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Comparative Effects of Certain Derivatives of Cyclohexanol

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COMPARATIVE EFFECTS OF CERTAIN DERIVATIVES OF CYCLOHEXANOL

BY

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Title Abstract

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OF

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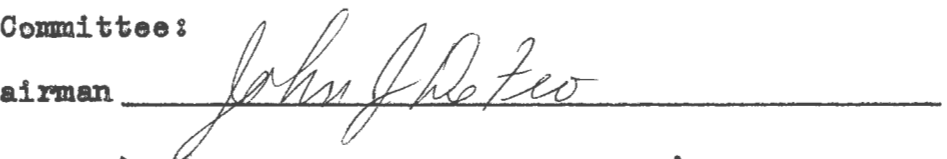
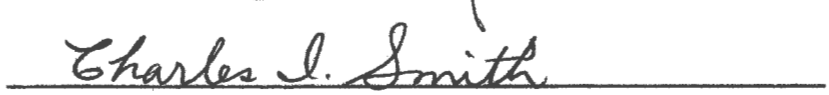
Chairman 



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ABSTRACT

Derivatives of cyclohexanol were screened for pharmacological effects on intact anesthetized rats, isolated guinea pig hearts, isolated rat atria and isolated rabbit small intestine. All the compounds tested produced a hypotensive response and cardio-inhibitory effects varying from slight depression to complete arrest. On the basis of recovery from induced cardiac arrest none of the compounds appeared to be cardio-toxic. All the compounds produced inhibitory effects on the isolated small intestine in varying degrees.

Methyl 3-hydroxy-2-o-tolylcyclohexylacetate and trans-3-oxo-2-o-tolylcyclohexylacetic acid were equally potent and the most effective in their cardiovascular effects whereas the ketal from glycerol and 2-o-tolylcyclohexanone was the most effective on the isolated rabbit small intestine.

Studies of mechanism, employing the isolated rat atria, indicate an antagonistic effect between these compounds and calcium.

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I. INTRODUCTION

The production of elective cardiac arrest has become of interest within the past decade with the advances made in open-heart surgery. During cardiopulmonary by-pass procedures for the correction of intracardiac lesions, it sometimes is expedient to utilize adjuvant measures for the temporary control of cardiac perfusion and activity. These are valuable assets to the cardiac surgeon and provide a quiet and dry field which facilitates the repair of complex cardiac defects.

Experimental and clinical cardioplegia has been achieved by various techniques. These consist chiefly of inducing cardiac arrest by anoxia, hypothermia, and the use of substances such as acetylcholine and potassium citrate. Other agents and techniques have been introduced and in turn received with varying degrees of enthusiasm, with none of them reliable for routine use. The major complications are the difficulty in restoring the heart beat, ventricular fibrillation and arrhythmias, resistance to electrical stimulation, and myocardial damage.

The ideal cardioplegic agent should be able to produce a prompt and effective cardiac arrest that will continue for a period of time sufficient for the repair of complicated intracardiac pathology. It should be nontoxic to the myocardium and other tissues and recovery from arrest should be rapid, safe and reliable (Fergulio, 1959). The synthesis of

new compounds that could bring about elective cardiac arrest has progressed at a rapid rate, but the ideal cardioplegic agent has yet to be found.

It is evident from various reports in the literature that several derivatives of cyclohexanol possess cardioplegic properties. The present investigation deals with the evaluation of newer cyclohexanol derivatives in an effort to further correlate molecular structure with cardio-inhibitory activity.

II. REVIEW OF THE LITERATURE

Elective cardiac arrest, induced in the course of open cardiac operations with cardiopulmonary bypass, has been found to be a valuable technical adjunct. In particular, the quiet and dry field it provides greatly facilitates the repair of more complex anomalies.

Since this investigation deals with the pharmacological approach to elective cardiac arrest the review of the literature concerns agents that produce this effect.

The concept of temporarily abolishing cardiac activity has been known since the latter part of the nineteenth century. Ringer in 1883 drew attention to the effect of the different cations on the heart beat and Hooker (1929) suggested that potassium inhibition induced by an excess of potassium chloride could be used to stop the heart when its beat was disorganized by ventricular fibrillation. He recommended a solution of calcium chloride as an antidote to potassium when re-starting the heart. This work was revived by Montgomery *et al.* (1954) in order to reverse ventricular fibrillation in hypothermic patients.

Senning (1952) attempted to inhibit cardiac contractions temporarily by the injection of acetylcholine, methacholine and potassium chloride, but he was apparently unsuccessful because he maintained coronary perfusion which immediately washed out or diluted the cardioplegic agent.

Based on these observations, Melrose et al. (1955) succeeded in evolving a method of stopping and re-starting the heart at both normal and reduced body temperatures. Thirty-three adult dogs were used to determine whether the induction of cardiac arrest by chemical means was a practical measure. While the vital centers were protected with the heart-lung machine or by reduction of the general body temperature, cardiac arrest was induced by an injection of potassium citrate into the root of the aorta. The arrest was maintained as long as an adequate level of potassium citrate remained in the coronary arteries. Normal beat was restored when simple perfusion of the coronary circulation reduced the level of potassium citrate. This technique provided an almost bloodless operative field with excellent visibility. The heart is completely motionless and flaccid so that it can be manipulated for the exact placing of sutures.

