	Normal Saline	1 cc/kg	12	32
Positive Control	Electroconvulsive shock	150 ma., 02. sec.	12	97
CNS Stimulant	d-Amphetamine Sulfate (405,329)	3 mg/kg	12	38
Anticholinergic	Atropine Sulfate (405,342)	30 mg/kg	12	39
Vasoconstrictor	400,386	2 mg/kg	12	64

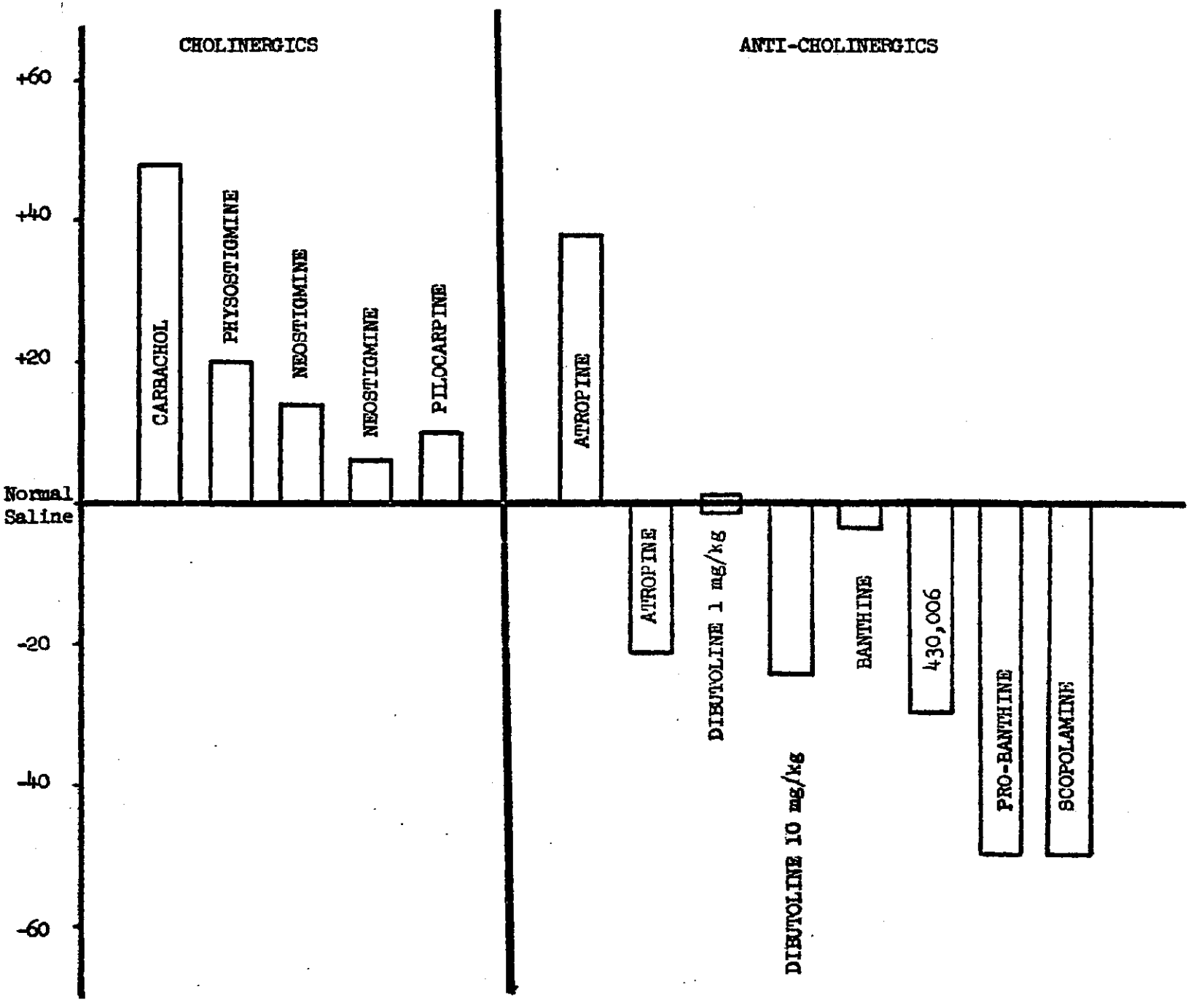


Figure 1. - Relative RA Index of Cholinergic and Anti-cholinergic Agents

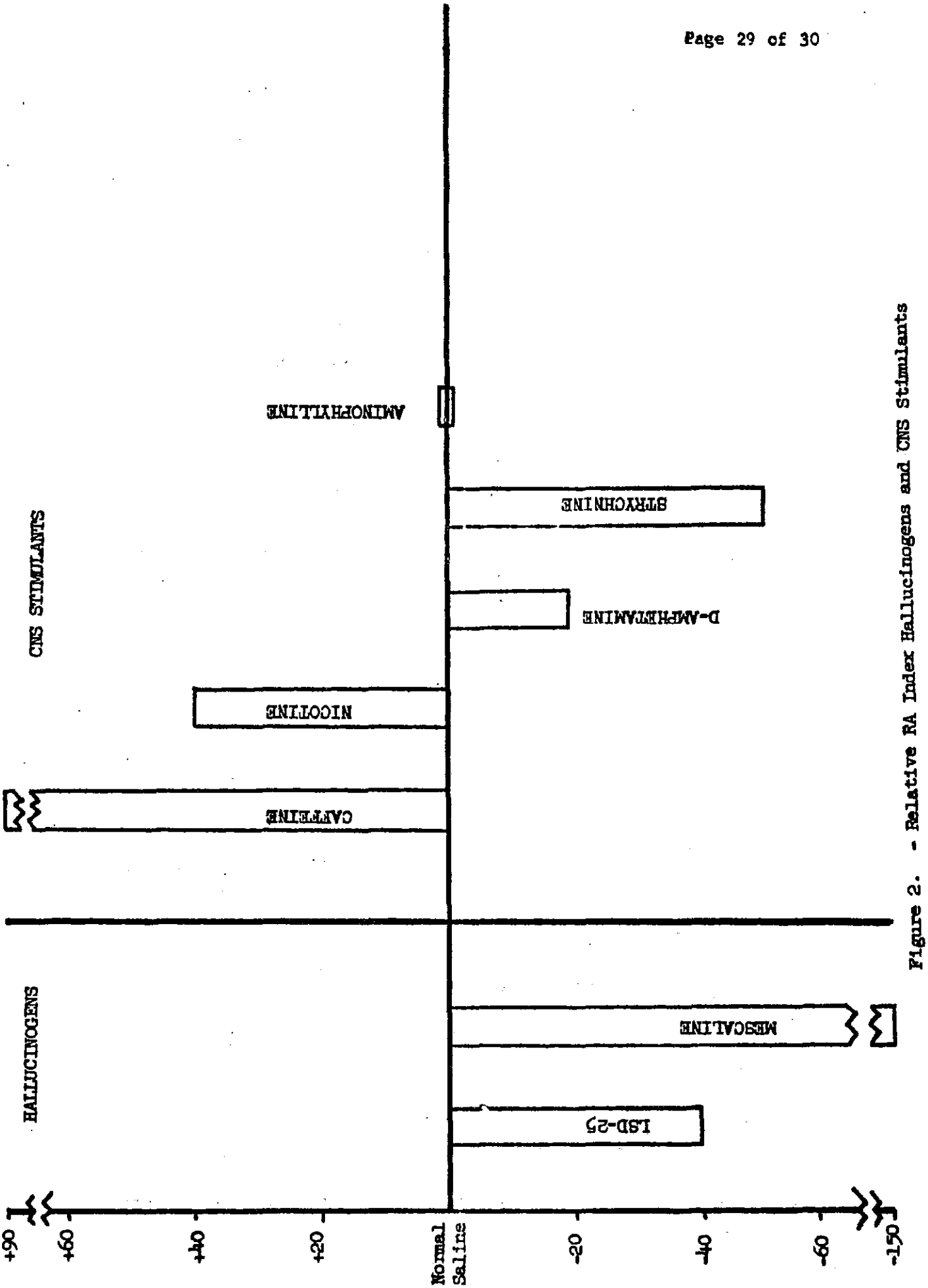


