1969

Pharmacology of Some 1-Alkyl-3-Benzoylpyridinium Halides

Angela Caretta Springfield
University of Rhode Island

PHARMACOLOGY OF SOME 1-ALKYL-3-BENZOYLPYRIDINIUM HALIDES

BY

ANGELA CARETTA SPRINGFIELD

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY

UNIVERSITY OF RHODE ISLAND

1969

ABSTRACT

Several 1-alkylbenzoylpyridinium halides containing alkyl side chains of one to fifteen carbons were screened for pharmacological activity. A comparison of potency was based on the ability to produce mortality in 50 percent of mice (LD50).

The following parameters were investigated in a select number of compounds representative of the series: acute intraperitoneal LD50 in rats; ED50 (hypotensive) in rats; effects on the autonomic nervous system of anesthetized cats and dogs as indicated by alterations in blood pressure; effects on the nictitating membrane in anesthetized cats; direct effects upon blood vessels employing hind quarter perfusion in intact rats; effects on the isolated perfused guinea pig heart.

In mice the trend of acute intraperitoneal toxicity was biphasic. The methyl salts were the least toxic, while toxicity sharply increased with the ethyl salts. As the carbon substitution increased to seven, toxicity decreased. Further carbon substitution led to a progressive increase in toxicity. Although fewer compounds were tested in rats, the LD50's approximately paralleled those of the mice.

3-Benzoyl-1-ethylpyridinium iodide (3-C2-I),

3-benzoyl-1-octylpyridinium bromide (3-C8-Br), and 3-benzoyl-1-pentadecylpyridinium bromide (3-C15-Br) produced hypotensive effects in anesthetized rats. The ED50 (hypotensive) increased in that order.

Demonstration of hypotensive responses in anesthetized rats led to an investigation of the mechanism of this action. Decreased responses to carotid occlusion and vagal stimulation and increased responses to injected epinephrine in anesthetized cats and dogs suggested that the 3-C2-I and 3-C8-Br exerted a hypotensive response through ganglioplegia. 3-C8-Br was more potent than 3-C2-I. Further testing of 3-C15-Br was discontinued at this time as respiratory failure followed by cardiac arrest occurred, apparently due to excessive quantities of fluid in the respiratory passages. 3-Benzoyl-1-dodecylpyridinium bromide also elicited this response.

The ability of 3-C2-I and 3-C8-Br to block the normal response of the nictitating membrane in cats by electrical stimulation of the preganglionic cervical sympathetic nerve was tested. 3-C8-Br was more potent than 3-C2-I in this test.

The flow rates of perfusions of the hind quarter vasculature of the intact rats were unaffected by doses up to the equivalent ED50 (hypotensive). 3-C2-I and 3-C8-Br showed cardiodepressant and cardiotoxic effects on the isolated guinea pig heart, although a direct dose

effect relationship could not be shown. 3-C8-Br was more potent than 3-C2-I in producing cardiac arrest.

The results of these experiments support the hypothesis that 3-C2-I and 3-C8-Br exert a hypotensive effect through ganglioplegia, unabetted by direct vasodilation. A depressant action on the heart may contribute to the hypotension induced by ganglioplegia.

MASTER OF SCIENCE THESIS

OF

ANGELA CARETTA SPRINGFIELD

Approved:

Thesis Committee:

Chairman _____

_____

_____

Dean of the Graduate School _____

UNIVERSITY OF RHODE ISLAND

1969

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

I. INTRODUCTION

Some 1-alkyl-3-benzoylpyridinium halides were synthesized in 1967 by the Department of Pharmaceutical Chemistry, University of Rhode Island, College of Pharmacy, Kingston, Rhode Island. The most distinctive chemical feature of pharmacological interest of these compcunds is the quaternary ammonium moiety. Although these compounds were designed as molluscicidal agents, it was felt that they might exhibit properties similar to other quaternary ammonium compounds, such as ganglioplegia, skeletal muscle relaxation, or other autonomic effects.

The possibility of effects on the cardiovascular system and autonomic nervous system led to the screening of these compounds with various tests designed to show such actions.

The results of this study may indicate a possible therapeutic value of these agents as well as relative danger or safety when employed as molluscicidal agents.

II. REVIEW OF THE LITERATURE

In the course of the nineteenth century, which encompasses the era of development and growth of the field of synthetic organic pharmaceuticals, many natural products were isolated, purified and their chemical structure determined. When it became apparent that certain similar structural moieties were sometimes present in compounds possessing related pharmacodynamic activity, this observation was seized upon as the only available guide for future research. The modification of the structure of a promising drug compound is still the main approach to producing new drugs. Since structural variations in a compound may not alter all actions of the drug equally, the therapeutic utility of a compound may be enhanced by developing a congener whose therapeutic index is greater than that of the parent compound. Physical changes accompanying structural variations may bring improvements in solubility, volatility, color and odor which may increase the utility of a given compound (Burger, 1960; Wilson and Gisvold, 1962). Therapeutically and experimentally valuable drugs have been developed which are structurally related antagonists of other drugs (Hart et al., 1944) or of chemicals important in biochemical or physiological function (Farber et al., 1948; Symposium, 1954).

It has been hoped that the action of different drugs might be explained on a unified basis thus allowing formation of general principles guiding the rational development of utilitarian new drugs with predicted and select pharmacological properties. Largely unsuccessful attempts were made to relate a particular functional group to a particular pharmacological effect (Burger, 1958). Many compounds unrelated chemically elicit the same pharmacological action, particularly among the CNS depressants (Burger, 1960). Conversely, many compounds containing the same functional group show a divergence of action, such as the sulfonamides which have bacteriostatic (Northey, 1948), hypoglycemic (Loubatieres et al., 1955; Editorial, 1956), diuretic (Krebs, 1948; Miller et al., 1950) and artificial sweetening properties (Schutz et al., 1957).

The difficulty in finding a relationship among compounds having similar biological actions but different chemical structures, and the converse, does not negate the significance of structure activity relationships. To the contrary, it emphasizes the complexities of biological systems, the limitations of our knowledge of the basic mechanisms of drug action and that drugs may produce overtly similar responses by more than one mechanism.

Even the more advanced theories of isosterism (Erlenmeyer et al., 1932; Friedman, 1951) and interference with drug metabolism (Brody, 1955) have not shown

regularities in structure activity relationships that could be transferred indiscriminantly from one group of compounds to the next. However, the most pronounced regularities could be expected in a series of closely analogous compounds. This is demonstrated in an homologous series since the regularities resulted from gradual physical changes (Ferguson, 1939; Hansch, 1966).

"In 1869, Crum-Brown and Fraser observed that tertiary amines with varying pharmacological properties tended to show similar properties when quaternized" (Wilson and Gisvold p. 4, 1962). Thus, although the literature reports no pharmacology of the 1-alkyl-3-benzoylpyridinium halides, the presence of a quaternary ammonium group might impart known characteristics of other quaternary compounds.

Among the quaternary ammonium compounds are antibacterial agents (Lawrence, 1958) (disinfectants, antiseptics or sanitizers) such as benzalkonium chloride and cetylpyridinium chloride. Other quaternary ammonium compounds have anticholinergic properties which have found use as antisecretory, antispasmodic and antiulcer drugs (Bachrach, 1958; Kirsner et al., 1953). Many of these drugs are quaternary ammonium derivatives of atropine, scopolamine, and papaverine. Curariform activity is elicited by certain quaternary ammonium compounds (Barlow et al., 1948; Castillo et al., 1950), while others, such as neostigmine, are cholinesterase inhibitors or curare

antagonists (Acheson et al., 1946; Wien et al., 1952).