Following the work of Melrose et al. a great deal of interest was aroused in this field. Baker and Dreyer (1956) were able to induce cardiac arrest by potassium citrate and demonstrated the restoration of the normal beat by simple perfusion.

Previous experiments left unanswered the degree of asphyxia to be expected following cessation of coronary circulation during induced cardiac arrest. To investigate this problem, Bentall and Melrose (1957) studied the production of lactic acid after varying periods of arrest, in

the isolated perfused hearts. The lactic acid concentration in the effluent perfusate rose after interruption of perfusion. The concentration increased progressively as the interruption was prolonged.

Elective cardiac arrest with potassium citrate has been applied clinically by different workers employing the Melrose technique. Effler et al. (1956) successfully treated two of three cases of congenital heart disease with the heart resuming normal sinus rhythm in all three cases. In 1957, these same investigators observed that ventricular asystole occurred frequently with other clinical cases.

Kolff et al (1957) reported that, in all of the thirty-seven cases in which the Melrose technique was applied, complete ventricular asystole was achieved and it was possible to restore effective heart action and adequate circulation. Ventricular fibrillation occurred in seven patients, but normal rhythm always returned. It was found that under the conditions of the experiment the heart could resume its normal action after periods of arrest as long as 40 minutes.

In a series of experiments on dogs, Helmworth and co-workers (1958, 1958a, 1959), found that no animals died following 30 minutes of total cardiopulmonary by-pass, whereas animals in which potassium citrate arrest was induced for 30 minutes had a mortality of nearly fifty per cent with evidence of some myocardial damage.

At the same time other investigators were using acetylcholine as a cardioplegic agent. Moulder et al. (1956) reported on the use of acetylcholine to inhibit cardiac contractions in dogs and humans under hypothermia. Lam et al. (1957, 1957a, 1958) reported clinical experiences with acetylcholine-induced cardiac arrest during intracardial surgical procedures. They found rapid resuscitation and low incidence of ventricular fibrillation during recovery. It was of interest to note that during acetylcholine-induced cardiac arrest the heart might beat if the ventricle was stimulated mechanically.

Sergeant et al. (1957) showed acetylcholine to be a safe and effective agent for both the hypothermic and the normothermic dog hearts. Similar success was obtained in eight human open-heart operations.

Other investigators have attained less success with acetylcholine as a cardioplegic agent. Shramel et al. (1957) could produce cardiac arrest with acetylcholine and methacholine for only short periods of time with no complete cessation of electrical activity during any of the arrests. Kenyon et al. (1960) and McKain (1961) reported failure to produce cardiac arrest with acetylcholine. Bramwald et al. (1959) found severe depression of myocardial activity after 20 to 30 minutes arrest with either acetylcholine or potassium citrate.

Meanwhile, Bigelow et al. (1950, 1950a) and Swan and

Zeavin (1954) had shown that hypothermia protected the heart from long periods of oxygen deprivation by decreasing its metabolic requirements. This fact stimulated its use as a method for inducing cardiac arrest. Various combinations of chemically induced arrest in conjunction with hypothermia have been reported in the literature. In a series of reports, Cooper et al. (1959) and Willman et al. (1959, 1959a), have shown marked depression of left ventricular myocardial function after a 30-minute arrest with either potassium citrate or acetylcholine. Partial protection of the heart was provided by previously cooling to 28°C. Weirich et al. (1959) had noted a similar effect on myocardial function with increasing deterioration as the period of arrest was prolonged from 5 to 20 minutes. McKain (1961) and Berne (1958) could not maintain cardioplegia with acetylcholine even under hypothermic conditions.

McFarland et al. (1960) reported myocardial necrosis in 79 per cent of the patients subjected to potassium citrate arrest who had died at various intervals following open-cardiac operations. The lesion was not observed on the hearts of patients who underwent similar procedures without arrest or with anoxic arrest.

Kenyon et al. (1959) administered potassium citrate and acetylcholine with the infusate temperature at 4° or 5°C. Cardiac arrest occurred in only 4 per cent of the animals treated with acetylcholine in comparison to 100 per cent of

those injected with potassium citrate.

An indication that, with potassium citrate arrest, the ATP, phosphocreatine, and glycogen levels fell much more rapidly than with selective cardiac hypothermia was first presented by Gott et al. (1959). These results have been confirmed by Hall et al. (1959) and Ellison et al. (1960). According to these workers hypothermia appeared to be superior to arrest by potassium citrate for the maintenance of the myocardial energy sources.

Kusinoki et al. (1960), have carried out an investigation on cardioplegic agents in both the normothermic and the hypothermic state. They concluded that cardiac arrest for fifteen minutes by any of the techniques studied resulted in depression of myocardial function to 50-70 per cent of the control function when measured 15 minutes post-arrest. Hypothermia to 25°C partially protected the heart rendered hypoxic for 15 minutes; and, in comparison with other methods studied, cardioplegia by this technique resulted in least depression and best recovery of myocardial function.

Several other chemical agents or combinations of these and other methods for inducing cardioplegia have been reported. Merrit et al. (1958) had reported that magnesium by itself was not a satisfactory cardioplegic agent. However, he pointed out that potassium and magnesium ions appeared to act synergistically.