Figure 2. - Relative RA Index Hallucinogens and CNS Stimulants

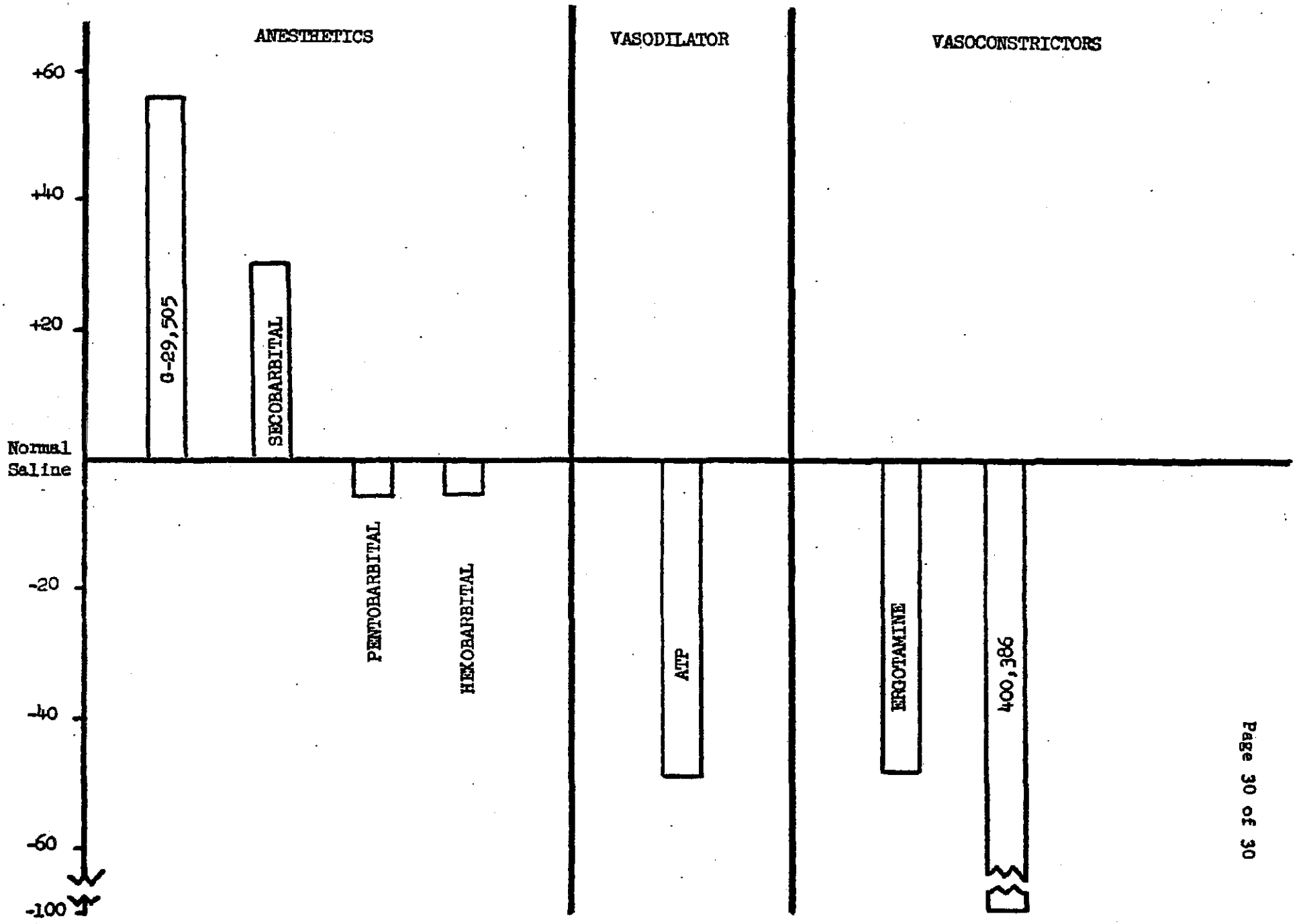


Figure 3. Relative RA Index of General Anesthetics, Vasodilators and Vasoconstrictors