Although a superficial inspection supports the assumption of a divergence of biological action among the quaternary ammonium salts, the common factor is the structural resemblance to acetylcholine, more specifically, the quaternary ammonium group. Since acetylcholine mediates nervous transmission at various sites in the body (Triggle, 1965), a structural congener of acetylcholine may mimic its action at these various sites, may antagonize acetylcholine or may inhibit cholinesterase, which deactivates acetylcholine. If its absorption and distribution permits, it may exhibit these actions at more than one site. Since multiplicity of pharmacodynamic behavior interferes with the utility of many of these drugs (Issekutz, 1954), structural modifications may lead to a higher specificity of action.

III. INVESTIGATION

A. OBJECTIVES

The overall objective of this investigation was to evaluate the pharmacology of some benzoylpyridinium salts in mammals.

The specific objectives of this investigation were:

1. To screen a series of synthetic benzoylpyridinium salts for pharmacological activity.

2. To relate the activity to the structural arrangement of the compounds screened.

3. To study in detail a select number of these compounds in an attempt to determine the pharmacological site of action.

B. COMPOUNDS

The following compounds were used in this investigation and will hereafter be denoted by the abbreviations shown in parenthesis: 3-benzoyl-1-methylpyridinium iodide (3-C1-I); 3-benzoyl-1-ethylpyridinium iodide (3-C2-I); 3-benzoyl-1-propylpyridinium iodide (3-C3-I); 3-benzoyl-1-butylpyridinium bromide (3-C4-Br); 3-benzoyl-1-pentylpyridinium bromide (3-C5-Br); 3-benzoyl-1-heptylpyridinium bromide (3-C7-Br); 3-benzoyl-1-octylpyridinium bromide (3-C8-Br); 3-benzoyl-1-nonylpyridinium bromide (3-C9-Br); 3-benzoyl-1-decylpyridinium bromide (3-C10-Br); 3-benzoyl-

1-dodecylpyridinium bromide (3-C12-Br); 3-benzoyl-1-pentadecylpyridinium bromide (3-C15-Br); 4-benzoyl-1-methylpyridinium iodide (4-C1-I); and 4-benzoyl-1-ethylpyridinium iodide (4-C2-I).

All of the compounds were solids and most of them were freely soluble in water. All compounds were administered in aqueous solution.

The compounds were reported to be pure except for 3-C2-I, which was 91 percent pure. The doses reported throughout the investigation were uncorrected for the impurity, i.e., the doses were reported as if the compound were pure.

C. ANIMALS

1. Mice: Male and female albino mice of the Charles River strain[1] were employed for the acute toxicity studies.

2. Rats: Male and female albino rats of the Charles River strain[2] were employed for the acute toxicity studies and the hind leg perfusion studies, while male albino rats of the same strain were employed for the blood pressure studies.

3. Guinea pigs: Mongrel guinea pigs of either sex were employed for all isolated heart studies.

4. Cats: Mongrel cats of either sex were employed for

---

[1] Charles River Breeding Farms, North Wilmington, Mass.

[2] Ibid.

blood pressure studies and for the nictitating membrane preparations.

5. Dogs: Mongrel dogs of either sex were employed for blood pressure studies.

D. INSTRUMENTS

The E&M Physiograph[1] and its accessories were used for all electronic recordings.

E. EXPERIMENTAL PROCEDURES AND RESULTS

The investigation was divided into six sections:

1. The acute intraperitoneal toxicity of the 1-alkyl-3- and 1-alkyl-4-benzoylpyridinium halides in mice; the acute intraperitoneal toxicity of 3-C2-I, 3-C4-Br, 3-C5-Br, 3-C8-Br and 3-C15-Br in rats.

2. The effects of 3-C2-I, 3-C8-Br and 3-C15-Br upon the blood pressure of rats.

3. The effects of 3-C2-I, 3-C8-Br, 3-C12-Br and 3-C15-Br upon the autonomic nervous system employing cardiovascular tests in cats and dogs.

4. The effects of 3-C2-I and 3-C8-Br upon the nictitating membrane of the cat.

5. The effects of 3-C2-I and 3-C8-Br upon the hind quarter vasculature of the intact rat.

6. The effects of 3-C2-I and 3-C8-Br upon the isolated guinea pig heart.

---

[1] E&M Instrument Company, Inc., Houston, Texas

1. Acute Intraperitoneal Toxicity of the 1-Alkyl-3- and 1-Alkyl-4-benzoylpyridinium Halides in Mice and Rats

a) Experimental Procedures

Fifty albino mice weighing 19g to 40 g and fifty albino rats weighing 150 g to 400 g were divided into five groups each containing equal numbers of both sexes, except where noted.

Individual drugs, 20 mg/ml as the salt or suitable dilutions thereof prepared in distilled water, were administered intraperitoneally. The animals were observed for 24 hours and general symptoms and time of death recorded. No animal was used more than once throughout the entire study.

The doses were spaced in approximately logarithmic intervals. Dosage was adjusted according to the results obtained, in order to determine the dose of a particular compound that would produce death in 50 percent of the animals (LD50). The dose and response were subjected to the Litchfield and Wilcoxon (1948) method of evaluating dose-effect experiments. The results were plotted and the LD50 graphically calculated.

The compounds were compared on the basis of their mg/kg LD50 and their mM/kg LD50.

b) Results

The results include the following:

(1) A comparison of the LD50 of the various

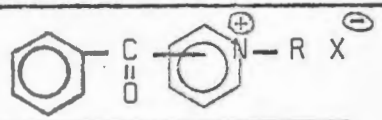
compcunds (Table 1).

(2) A comparison of the mM LD50 versus the number of carbons in the side chain (Fig. 1).

(3) The compounds containing from one to eight carbors in the alkyl side chain were found to be short acting, as death or recovery occurred in 1 hour. Higher members of the series were found to be longer acting, with death or recovery requiring a 24 hour period.

The general symptoms produced by all the drugs in both species were the same, i.e., sluggishness, decreased activity, mydriasis, loss of righting reflex as death approached, and dyspnea followed by convulsions and death.

TABLE 1. A comparison of the LD50 of some 1-alkyl-3- and 1-alkyl-4-benzoylpyridinium halides in mice and rats



Isomer	R	X	Mice LD50 + 0.95 Confidence Limits[a] (mg/kg)	Mice (mM/kg)	Rats LD50 + 0.95 Confidence Limits[a] (mg/kg)	Rats (mM/kg)
3	CH_3	I	84 (73-104)	0.285		
3	C_2H_5	I	46 (39- 55)	0.136	127 (113-142)	0.375
3	C_3H_7	I	55 (51- 60)	0.156		
3	C_4H_9	Br	86 (79- 94)	0.269	180 (158-205)[b]	0.563
3	C_5H_{11}	Br	90 (84- 96)	0.270	227 (207-249)[b]	0.680
3	C_7H_{15}	Br	103 (97-109)	0.284		
3	C_8H_{17}	Br	90 (78-110)	0.244	40 (36- 44)	0.106
3	C_9H_{19}	Br	67 (59- 76)	0.156		
3	$C_{10}H_{21}$	Br	46 (48- 53)	0.114		
3	$C_{12}H_{25}$	Br	38 (33- 44)	0.088		
3	$C_{15}H_{31}$	Br	38 (33- 44)	0.080	44 (31- 63)	0.092
4	CH_3	I	110 (94-129)	0.338		
4	C_2H_5	I	15 (11- 21)	0.044		

[a]Calculated from the Litchfield and Wilcoxon (1948) method.

[b]Three groups of 6 animals were used instead of usual 5 groups of 10 animals.