Young et al. (1956) utilized a combination of magnesium

sulfate, potassium citrate and neostigmine methylsulfate to induce cardioplegia. The effects obtained were apparently superior to potassium citrate alone in terms of post-arrest recoveries. Similar results were obtained by Sealy et al. (1958) and Milnes et al. (1958).

Mondini et al. (1957), demonstrated that isolated guinea pig hearts were arrested in diastole for 3 to 5 minutes with a combination of acetylcholine and procaine hydrochloride.

Clark et al. (1959) devised a method for inducing cardiac arrest by passing blood through a selectively charged magnesium ion exchange resin. The resulting hypocalcemic blood was then perfused into the coronary system producing arrest. Recoveries after forty-five minutes were obtained with no gross evidence of damage.

Yashar et al. (1960) subjected thirty dogs to cardiopulmonary by-pass and cardiac arrest was achieved by means of various cardioplegic agents. They observed that cardiac arrest could be achieved with sodium citrate, sodium phytate, methacholine, and potassium chloride, in addition to potassium citrate and acetylcholine. The rate of recovery after cardiac arrest and perfusion of the coronary arteries with arterialized blood was not predictable. There was no relationship between the dose injected and the duration of the cardiac arrest or the rate of recovery. None of the agents tested appeared safe for routine clinical use.

Advances in the correction of stenotic and insufficient aortic valves have prompted the use of a retrograde perfusion technique in order to facilitate the direct vision of the diseased valve. This technique has been employed by Gott et al. (1959), Lillehei et al. (1958), and other investigators.

In recent years it appears that hypothermia is favored over all other methods used to arrest the heart as an adjunct in open heart surgery.

Reports of adverse effects of prolonged elective cardiac arrest upon myocardial structure and function led Miller et al. (1961) to undertake a comparative study of methods of arrest. Arrest was achieved by potassium citrate, anoxia, and a combination of anoxia and local hypothermia. The changes produced in the heart function following arrest by potassium citrate were less than those observed in other groups. Extensive myocardial necrosis was observed following one hour of potassium citrate arrest. Again in 1964, these investigators studied the progression or reversibility of acute changes in myocardial function and structure after periods of elective cardiac arrest from 30 to 60 minutes. They found that cardiac function was variably impaired following the arrest period. Gross and microscopic areas of necrosis were observed in dog hearts following recovery from potassium citrate arrest. No morphological changes were observed following cardiac arrest

by hypothermia with or without coronary perfusion.

Bjork and Fors (1961) performed an experimental study on dogs to compare the different methods of inducing cardiac arrest. The arrest was maintained for 30 to 40 minutes. Little alteration in the myocardium was found after hypothermic and acetylcholine arrest whereas the most pronounced changes were found after potassium arrest. They concluded that deep hypothermia was preferred over the other methods because of the diminished oxygen demands during this type of arrest.

Brewer et al. (1963) upon evaluating cardioplegics in patients undergoing open heart surgery, found hypothermic arrest to be superior. The mortality following this type of arrest was lower than with any of the others and myocardial degeneration was less severe in the hearts of the patients that died.

Redo (1962) evaluated the effects of prolonged periods of cardioplegia on the mechanical and electrical activity of the isolated guinea pig heart. Arrest was achieved using potassium citrate, Sealy's mixture, acetylcholine, anoxia and hypothermia. Arrest up to periods of 60 minutes by the use of potassium citrate, Sealy's mixture, or potassium citrate were followed by mechanical recovery of 83 to 100 per cent. Anoxic arrest led to the poorest return of cardiac action as compared with the other methods used. The results indicated that the longer the duration of arrest, the longer

the heart takes to recover and the poorer is the mechanical recovery. Fibrillation occurred in all instances after prolonged periods of cardioplegia except when the arrest was achieved by hypothermia. Fibrillation reverted to normal rhythm spontaneously within several minutes.

Burdette and Al Shamma (1962) reported a decrease in myocardial ATP and concomitant increase in ADP and AMP during normothermic hypoxic or potassium citrate arrest. Hypothermia did not prevent the metabolic changes but appeared to facilitate recovery.

More recently, Berne *et al.* (1964) were unable to show any significant differences between the potassium citrate arrest and the hypothermic arrest. These findings are in contrast to those reported by Gott *et al.* (1959), Hall *et al.* (1959), and Ellison *et al.* (1960).

From various reports in the literature it appears that certain structural substitutions in the cyclohexanol ring can bring about cardioplegic properties.

Nelson *et al.* (1958) reported that in certain preliminary experiments *trans*-2-*o*-tolylcyclohexanol had been able to arrest the heart of a dog but recovery was not successful.

Huitric (1959) published a note concerning the production of reversible mechanical arrest on isolated cat heart with this same compound. This prompted Huitric to synthesize a series of cyclohexanol derivatives.

Smookler and DeFeo (1962) extensively investigated cyclohexanol and certain of its 2-tolyl and 2-chlorophenyl substitution products for cardioplegic effects. The compounds were found to be capable of lowering the blood pressure of intact rats and depressing myocardial contractility of isolated guinea pig hearts. The inhibitory effects varied from slight depression to complete arrest. Evaluation of potency was based on the ability to produce cardiac arrest and the duration of the arrest. In both series of substitutions the trans-2-o-tolyl derivatives were the most potent and the cis derivatives the least potent. Mechanism studies indicated an antagonism between these compounds and calcium.

In unpublished work, Smookler (1961) showed that trans-2-o-tolylcyclohexanol, trans-2-o-chlorophenylcyclohexanol and cyclohexanol were capable of inducing cardiac arrest in dogs undergoing cardiopulmonary by-pass. There appeared to be no relationship between the dose employed and the production of cardiac arrest. Recovery was faster and more consistent with trans-2-o-tolylcyclohexanol.

Unpublished work by Smith (1963) showed that other derivatives of cyclohexanol produced similar effects in the cardiovascular system. The compounds tested were trans-2-o-tolyl, cis-2-o-tolyl, trans-2-p-tolyl, and cis-2-p-tolyl derivatives of cyclohexylamine hydrochloride and other miscellaneous cyclohexane derivatives. Trans-2-o-tolylcyclohexylamine hydrochloride was the most potent of the cyclohexylamine series tested.

Other derivatives of cyclohexanol and cyclohexylamine were evaluated similarly. Unpublished work by Kosegarten (1966) showed that the non-ethynyl cyclohexanol derivatives were more potent in producing a hypotensive response than were the derivatives of cyclohexylamine. Trans-2-o-tolyl-cis-1,4-cyclohexanediol and trans-2-o-tolyl-trans-1,5-cyclohexanediol were equally effective and the most active. Conversely, the cyclohexylamines were more potent cardioplegic agents. An antagonistic effect between calcium and the negative inotropic action of these compounds was demonstrated on isolated rat atria.

Based on these reports, there appears to be justification in assessing the comparative cardioplegic effects of other cyclohexanol derivatives.

III. THE INVESTIGATION

A. Objectives

It is evident from recent reports in the literature that certain substituted cyclohexanols show cardioplegic properties. Smookler and DeFeo (1962) reported that certain cyclohexanol derivatives in a series synthesized by Huitric were also capable of producing cardiac arrest. Similar results were obtained by Smith (1963) with substituted cyclohexylamines and miscellaneous cyclohexanol derivatives. Since that time Huitric has synthesized another group of cyclohexanol derivatives.

This investigation was undertaken to determine the comparative effects of these substitution products on the cardiovascular system and to attempt to relate the various effects of the compounds to their molecular structures.

The objectives of the investigation were as follows:

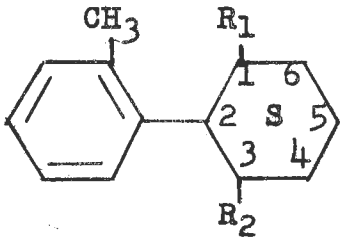
1. To screen pharmacologically the various compounds for their effects on the animals or tissues used.

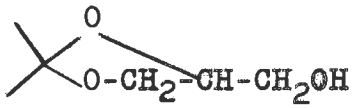
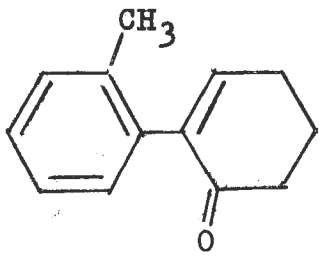
2. To attempt to determine the mechanism through which the compounds' effects are brought about.

B. The Compounds

The substituted cyclohexanols used in this investigation (Figure 1) were synthesized by Dr. Alain C. Huitric of the Department of Pharmaceutical Chemistry, University of Washington, College of Pharmacy, Seattle, Washington. Since

Figure 1. Cyclohexanol Derivatives



R_1	R_2	Name
$-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_3$	-OH	methyl 3-hydroxy-2-o-tolyl-cyclohexylacetate (SH-24)
$-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_3$	$-O-\overset{\overset{O}{\|}}{C}-CH_3$	methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26)
$-CH_2COOH$	-OH	3-hydroxy-2-o-tolylcyclo-hexylacetic acid (SH-23)
$-CH_2COOH$	>=O	trans-3-oxo-2-o-tolylcyclo-hexylacetic acid (SH-16)
H		ketal of glycerol and 2-o-tolylcyclohexanone (#5)
		2-o-tolylcyclohexen-2-one (SH-14)

the solubility of the compounds was not reported by Dr. Huitric, the water solubilities were determined according to the following procedure:

Ten to 25 mg of the compound was weighed into a vial on a Mettler analytical balance. Deionized water was added dropwise from a pipette. After each addition the vial was thoroughly agitated. It was found necessary in all cases to employ heat to effect the solution. The procedure was repeated three times for each compound, and the average of the three determinations was taken as the solubility. Once the compound had dissolved, it remained in solution for at least several hours.

Some of the compounds were practically insoluble in water. The water solubilities of these compounds were not determined. They were dissolved in a mixture of polyethylene glycol (400) and water. Table I lists the physical state of the compounds and the water solubilities.

C. Animals Used

Rats--Albino rats of the Wistar (MW-2) strain were employed for all the rat experiments.

Guinea pigs--The guinea pigs used were of a heterogeneous group.

Rabbits--All rabbits were albino.

D. Experimental Procedures and Results

This investigation was divided into four sections:

1. The effects of the compounds on the blood pressure of intact anesthetized rats.
2. The effects of the compounds on isolated guinea pig hearts.
3. The effects of the compounds on isolated rat atria.
4. The effects of the compounds on rabbit intestinal smooth muscle.

TABLE I

Physical state and water solubility of various derivatives of cyclohexanol.