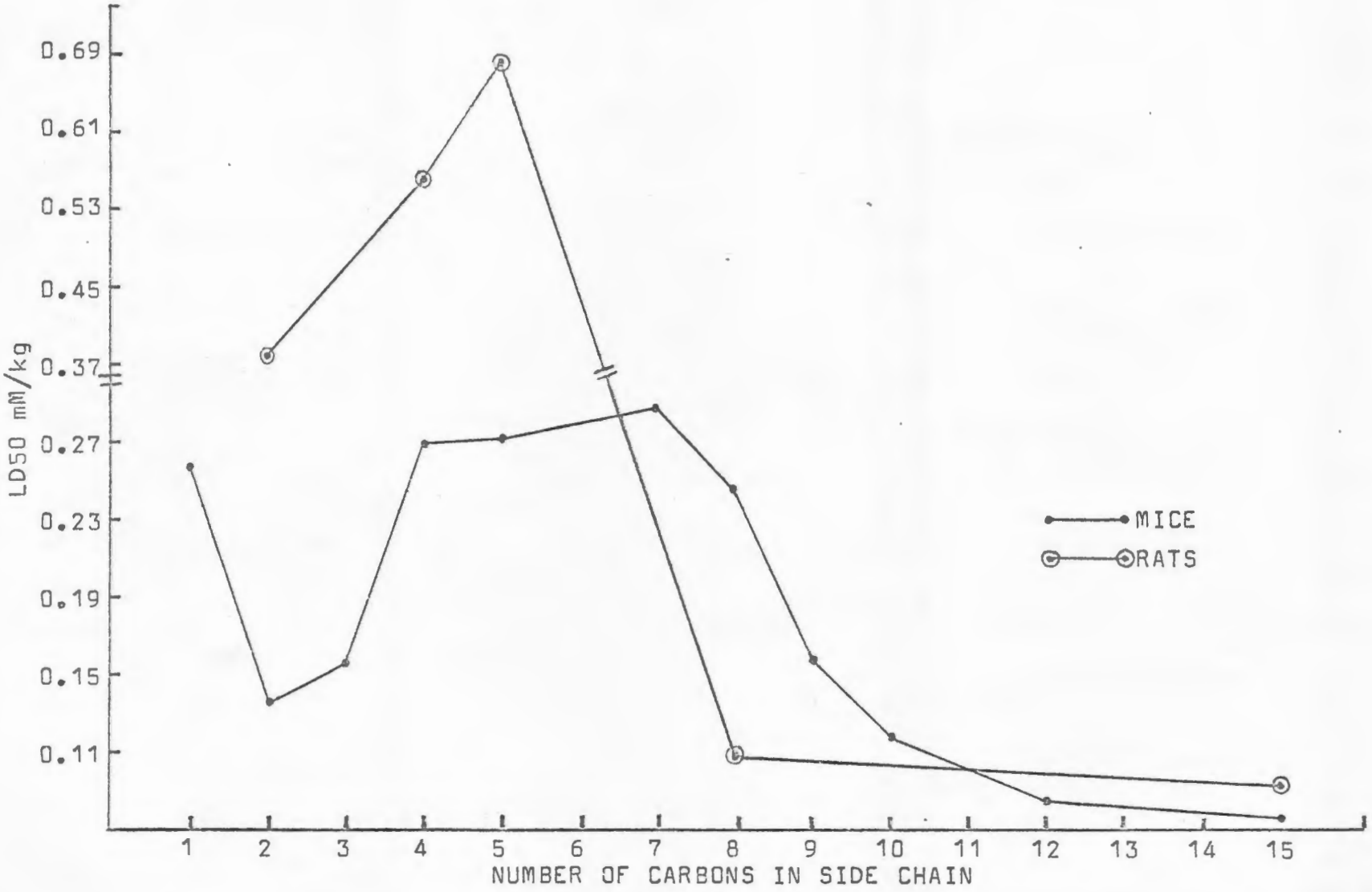


Fig. 1. Structure Activity Relationship of the 1-Alkyl-3-benzoylpyridinium Halides

2. Effects of 3-Benzoyl-1-ethylpyridinium Iodide (3-C2-I), 3-Benzoyl-1-octylpyridinium Bromide (3-C8-Br) and 3-Benzoyl-1-pentadecylpyridinium Bromide (3-C15-Br) on the Blood Pressure of Rats

a) Experimental Procedures

The effects of the compounds on the blood pressure of male albino rats weighing between 300 g and 475 g were recorded manometrically by direct cannulation of the left carotid artery. A normal saline, fluid bridge in tygon tubing connected the cannula to a mercury manometer. Blood pressure responses were recorded on a slowly moving smoked kymograph.

Rats were anethetized with urethane, 1.2 g/kg of a freshly prepared 20 percent solution, administered intraperitoneally via the right femoral vein. To prevent clotting, heparin sodium, 0.1 ml of a 1:10,000 solution, was administered to each rat prior to cannulation. A similar concentration of heparin sodium was placed into the cannula.

A minimum of 10 minutes was allowed to elapse prior to drug administration to permit stabilization of the blood pressure. One drug was administered per animal and no more than a total of 4 injections were administered to one animal. Following each injection the blood pressure was allowed to return to normal before a second injection was made.

Doses were adjusted to determine the dose of a compound that would produce an approximate 50 percent decrease in blood pressure. The dose and response was subjected to the Litchfield and Wilcoxon (1948) method of evaluating dose-effect experiments. The results were plotted and the ED50 (hypotensive) calculated.

The compounds were compared using the following indices of activity:

(1) The percent decrease in blood pressure produced by different doses of the compounds.

(2) The duration of the hypotensive effect, i.e., time elapsed before the arterial blood pressure returned to 90 percent of the normal blood pressure.

(3) The dose of the compound required to produce a 50 percent decrease in arterial blood pressure, i.e., ED50 (hypotensive).

b) Results

The results include the following:

(1) Effects of 3-C2-I, 3-C8-Br and 3-C15-Br on the arterial blood pressure of male albino rats (Tables 2 to 4).

(2) A comparison of the effects of 3-C2-I, 3-C8-Br and 3-C15-Br on the arterial blood pressure of male albino rats (Table 5).

TABLE 2. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on the blood pressure of rats

Dose (mg/kg)	Number of Trials	Mean % Drop in Blood Pressure ± S.D.	Duration of Drop (Mean and Range) (Min.)
2.0	2	12 ± 2	3.0 (2.7-3.3)
3.0	2	15 ± 5	3.2 (3.0-3.4)
6.0	1	22	3.2
10.0	3	26 ± 3	3.4 (2.7-4.0)
20.0	4	34 ± 2	3.3 (3.0-4.2)
25.0	4	40 ± 7	3.6 (3.3-4.0)
30.0	4	45 ± 4	3.3 (2.5-3.6)

TABLE 3. Effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on the blood pressure of rats

Dose (mg/kg)	Number of Trials	Mean % Drop in Blood Pressure ± S.D.	Duration of Drop (Mean and Range) (Min.)
2.5	3	34 ± 1	3.6 (3.0-4.2)
5.0	6	39 ± 3	4.4 (3.2-5.2)
7.5	3	43 ± 3	5.0 (4.0-6.0)
10.0	4	48 ± 3	6.9 (6.0-8.2)

TABLE 4. Effects of 3-benzoyl-1-pentadecylpyridinium bromide (3-C15-Br) on the blood pressure of rats

Dose (mg/kg)	Number of Trials	Mean % Drop in Blood Pressure ± S.D.	Duration of Drop (Mean and Range) (Min.)
1.0	2	24 ± 1	3.5 (3.0- 4.0)
2.0	2	32 ± 1	7.3 (4.6-10.0)
3.0	2	42 ± 1	10.8 (5.6-16.0)
5.0	1	43	6.2
7.0	2	48 ± 1	13.5 (9.0-18.0)
10.0	1	54	20.0[a]

[a]Did not recover to 90 percent of normal within 20 minutes; terminated.