Compound	Physical State	Water Solubility (mg /ml.)
methyl 3-hydroxy-2-*o*-tolyl-cyclohexylacetate (SH-24)	solid	6.3
trans-3-oxo-2-*o*-tolylcyclo-hexylacetic acid (SH-16)	solid	4.0
3-hydroxy-2-*o*-tolylcyclo-hexylacetic acid (SH-23)	solid	-
methyl 3-acetoxy-2-*o*-tolyl-cyclohexylacetate (SH-26)	solid	-
2-*o*-tolylcyclohexen-2-one (SH-14)	solid	-
ketal of glycerol and 2-*o*-tolylcyclohexanone	liquid	-

1. Effects of Various Derivatives of Cyclohexanol on the Blood Pressure of Rats

a. Experimental Procedures

Wistar rats of either sex weighing approximately 200 g were anesthetized by an intraperitoneal injection of 1.2g/kg of urethan and secured to an animal board, ventral side up. A midline incision was made into the neck, and the trachea was cannulated to insure freedom of respiration. The right carotid artery was isolated and cannulated with a fine glass cannula connected to a U-tube mercury manometer via polyethylene and rubber tubing.

The entire system was filled with normal saline (0.9% NaCl) and the pressure in the system was increased to approximately 90 mm Hg to prevent undue loss of the animal's blood into the saline bridge. To prevent clotting a solution of 0.1 ml of heparin sodium was added to the cannula prior to cannulation. The blood pressure was recorded by means of a glass capillary float from the manometer onto the smoked paper of a slowly moving kymograph. The time was recorded simultaneously with the blood pressure. The blood pressure was allowed to stabilize for a period of 30 minutes prior to administration of any of the experimental compounds.

The compounds were administered intravenously in either femoral vein. The various injections were carried out at

intervals which allowed return of the blood pressure to a steady state level. The total number of injections did not exceed four per experiment. Dosage was adjusted according to the results obtained. In order to determine the dose of a particular compound that would produce a drop in blood pressure of approximately 50 per cent, the dose used and the response obtained were subjected to linear regression computations carried out by the IBM 1410 computer.

In these experiments only methyl 3-hydroxy-2-o-tolyl-cyclohexylacetate and trans-3-oxo-2-o-tolycyclohexylacetic acid were tested, since the other compounds were, for all practical purposes, insoluble in water and the solvent employed for their administration interfered with their response.

The activity of the compounds was evaluated as follows:

1. The per cent change from the normal blood pressure produced by the compounds.
2. The duration of the hypotensive effect.
3. The dose of the compounds needed to produce a 50 per cent drop in blood pressure.

b. Results

The results include the following:

1. Effects of the compounds on the blood pressure of rats (Tables II and III).
2. A comparison of the compounds tested on the blood pressure of rats (Table IV and Figure 2).

TABLE II

Effects of methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) on the blood pressure of rats.

Dose (mg/Kg)	Number of Trials	Mean % Drop in Blood Pressure ± S.D.	Duration of Drop in Blood Pressure (Mean and Range) (sec)
2	4	9 ± 3.0	41 (30-50)
4	3	15 ± 7.0	213 (160-300)
8	4	30 ± 9.0	423 (250-600)
12	3	49 ± 4.0	1020 (900-1140)
16	4	60 ± 2.0	1195 (1000-1440)
18	3	75 ± 3.0	920 (860-1000)

TABLE III

Effects of trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) on the blood pressure of rats.

Dose (mg/Kg)	Number of Trials	Mean % Drop in Blood Pressure ± S.D.	Duration of Drop in Blood Pressure (Mean and Range) (sec)
4	4	17 ± 6	187 (100-300)
8	2	36 ± 0.7	555 (450-660)
10	3	40 ± 4.0	223 (100-370)
12	4	48 ± 9.0	975 (810-1140)
15	5	55 ± 8.0	1652 (1000-2160)
18	2	78 ± 2.0	950 (900-1000)

TABLE IV

A comparison of the ED_{50} (hypotensive) of the cyclohexanol derivatives tested on the blood pressure[a].

Compound	Total Trials	Calculated ED_{50}[b] mg/kg
methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24)	21	11.2
trans-3-oxo-2-o-tolyl-cyclohexylacetic acid (SH-16)	17	11.0

[a]See Figure 2.

[b]Derived from the regression line formula obtained from the analysis of results on the IBM 1410 Computer.