TABLE 5. A comparison of the ED50 (hypotensive) of the 1-alkyl-1-benzoylpyridinium halides on the blood pressure of rats

Compound	Total Trials	Calculated ED50[a] (mg/kg)	ED50 (mM/kg)
3-Benzoyl-1-ethylpyridinium iodide (3-C2-I)	20	52.0	0.153
3-Benzoyl-1-octylpyridinium bromide (3-C8-Br)	16	14.0	0.037
3-Benzoyl-1-pentadecylpyridinium bromide (3-C15-Br)	10	8.1	0.017

[a]Derived from the Wilcoxon and Litchfield (1948) method of evaluating dose-effect experiments.

3. Effects of 3-Benzoyl-1-ethylpyridinium Iodide (3-C2-I), 3-Benzoyl-1-octylpyridinium Bromide (3-C8-Br), 3-Benzoyl-1-dodecylpyridinium Bromide (3-C12-Br) and 3-Benzoyl-1-pentadecylpyridinium Bromide (3-C15-Br) on Four Cardiovascular Tests in the Cat and the Dog

a) Experimental Procedures

Mongrel cats of either sex weighing 2.5 to 3.5 kg and mongrel dogs of either sex weighing 17.5 to 19.5 kg were anesthetized with sodium pentobarbital, 35 mg/kg of a 3.5 percent solution, administered intraperitoneally and intravenously, respectively. Following cannulation of the left femoral artery, the blood pressure was recorded on a calibrated Physiograph. The cannula was connected via a fluid bridge of normal saline to a Bourdon type pressure transducer. All injections were made into the right femoral vein via a cannula connected to a buret containing normal saline used to displace the drug. A tracheotomy was performed as part of thr rormal procedure. The common carotid arteries were isolated and a loose ligature passed underneath each. When performing a bilateral carotid occlusion, the ligature was lifted and a taped bulldog clamp secured caudad to the bifurcation. Care was taken to avoid unnecessary stretching of the artery. The cervical portions of the vagal nerves were then isolated, ligated and severed craniad to the ligature. Following stabilization of the

preparation the following tests were performed:

(1) bilateral occlusion of the common carotids-30 sec.

(2) bilateral vagal stimulation- the intensity was adjusted to obtain certain percent responses-20 sec.

(3) epinephrine-the dose was adjusted to obtain certain percent responses.

(4) acetylcholine-the dose was adjusted to obtain certain percent responses.

(5) tests compound-the dose was adjusted to obtain certain percent responses.

(6) repeat test (1).

(7) repeat test (2).

(8) repeat test (3).

(9) repeat test (4).

It should be noted that additional doses of a compound were given when the effect was not of sufficient duration to complete the series of tests.

The percent change in blood pressure produced by each of the test procedures before and after administration of a compound was the index of activity studied.

b) Results

The results include the following:

(1) Changes induced in four cardiovascular tests by 3-C2-I and 3-C8-Br in the cat and the dog (Tables 6 to 11).

(2) A comparison of the changes induced in four cardiovascular tests by these compounds (Table 12).

(3) When 3-C12-Br and 3-C15-Br were administered to cats, the blood pressure was lowered. Death soon followed, apparently due to respiratory impairment from large amounts of fluid in the lungs. The secretion of fluid did not result from the use of acetylcholine as it also appeared when these compounds were administered to cats prepared in the same surgical manner but which had not received any prior drugs.

TABLE 6. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on four cardiovascular tests in the cat: cat 1

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg.)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		120-190	+58
Bilateral vagal stimulation (20 sec.)		120- 80	-33
Epinephrine HCl	6.6 ug	120-170	+42
Acetylcholine HBr	0.3 ug	130- 75	-42
3-C2-I	20.0 mg	125-105	-16
Bilateral carotid occlusion (30 sec.)		105-110	+ 5
Bilateral vagal stimulation (20 sec.)		105-105	0
Epinephrine HCl	6.6 ug	105-160	+52
Acetylcholine HBr	0.3 ug	110- 70	-36

TABLE 7. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on four cardiovascular tests in the cat: cat 2

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		160-210	+ 32
Bilateral vagal stimulation (20 sec.)		160-130	- 20
Epinephrine HCl	0.8 ug	160-210	+ 32
Acetylcholine HBr	0.2 ug	160-110	- 31
3-C2-I	6.5 mg	160-120	- 25
Bilateral carotid occlusion (30 sec.)		110-120	+ 9
Bilateral vagal stimulation (20 sec.)		130-110	- 8
3-C2-I	13.1 mg	160- 80	- 50
Epinephrine HCl	0.8 ug	80-160	+100
3-C2-I	13.1 mg	150- 75	- 50
Acetylcholine HBr	0.2 ug	70- 50	- 29

TABLE 8. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on four cardiovascular tests in the dog: dog 1

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		150-185	+23
Bilateral vagal stimulation (20 sec.)		140-100	-28
Epinephrine HCl	2.5 ug	150-170	+13
Acetylcholine HBr	0.5 ug	130-105	-19
3-C2-I	8.5 mg	135-105	-22
Bilateral carotid occlusion (30 sec.)		130-130	0
Bilateral vagal stimulation (20 sec.)		130-130	0
Epinephrine HCl	2.5 ug	130-175	+25
Acetylcholine HBr	0.5 ug	125-110	-12

TABLE 9. Effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on four cardiovascular tests in the cat: cat 3

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		190-280	+47
Bilateral vagal stimulation (20 sec.)		190-130	-32
Epinephrine HCl	0.7 ug	180-210	+17
Acetylcholine HBr	0.3 ug	170-100	-41
3-C8-Br	10.6 mg	170- 80	-53
Bilateral carotid occlusion (30 sec.)		90-110	+22
Bilateral vagal stimulation (20 sec.)		90- 75	-17
Epinephrine HCl	0.7 ug	110-180	+63
Acetylcholine HBr	0.3 ug	110- 60	-45

TABLE 10. Effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on four cardiovascular tests in the cat: cat 4

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		120-170	+42
Bilateral vagal stimulation (20 sec.)		120- 80	-33
Epinephrine HCl	0.8 ug	130-190	+46
Acetylcholine HBr	0.3 ug	150- 80	-47
3-C8-Br	13.5 mg	150- 80	-47
Bilateral carotid occlusion (30 sec.)		80- 85	+ 6
Bilateral vagal stimulation (20 sec.)		80- 75	- 6
Epinephrine HCl	0.8 ug	80-140	+75
Acetylcholine HBr	0.3 ug	80- 40	-50

TABLE 11. Effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on four cardiovascular tests in the dog: dog 2

Test	Dose (units/kg)	Change in Blood Pressure (Original-Peak) (mm Hg)	% Change in Blood Pressure
Bilateral carotid occlusion (30 sec.)		140-175	+ 25
Bilateral vagal stimulation (20 sec.)		135- 85	- 37
Epinephrine HCl	2.2 ug	135-160	+ 19
Acetylcholine HBr	0.6 ug	130-100	- 23
3-C8-Br	6.7 mg	135-100	- 30
Bilateral carotid occlusion (30 sec.)		100-105	+ 5
Bilateral vagal stimulation (20 sec.)		95- 95	0
Epinephrine HCl	2.2 ug	95-200	+100
Acetylcholine HBr	0.6 ug	90- 70	- 22

TABLE 12. A comparison of the effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) and 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on four cardiovascular tests in the cat and the dog

Animal	Drug	Dose (mg/kg)	Blood Pressure Changes[a,b]			
			BCO	BVS	EPI	ACH
Cat 1	None		++	--	++	--
	3-C2-I	20.0	0	0	++	--
Cat 2	None		++	--	++	--
	3-C2-I	6.5	0	0	+++	--
Dog 1	None		++	--	++	--
	3-C2-I	8.5	0	0	+++	--
Cat 3	None		++	--	++	--
	3-C8-Br	10.6	+	-	+++	--
Cat 4	None		++	--	++	--
	3-C8-Br	13.5	0	0	+++	--
Dog 2	None		++	--	++	--
	3-C8-Br	6.7	0	0	+++	--

[a]Symbols: +, rise; ++, marked rise; +++, severe rise; -, fall; --, marked fall; 0, no change.

[b]Abbreviations: BCO, bilateral carotid occlusion; BVS, bilateral vagal stimulation; EPI, epinephrine; ACH, acetylcholine.

4. Effects of 3-Benzoyl-1-ethylpyridinium Iodide (3-C2-I) and 3-Benzoyl-1-octylpyridinium Bromide on the Nictitating Membrane of the Cat

a) Experimental Procedures

Mongrel cats of either sex weighing 2.5 to 3.5 kg were anesthetized with a 3.5 percent solution of pentobarbital 35 mg/kg, administered intraperitoneally. A tracheotomy was performed as part of the normal procedure. Following cannulation of the left femoral artery, the blood pressure was recorded on a calibrated Physiograph. The cannula was connected via a fluid bridge of normal saline to a Bourdon type pressure transducer. All injections were made into the right femoral vein via a cannula connected to a buret containing normal saline used for displacement of the drug.

The preganglionic cervical sympathetic nerve was isolated, ligated and severed caudad to the ligature. The superior cervical ganglion together with its postganglionic fibers was isolated and a ligature passed underneath the postganglionic fibers. The responses of the nictitating membrane to electrical stimulation of the cervical sympathetic nerve were recorded on a Physiograph by means of a myograph B transducer attached by silk thread, passing over a pulley to the nictitating membrane. The minimal intensity of electrical stimulation needed to produce a contraction of the nictitating membrane via stimulation of the pre- and postganglionic sympathetic fibers was determined. The test

compound was then administered. The pre- and postganglionic fibers were stimulated during the effects of the drug as determined by changes in blood pressure. The blood pressure was allowed to return to normal. Responses of the nictitating membrane to pre- and postganglionic nerve stimulation were re-established to insure the integrity of the preparation.

b) Results

The results include the following:

(1) The effects of 3-C2-I and 3-C8-Br on the nictitating membrane of the cat (Table 13).

TABLE 13. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) and 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on the nictitating membrane of the cat

Animal	Drug	Dose (mg/kg)	Change in Blood Pressure Original-Peak (mm Hg)	% Change	Response to Stimulation of Cervical Sympathetic Nerve[a] Preganglionic	Postganglionic
1	3-C2-I	0.0	0- 0	0	++	++
		10.0	120- 60	-50	0	++
2	3-C2-I	0.0	0- 0	0	++	++
		20.0	110- 70	-36	+	++
3	3-C2-I	0.0	0- 0	0	++	++
		20.0	145- 90	-38	0	++
4	3-C8-Br	0.0	0- 0	0	++	++
		8.0	180-120	-33	0	++
5	3-C8-Br	0.0	0- 0	0	++	++
		10.0	80- 50	-38	0	++

[a]Symbols: +, rise; ++, marked rise; 0, no change.

5. Effects of 3-Benzoyl-1-ethylpyridinium Iodide (3-C2-I) and 3-Benzoyl-1-octylpyridinium Bromide (3-C8-Br) on the Hind Quarter Vasculature of the Intact Rat

a) Experimental Procedures

Male albino rats weighing 330 g to 430 g were anesthetized with sodium pentobarbital, 35 mg/kg of a 3.5 percent solution, administered intraperitoneally. A midline incision was made from the pelvis to the sternum. The intestines were lifted out of the body cavity and covered with gauze moistened in normal saline. The abdominal aorta and vena cava were isolated at the level of the renal veins and ligated caudad to them. The aorta and vena cava were cannulated.

The cannulated aorta was connected by gum tubing, capacity 6 ml, to a modified Anderson Craver[1] heart perfusion apparatus. The preparation was stabilized at a constant temperature of 35.5 degrees centigrade, maintained by a circulating water bath. A constant perfusion head pressure of approximately 60 cm of water was maintained. The perfusion fluid was aerated Locke-Ringer solution. During each experiment the perfusate was collected and discarded.

Heparin sodium, 0.1 ml of a 1:1000 solution, was perfused through the preparation. When the perfusate was clear, the counting was begun.

An electronic drop counter was used to record the

---

[1]Metro Industries, Long Island City, New York

rate of effluence. Following the recording of normal effluence, drug administration was effected via an injection spiral (1.5 ml volume) connected to the perfusion canal. 2.0 ml of perfusate was used to displace the drug.

The experimental procedure was standardized with a 2 minute control count. When the volume of the effluent equaled the total volume of the tubing, a 4 minute drug effect count was begun. The highest dose used was chosen to equal or exceed the intraperitoneal ED50 in rats. The highest dose used in the above experiments was also tested with a 15 minute drug effect interval and a 2 minute control. A two tailed Student's t test was used to test the significance of the data.

Only one drug was used per animal. Not more than a total of 5 doses was administered per preparation. Each animal was used for a period not exceeding 2 hours, due to severe edema occurring after this period of time.

b) Results

The results include the following:

(1) The effects of 3-C2-I and 3-C8-Br on the hind quarter vasculature of the intact rat (Tables 14 and 15).

TABLE 15. Effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on the hind quarter vasculature of the intact rat

Animal	Dose (mg)	Dose Equivalent (mg/kg)	Control Drops/Min. ± S.D.	Drug Effect Drops/Min. ± S.D.	P[b]
1	20	60.6	32.0 ± 2.0	37.8 ± 3.9	n.s.[b]
	20	60.6	35.0 ± 1.0	41.0 ± 9.6	n.s.
2	10	30.3	18.0 ± 1.0	17.3 ± 1.1	n.s.
	10	30.3	18.5 ± 0.5	19.0 ± 2.4	n.s.
	20	60.6	17.1 ± 1.0	19.5 ± 1.1	n.s.
3[a]	20	55.5	31.5 ± 0.5	39.0 ± 6.1	n.s.
	20	55.5	47.0 ± 0.0	45.0 ± 1.7	n.s.

[a]Drug effect count taken for 15 minutes after a 2 minute control count.

[b]Symbols: P, 0.01 level of significance; n.s., not significant.

TABLE 14. Effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on the hind quarter vasculature of the intact rat

Animal	Dose (mg)	Dose Equivalent (mg/kg)	Control Drops/Min. ± S.D.	Drug Effect Drops/Min. ± S.D.	P[b]
1	10	23.3	251.0 ± 1.0	247.3 ± 6.2	n.s.[b]
	20	46.5	132.0 ± 10.0	121.0 ± 5.3	n.s.
2	5	13.0	84.0 ± 1.0	81.3 ± 2.6	n.s.
	10	26.0	125.5 ± 9.5	110.0 ± 10.7	n.s.
	20	52.0	123.5 ± 1.5	124.0 ± 11.7	n.s.
3[a]	20	54.8	114.5 ± 7.5	112.7 ± 3.6	n.s.
	40	109.6	71.0 ± 5.0	78.6 ± 3.3	>0.05

[a]Drug effect count taken for 15 minutes after a 2 minute control count.

[b]Symbols: P, 0.01 level of significance; n.s., not significant.