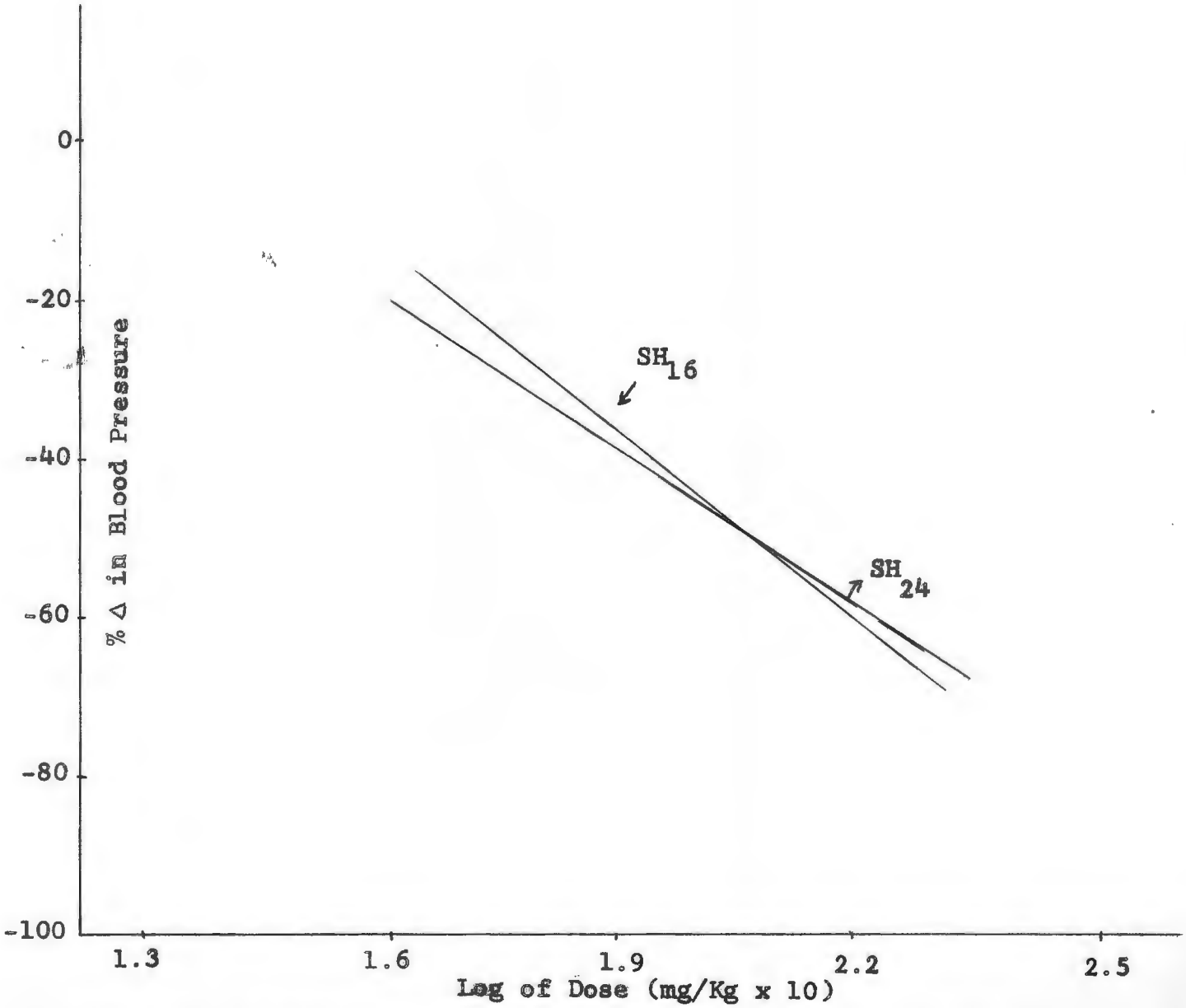


Figure 2. A comparison of the blood pressure effects produced by the cyclohexanol derivatives tested.

2. The Effects of Various Cyclohexanol Derivatives on the Isolated Guinea Pig Heart

a. Experimental Procedures

Guinea pigs of either sex were sacrificed by cervical dislocation. The thoracic cavity was opened and the aorta was clamped. The heart was removed and transferred to a container of oxygenated perfusion solution. The aorta was immediately cannulated via an incision made in the ventral surface. A few milliliters of warm perfusion solution was forced into the cannula to remove residual blood from the coronary vessels and to determine whether the tip of the cannula was occluding the coronary vessels.

The excised cannulated heart was quickly suspended in an Anderson-Craver Apparatus[1] for the continuous perfusion of the heart. Chenoweth's solution (1946) as modified by Smookler and DeFeo (1962) was used as the perfusion fluid. The composition of the perfusion solution expressed in grams per liter is as follows:

	g/l
sodium chloride	7.00
potassium chloride	0.42
calcium chloride	0.24
magnesium sulfate	0.52
dextrose	1.80
sodium bicarbonate	2.10

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

Three liters of stock solution containing 350 g sodium chloride, 21 g potassium chloride, 12 g calcium chloride, and 26 g magnesium sulfate were prepared and stored at room temperature. The final perfusion solution was prepared the day of the experiment by taking 120 ml of the stock solution, 3.6 g of dextrose, and 4.2 g of sodium bicarbonate and diluting to 2 l of solution.

A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled continuously through the solution in the reservoir bottle. A constant temperature of 37.5°C was maintained throughout the experiment. The effluent fluid was recirculated during the experiment, except after the injection of the drug when at least 40 ml was withdrawn to insure complete removal of the drug.

After the preparation had equilibrated, any remaining extraneous tissue was removed and a stainless steel hook was inserted through the apex of the heart. The amplitude of contractions of the heart was recorded on a standard kymograph by passing a thread from the hook to the recording lever via a pulley system.

Injections were made via an injection spiral connected to the perfusion canal. Since the calculated volume of the spiral was 1.5 ml, this same amount of the perfusion solution was injected in the same manner after each injection, to insure complete administration of the drug.

The number of injections performed in each preparation

depended on the ability of the heart to recover from a previous dose and was always limited to five trials. Only one compound was tested in each experiment. A depression of 95 per cent or more was considered to be cardiac arrest.