6. Effects of 3-Benzoyl-1-ethylpyridinium Iodide (3-C2-I) and 3-Benzoyl-1-octylpyridinium Bromide (3-C8-Br) on the Isolated Guinea Pig Heart

a) Experimental Procedures

Guinea pigs of either sex weighing 450 g to 950 g were sacrificed by cervical dislocation. Following a mid-thoracic incision and retraction of the ribs, the heart was prepared as follows:

(1) The descending aorta was clamped and cut distally in the area of the diaphragm.

(2) The major arteries, innominate, left subclavian, and left common carotid, were severed thus freeing the aorta.

(3) The inferior and superior venae cavae were severed.

(4) The trachea was severed.

The heart with lungs attached, was then rapidly removed and transferred to a dish of oxygenated perfusion solution at room temperature. Several ml of warm oxygenated perfusion solution containing 0.1 ml of a 1:1000 solution of sodium heparin were gently passed through the coronary arteries via injection into the aorta to remove residual blood and to prevent formation of clots within these small blood vessels. The aorta was cannulated with a glass cannula with its tip proximal to the innominate artery and distal to the coronary ostia and secured. The cannulated heart was then transferred to an Anderson Craver heart perfusion apparatus. A constant fluid level was maintained in the perfusion well;

the siphoned fluid was collected by means of suction and discarded.

A constant temperature of 37.5 degrees centigrade was maintained by use of a circulating water bath. Following stabilization of the preparation under a perfusion head pressure of 55 cm of water, all extraneous tissue including the pericardium was dissected free. A stainless steel hook was inserted into the ventricular musculature at the heart's apex and the glass chamber secured. A silk thread connected the hook, through a pulley system, to a recording lever that recorded the cardiac contractions on a slowly moving smoked kymograph.

The perfusion solution used was that recommended by Chenoweth and Koelle (1946) modified by Smookler and DeFeo (1962).

Five liters of stock solution containing 350 g sodium chloride, 21.0 g potassium chloride, 15.9 g calcium chloride dihydrate and 17.8 g magnesium sulfate monohydrate were prepared using deionized water and stored at room temperature.

The final perfusion fluid was prepared for each experiment by taking 3.6 g dextrose, 4.2 g of sodium bicarbonate, 200 ml of stock solution and sufficient deionized water to make 2 liters of solution. The perfusion fluid was oxygenated with a mixture of 95 percent oxygen and 5 percent carbon dioxide. The pH of the final solution was

7.8. During each experiment the perfusate was collected and discarded.

Following the recording of normal cardiac contractions drug administration was effected via an injection spiral connected to the perfusion canal, with a volume of perfusate (2.0 ml) greater than that held by the spiral (1.5 ml). One compound was tested per preparation and not more than 6 injections were made on one preparation. Succeeding doses were administered after a lapse of 10 minutes from the time of recovery from the previous dose.

A depression of 95 percent or more from the normal was considered to be cardiac arrest. A depression of 50 to 95 percent was considered to be severe cardiac depression (SD), while 20 to 50 percent, mild cardiac depression (MD), 0 to 20 percent, no effect. Cardiac stimulation was considered to have occurred if there was an increase of amplitude of 50 percent over the normal.

The indices of activity used to compare the compounds were as follows:

(1) The incidence of cardiac arrest at a particular dose.

(2) The incidence and severity of cardiac depression if no arrest occurred.

(3) The incidence of cardiac stimulation if no arrest occurred.

b) Results

The results include the following:

(1) A profile of the effects of 3-C2-I and 3-C8-Br on the isolated guinea pig heart (Tables 16 and 17).

(2) 3-C8-Br is considered to be more toxic than 3-C2-I; at 2.0 mg, 3-C8-Br produced three out of three cardiac arrests with no recoveries, while 3-C2-I at the same dose produced one out of seven cardiac arrests with no recovery and five out of seven severe cardiac depressions with full recoveries.

(3) In most trials resulting either in cardiac arrest or severe depression, a 2 to 3 second interval of cardiac stimulation preceded the former events.

(4) In one experiment using 3-C2-I at 1.0 mg, there was one occurrence of cardiac stimulation only.

(5) Employing 3-C8-Br there were 3 occurrences of cardiac stimulation only, two at 0.6 mg, and one at 1.0 mg.

TABLE 16. Profile of the effects of 3-benzoyl-1-ethylpyridinium iodide (3-C2-I) on the isolated guinea pig heart

Dose (mg/kg)	Cardiac Arrest Arrests/Trials	Recovery to 50% Normal Recoveries/ Arrests	Cardiac Depression Depressions/Trials (if no arrest)	Recovery to 50% Normal Recoveries/ Depressions	No Effect No Effects/ Trials
0.02					2/2
0.1					1/1
0.2					3/3
0.5			1/2 SD[a]	1/1	1/1
1.0	1/4	0/1			2/4[b]
2.0	1/7	0/1	5/7 SD	5/5	1/7
4.0			1/1 SD	1/1	

[a]Symbol: SD, severe depression, 50-95 percent.

[b]One occurrence of cardiac stimulation, >50 percent.

TABLE 17. Profile of the effects of 3-benzoyl-1-octylpyridinium bromide (3-C8-Br) on the isolated guinea pig heart

Dose (mg/kg)	Cardiac Arrest Arrests/Trials	Recovery to 50% Normal Recoveries/ Arrests	Cardiac Depression Depressions/Trials (if no arrest)	Recovery to 50% Normal Recoveries/ Depressions	No Effect No Effects/ Trials
0.02					1/1
0.2	1/5	1/1	1/5 MD[a]	0/1	3/5
0.5			1/1 SD[a]	0/1	
0.6	4/7	2/4	1/7 MD	0/1	2/7[b]
1.0	3/5	2/3	1/5 SD	0/1	1/0[c]
2.0	3/3	0/3			
20.0	1/1	0/1			

[a]Symbols: SD, severe depression, 50-95 percent; MD, mild depression, 20-50 percent.

[b]Two occurrences of cardiac stimulation, >50 percent increase.

[c]One occurrence of cardiac stimulation, >50 percent increase.

IV. DISCUSSION

A. ACUTE TOXICITY STUDIES

The acute intraperitoneal toxicity of several 1-alkyl-3-benzoylpyridinium halides was determined in albino mice in order to test the possible existence of a trend in toxicity paralleling a change in structure. At the same time, the gross syndrome was observed in order to provide information concerning a possible mode of action.

The methyl salts of the 3 and 4 isomers were the least toxic and were comparable to each other. Toxicity increased with the ethyl salts of both isomers, although the ethyl salt of the 4 isomer was 3 times more toxic than its counterpart. As the carbon substitution increased from two to seven carbons, the toxicity of the 1-alkyl-3-benzoyl-pyridinium halides decreased. From eight to fifteen carbons the toxicity of these compounds rapidly increased. Physical changes in the compounds, decreasing water solubility and increasing ability of an aqueous solution to foam when agitated, paralleled the increasing toxicity.

The symptoms of toxicity appeared to be the same in all cases: decreased activity, mydriasis, dyspnea, rapidly followed by convulsions and death. The major change in toxicity was the period of time required for death or recovery to occur. Compounds containing one to eight

carbons caused death or recovery in one hour, while compounds containing nine to fifteen carbons required a 24 to 48 hour period for death or recovery.

The change from decreasing to increasing toxicity paralleled by physical changes in the compounds and the change in mortality time accompanying the above, suggest that there are two mechanisms of action for these compounds. The first occurs with the compounds containing one to seven or eight carbons. The second occurs with the compounds containing more than eight carbons and may be due in part to surface active properties.

Based on certain pharmacological principles, three compounds representative of the 1-alkyl-3-benzoylpyridinium halides were chosen for further study: 3-C2-I, 3-C8-Br, and 3-C15-Br. Acute intraperitoneal toxicities were determined in albino rats. Although the gross syndrome was similar to that of the mice, it was interesting to note the apparent change in the trend of toxicity. 3-C2-I was three times more toxic in rats while 3-C8-Br was two times more toxic in mice.

However, since only three compounds had been tested in rats, the possibility existed as in the mice that between 3-C2-I and 3-C8-Br the toxicity might increase before decreasing. Therefore, two more compounds, 3-C4-Br and 3-C5-Br, were each tested in 18 male albino rats employing three doses. More doses and larger groups were

not employed due to the inavailability of sufficient amounts of the compcurds. These two compounds were less toxic than 3-C2-I, indicating that the trend in toxicity in both species was somewhat similar but occurred at a slightly different number of carbons.

B. BLOCD PRESSURE STUDIES-Rats

Direct blood pressure studies were conducted in male albino rats because changes in blood pressure may indicate a drug action on the autonomic nervous system, on blood vessels, on the heart, or the central nervous system.

Intravenous administration of 3-C2-I, 3-C8-Br, and 3-C15-Br resulted in a rapid but transient hypotension. The magnitude of the response was dose related while the duration of the response was dose related for the latter two compounds only. The order of increasing hypotensive effect was 3-C2-I, 3-C8-Br, and 3-C15-Br; the duration of the response also increased in this order.

These compounds produced periods of dyspnea partic- at higher doses and in some instances, normal function was restored with artificial respiration. At higher doses of 3-C15-Br, the respiratory passages were filled with fluid and artificial respiration was ineffective. This fluid phenomenon did not occur with the other compounds tested. Respiratory failure preceded cardiac arrest and death.

C. BLOOD PRESSURE STUDIES-Cats and Dogs

Observation of the hypotensive effects of these

compounds led to the investigation of the mode of this action. The possibility of a central action was not pursued because quaternary ammonium salts do not readily traverse the blood brain barrier. Therefore, investigation of the hypotensive effects of these compounds was directed toward peripheral actions.

The first possible site of action investigated was the autonomic nervous system, since this system exerts a significant controlling influence on blood pressure. Four cardiovascular tests, mediated through the autonomic nervous system, were performed in cats: carotid occlusion, vagal stimulation, administration of acetylcholine and epinephrine. Carotid occlusion reflexly stimulates the sympathetic nerve leading to the heart through a central mechanism, which then causes acceleration. The blood vessels also constrict and a rise in blood pressure ensues. Electrical stimulation of the peripheral portion of the severed vagus nerve is a presynpatic stimulation of a parasympathetic nerve, whose impulses cause the heart rate to decrease and blood vessels to dilate. A fall in blood pressure ensues. Acetylcholine causes a fall in blood pressure, epinephrine a rise in blood pressure, due to their actions at the neuro-effector junction of the parasympathetic system and sympathetic system, respectively.

Concurrent to the peak hypotensive effect of 3-C2-I or 3-C8-Br, the responses to carotid occlusion and vagal

stimulation were either decreased or abolished. In most instances the response to epinephrine was increased. This suggested that the hypotensive effects of these two compounds resulted from the interference of transmission at autonomic ganglia. 3-C8-Br was more effective in this respect.

Intravenous administration of 3-C15-Br to cats resulted in death from respiratory failure probably due in part to large amounts of red fluid in the respiratory passages. Aspiration of the fluid and artificial respiration were not effective. It was possible that this secretion of fluid might be the result of excessive use of acetylcholine. Therefore, two additional cats were prepared by the same surgical procedure but were administered only the test compound. The same response was noted. 3-C12-Br was tested in this manner with the same results. With all four compounds, respiratory failure preceded cardiac arrest and death.

Some of the higher molecular weight quaternary ammonium salts are surface active agents and have found use as antibacterial agents (disinfectants, antiseptics, or sanitizers). Since aqueous solutions of 3-C12-Br and 3-C15-Br produce foam when agitated, it suggests that these two compounds have similar properties. It is possible that the excess fluid in the respiratory passages resulted from an effect on membrane permeability concomitant

with a hemolysis of red blood cells.

Although 3-C12-Br and 3-C15-Br exhibited hypotensive effects preceding the appearance of fluid in the respiratory passages, no positive conclusions were drawn concerning their mechanism of action. Further study of these compounds was discontinued at this time.

D. NICTITATING MEMBRANE

The nictitating membrane is a third eyelid present in certain animals. It is composed of smooth muscle and is innervated by sympathetic fibers, only. The pre- and postganglionic fibers innervating this membrane are readily isolated, thereby making it an ideal preparation for testing sympathclytic and ganglioplegic agents.

Prior testing had suggested that 3-C2-I and 3-C8-Br might be ganglioplegic agents. This preparation was employed to further test this supposition.

3-C2-I and 3-C8-Br were found to be hypotensive agents when administered intravenously to cats. The increasing hypotension was accompanied by a decreased and finally abolished response of the nictitating membrane to stimulation of the preganglionic cervical sympathetic nerve. The return of blood pressure to normal was accompanied by a recovery of the response of the nictitating membrane to preganglionic stimulation. The response to postganglionic stimulation was unaffected. These observations support the hypothesis that these two compounds

exert a hypotensive effect through ganglioplegia.

E. HINDQUARTER PERFUSION

Although previous tests had shown that 3-C2-I and 3-C8-Br have ganglioplegic properties, these procedures had not established whether direct vasodilation comprised a possible or significant component of the hypotensive effect. In order to test for vasodilator activity, hindquarter perfusions were performed in intact rats. The method employed permitted direct contact of the compounds with the hindquarter vasculature but precluded reflex control of the autonomic nervous system. Thus any change in flow rate would be due to a direct drug action upon the vasculature.

The compounds were employed in doses up to the equivalent ED50. A two tailed Student's t test at the 95 percent level rejected the hypothesis of a significant change in flow rate following intravenous administration of the test compound. Thus these experiments reject direct vasodilation as a significant component of the hypotensive effect.

F. ISOLATED HEART PERFUSION

Prior experimentation had established that 3-C2-I and 3-C8-Br exerted a hypotensive effect resulting from ganglioplegia unabetted by significant direct vasodilation. However, the possibility of a direct action upon the heart contributing significantly to the hypotensive effect existed

and was tested by employing an isolated heart preparation. Perfusion of the coronary system of an isolated heart has the advantage of supplying the drug directly to the cardiac muscle. However, this method is disadvantageous because of the inability to correlate the dose producing an effect on the isolated heart with the dose producing an effect in the intact animal. It can establish whether there is a depressant effect but not whether the cardiac depression contributed significantly to the overall hypotensive effect.

The principal effect of 3-C2-I on the isolated heart was severe depression occurring from 0.5 to 4.0 mg. The principal effect of 3-C8-Br on the isolated heart was cardiac arrest occurring from 0.6 to 1.0 mg. At 2.0 mg the compound became cardiotoxic. Neither the magnitude nor the duration of the response to either compound were dose related.

Evaluated on the basis of cardiotoxicity, 3-C8-Br was more toxic than 3-C2-I. At 2.0 mg, the former produced three out of three cardiac arrests with no recoveries, while the latter at the same dose produced one out of seven cardiac arrests with no recovery and five out of seven severe cardiac depressions with full recoveries. Also noted in several instances with these compounds was an initial increased amplitude of contraction, lasting 2 to 3 seconds preceding cardiac depression or arrest.

The results of these experiments indicated that these compounds have an effect on the heart. No positive

conclusions were drawn concerning this mechanism of action or concerning the contribution and significance of these effects to the overall hypotension.