When testing the compounds soluble in a mixture of polyethylene glycol and water, an equivalent amount of the solvent was employed as a control, prior to each injection, in order to determine its effects.

The dose and response were subjected to linear regression computations carried out by the IBM 1410 computer. The dose producing 50 per cent negative inotropic response was calculated from a graph.

The activity of the compounds was evaluated as follows:

1. The incidence of cardiac arrest at a particular dose, and the duration of arrest at that dose.

2. The incidence and per cent of cardiac depression, if no arrest occurred.

3. The time to return to 50 per cent of the normal contractions.

b. Results

The results include the following:

1. The effects of the various cyclohexanol derivatives on the isolated guinea pig hearts (Tables V to X).
2. A comparison of the effects of the various compounds on the isolated guinea pig hearts (Table XI and Figure 3).

TABLE V

Effects of methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (sec)	Mean % Depression ± S.D. (if no arrest)	Time of 50% Return Mean and Range (sec)
0.5	0/3	-	34 ± 2	55 (50-60)
1.0	0/3	-	57 ± 1	85 (80-94)
1.5	0/3	-	78 ± 12	126 (100-150)
2.0	2/3	200 (120-280)	89 ± 5	297 (180-430)

TABLE VI

Effects of trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (sec)	Mean % Depression (if no arrest) ± S.D.	Time of 50% Return (Mean and range) (sec)
0.5	0/2	-	18 ± 2	100 (80-120)
0.75	0/2	-	38 ± 4	130 (110-150)
1.0	0/2	-	53 ± 4	490 (480-500)
2.0	0/3	-	82 ± 7	217 (190-250)
2.5	2/2	78 (70-85)	-	220 (200-240)

TABLE VII

Effects of methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26) on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (sec)	Mean % Depression ± S.D. (sec) (if no arrest)	Time of 50% Return (Mean and range) (sec)
0.05	0/2	-	35 ± 4	110 (100-120)
0.10	0/2	-	39 ± 5	170 (150-190)
0.25	1/4	150	85 ± 8	427 (320-500)
0.50	2/2	125 (120-130)	-	285 (270-300)

TABLE VIII

Effects of 2-o-tolylcyclohexen-2-one (SH-14) on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (sec) (Mean and range)	Mean % Depression (if no arrest) ± S.D.	Time of 50% Return (Mean and range) (sec)
0.5	0/3	-	26 ± 3	87 (70-110)
1.0	0/3	-	28 ± 3	92 (80-105)
2.0	0/3	-	57 ± 2	133 (125-150)
2.5	2/4	137 (130-145)	92 ± 6	212 (150-320)

TABLE IX

Effects of 3-hydroxy-2-o-tolylcyclohexylacetic acid (SH-23) on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (Mean and range)	Mean % Depression (if no arrest) ± S.D.	Time of 50% Return (Mean and range)
2.5	0/2	-	10 ± 7	9 (7-11)
5.0	0/2	-	12 ± 8	9 (6-12)
6.0	0/2	-	8 ± 3	12 (11-13)
7.0	0/2	-	23 ± 11	18 (16-20)
8.0	0/2	-	30 ± 13	24 (21-27)

TABLE X

Effects of ketal of glycerol and 2-o-tolylcyclohexanone on the isolated guinea pig heart.

Dose (mg)	Cardiac Arrest Arrest/Trial	Duration of Arrest (sec) (Mean and range)	Mean % Depression ± S.D. (if no arrest)	Time of 50% Return (Mean and range) (sec)
1.46	0/3	-	29 ± 7	77 (46-104)
2.19	0/2	-	57 ± 3	105 (90-120)
2.92	1/3	65	78 ± 3	305 (180-330)
5.85	3/3	73 (40-100)	-	110 (100-120)

TABLE XI

A comparison of the ED_{95} (cardio-inhibitory) of the cyclohexanol derivatives on the isolated guinea pig heart[a].

Compound	Total Trials	Calculated ED_{95}[b] (mg)
methyl 3-hydroxy-2-o-tolyl-cyclohexylacetate (SH-24)	12	2.11
trans-3-oxo-2-o-tolylcyclo-hexylacetic acid (SH-16)	11	2.41
2-o-tolylcyclohexen-2-one (SH-14)	13	3.89
methyl 3-acetoxy-2-o-tolyl-cyclohexylacetate (SH-26)	10	4.17
ketal of glycerol and 2-o-tolylcyclohexanone	11	5.52
3-hydroxy-2-o-tolylcyclohexyl-acetic acid (SH-23)	10	c

[a]See Figure 3.

[b]Derived from the regression line formula which was obtained from the analysis of results on the IBM 1410 Computer.

[c]Compound did not produce more than 30 per cent depression in the dosage range tested.