V. SUMMARY AND CONCLUSIONS

1. A series of synthetic 1-alkyl-3- and 1-alkyl-4-benzoylpyridinium halides were screened for pharmacological activity with the purpose of relating such activity to their structural arrangement. An attempt was made to determine their mode of action.

2. In mice, the trend of the acute intraperitoneal toxicity for the 1-alkyl-3-benzoylpyridinium halides was biphasic. The methyl halides of the 3 and 4 isomers were found to be the least toxic and comparable to each other. The toxicity of the ethyl halides of these isomers was increased over that of the methyl halides, although the 4 isomer was three times more toxic than its counterpart. As the carbon substitution increased to seven in the 3 isomer, the toxicity decreased. Increasing carbon substitution resulted in increasing toxicity. This increasing toxicity was paralleled by an increasing ability of aqueous solutions of these compounds to foam and a decreasing water solubility.

3. In rats the acute intraperitoneal toxicity decreased as the carbon substitution increased from two to five. From 3-C5-Br to 3-C8-Br, the toxicity rapidly increased.

4. In rats, the ED50 (hypotensive) increased from 3-C2-I to 3-C8-Br to 3-C15-Br. A dose-effect relationship

existed. Higher doses of 3-C15-Br resulted in death from respiratory failure, which may have been partly due to fluid in the respiratory passages.

5. In cats and dogs, 3-C2-I and 3-C8-Br demonstrated hypotensive effects. The normal response to carotid occlusion and vagal stimulation was decreased and the response to injected epinephrine was increased suggesting ganglioplegia. 3-C8-Br was more potent than 3-C2-I in this ability. In cats, 3-C12-Br and 3-C15-Br in higher doses resulted in respiratory failure probably due to excessive amounts of red fluid in the respiratory passages. The ability of aqueous solutions of these compounds to foam suggests death resulted from a change in membrane permeability concomitant with a hemolysis of red blood cells.

6. 3-C2-I and 3-C8-Br blocked the normal response of the nictitating membrane in cats to electrical stimulation of the preganglionic cervical sympathetic nerve. The response of this membrane to postganglionic electrical stimulation was unaffected. 3-C8-Br was more potent in this ability than 3-C2-I.

7. Employing doses up to the equivalent ED50 (hypotensive) in rats did not affect the flow rate of perfusions of the hind quarter vasculature of the intact rat.

8. These two compounds showed cardiodepressant and cardiotoxic effects on the isolated guinea pig heart.

A dose-effect relationship was not apparent. 3-C8-Br was more potent than 3-C2-I.

9. The results of these experiments support the hypothesis that 3-C2-I and 3-C8-Br exert a hypotensive effect through ganglioplegia unabetted by direct vasodilation. A depressant action on the heart may contribute to the hypotension induced by ganglioplegia.

VI. REFERENCES

Acheson, G.H., and S.A. Pereira: The blocking effect of tetraethylammonium ion on the superior cervical ganglion of the cat. J. Pharmacol. Exptl. Therap. 87:273-280, 1946.

Bachrach, W.H.: Anticholinergic drugs-Survey of the literature and some experimental observations. Am. J. Digest, Dis. 3:743-799, 1958.

Barlow, R.B. and H.R. Ing: Curare-like action of polymethylene bis-quaternary ammonium salts. Brit. J. Pharmacol. 3:298-304, 1948.

Brody, T.M.: The uncoupling of oxidative phosphorylation as a mechanism of drug action. Pharmacol. Rev. 7:335-364, 1955.

Burger, A.: Chemical structure and biological activity. J. Chem. Educ. 35:142-145, 1958.

Burger, A.: Medicinal Chemistry, Interscience, New York, 1960.

Castillo, J.C. and E.J. DeBeer: The neuromuscular blocking action of succinylcholine (diacetylcholine). J. Pharmacol. Exptl. Therap. 99:458-464, 1950.

Chenoweth, M.B. and E.S. Koelle: An isolated heart perfusion system adapted to the determination of non gaseous metabolites. J. Lab. Clin. Med. 31:600-604, 1946.

Editorial, New oral insulin-like agent. Science 123:258, 1956.

Erlenmeyer, H. and M. Leo: Über Pseudoatome. Helv. Chim. Acta. 15:1171-1179, 1932.

Farber, S., L.K. Diamond, R.D. Mercer, R.F. Sylvester, Jr., and J.A. Wolff: Temporary remissions in acute leukemia by folic acid antagonist, 4-aminopteroyl-glutamic acid. New Eng. J. Med. 238:287-793, 1948.

Ferguson, J.: The use of chemical potential as indices of toxicity. Proc. Roy. Soc. (London) B127:387-402, 1939.

Friedman, H.L.: Influence of isosteric replacements upon biological activity. Symposium on Chemical-Biological Correlation, Natl. Acad. Sci. Natl. Research Council, publ. No. 206, Washington D.C. 295, 1951.

Hansch, C.: The use of substituent constants in drug design, Annual Reports in Medicinal Chemistry, Chapter 34, 1966.

Hart, E.R. and E.L. McCawley: The pharmacology of N-allylnormorphine as compared with morphine. J. Pharmacol. Exptl. Therap. 82:339-348, 1944.

Issekutz, B.: Über die chemische Struktur and pharmakologische Wirkung der die vegetative Ganglion lähmenden Verbindungen. Pharmazie 9:124-131, 1954.

Kirsner, J. and W. Palmer: Newer gastric antisecretory compounds. J. Am. Med. Assoc. 151:798-805, 1953.

Krebs, H.A.: Inhibition of carbonic anhydrase by sulfonamides. Biochem. J. 43:525-528, 1948.

Lawrence, C.A.: Surface-Active Quaternary Ammonium Germicides, Academic Press, N.Y. 1958.

Litchfield, Jr., J.T., and Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. and Exptl. Ther. 96:99, 1949.

Loubatieres, A., P. Bouyard and C. Fruteau de Laclos: Actions du para-aminobenzene-sulamido-isopropylthiodiazol sur la glycemie, la structure des ilots de Langerhans et le metabolisme de l'animal normal ou diabetique. Compt. rend. soc. biol. 149:1642-1645, 1955.

Miller, W.H., A.M. Dessert and R.O. Roblin, Jr.: Heterocyclic sulfonamides as carbonic anhydrase inhibitors. J. Am. Chem. Soc. 79:2028-2030, 1950.

Northey, E.H.: The Sulfonamides and Allied Compounds, Reinhold Publishing Corporation, New York, 1948.

Schutz, H.G. and F.J. Pilgrim: Sweetness of various compounds and its measurement. Food Research 22:206-213, 1957.

Smith, C.M., H.L. Cohen, E.W. Pelikan and K.R. Unna: Mode of ation of antagonists to curare. J. Pharmacol. Exptl. Therap. 105:391-399, 1952.

Smookler, H.H. and J.J. DeFeo: Comparative cardio-inhibitory effects of substituted cyclohexanols. J. Pharm. Sci. 51:736-740, 1962.

Symposium on 6-mercaptopurine: Ann. N.Y. Acad. Sci. 60:183-508, 1954.

Triggle, D.J.: Chemical Aspects of the Autonomic Nervous System, Academic Press, New York, 1963.

Wien, R., D.F.J. Mason, N.D. Edge and G.T. Langston: The ganglionic blocking properties of homologous compounds in the methonium series. Brit. J. Pharmacol. 7:534-541, 1952.

Wilson, C.O. and O. Gisvold: Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott, Philadelphia, 1960.

VII. ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation for the patience and guidance given throughout this investigation by Dr. John J. DeFeo and the members of the graduate committee.

The author is sincerely grateful for the inspiration and continued empathy of her parents, Mr. and Mrs. James Branyon Springfield.

The author acknowledges that a National Defence Education Act Fellowship helped support this investigation.