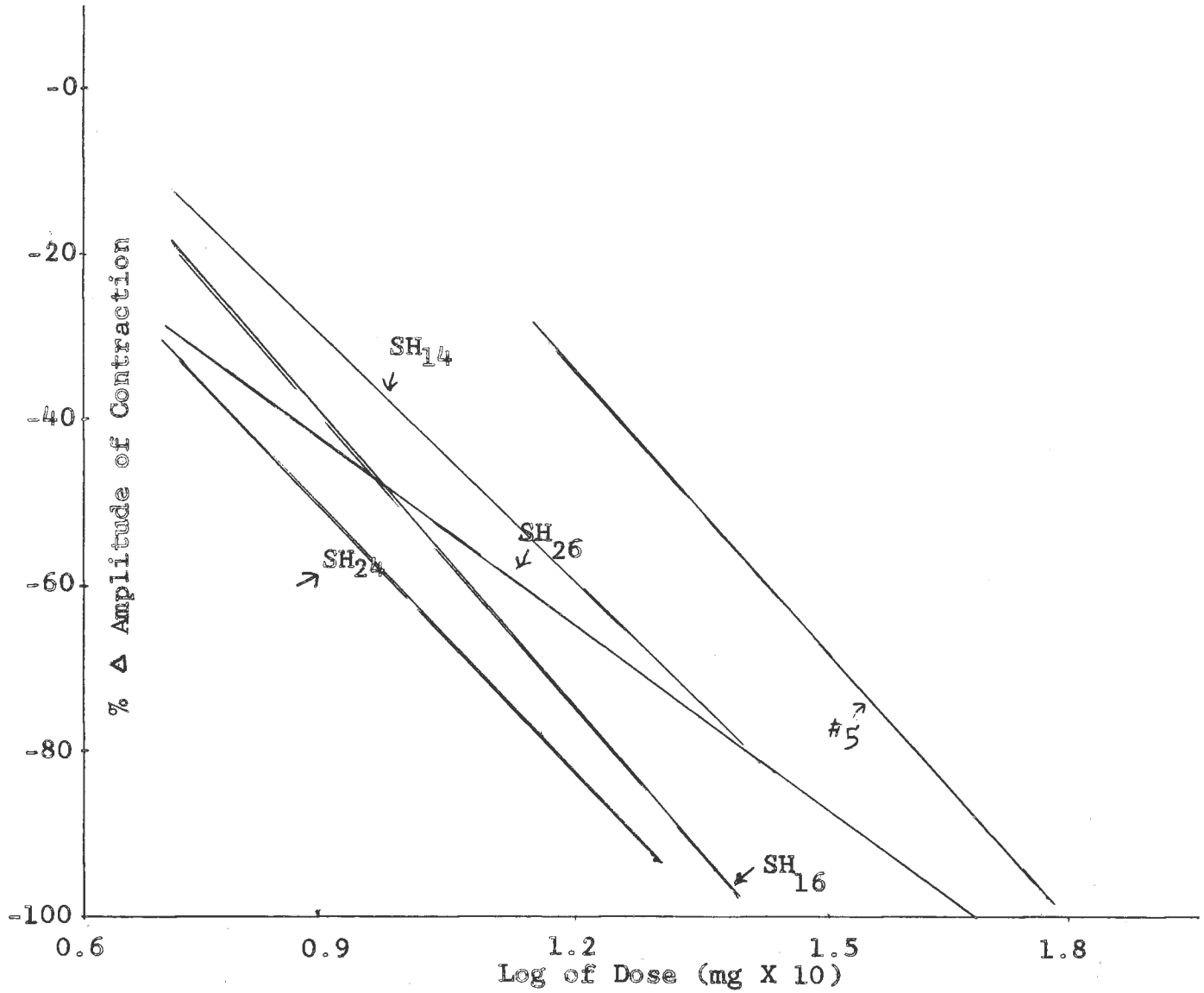


Figure 3. A comparison of the cardio-inhibitory effects produced by the various cyclohexanol derivatives tested.

3. The Effects of Various Cyclohexanol Derivatives on the Isolated Rat Atria

a. Experimental Procedure

Albino rats, either male or female, were sacrificed by cervical dislocation. The thoracic cavity was opened by removal of the sternum and the heart was rapidly removed and immersed in a container filled with warm oxygenated solution. After 20 to 30 minutes the atria were freed of ventricular muscle, fat, and connective tissue and suspended in a tissue bath containing the same nutrient solution as used in the perfusion experiments.

The temperature of the bath was maintained at 32°C as suggested by Webb (1950) in order to reduce the rate and increase the amplitude of contractions. The solution in the bath was continuously aerated with a mixture of oxygen (95%) and carbon dioxide (5%).

The tip of the right atrium was then attached to a stainless steel hook attached to a recording lever with a thread. A second hook attached to a removable glass oxygen bubbler in the organ bath was then inserted to the left atrial appendage. The lever was adjusted for maximum magnification of contractions. The amplitude of contractions and the rate of spontaneous contractions were recorded on a slowly moving kymograph. In order to obtain a constant amplitude of contraction the atrial preparation was kept in the bath for at least 30 minutes before starting the experiment.

Methyl 3-acetoxy-2-o-tolylcyclohexylacetate was selected for mechanism studies on the isolated rat atria. A 4 per cent solution of calcium chloride was used to determine the possible interactions with the compound. All compounds were allowed to act for 3 minutes before washing or adding other agents.

The activity of the compounds was evaluated as follows:

1. The per cent change in the amplitude of contraction.

2. The change in the rate of the preparation.

b. Results

The results include the following:

1. The effects of the various cyclohexanol derivatives on the isolated rat atria (Tables XII to XVI).
2. A comparison of the effects of the various compounds on the isolated rat atria (Table XVII and Figure 4).
3. Calcium chloride (4.0 mg) produced an increase in the amplitude of contraction of approximately 80 per cent at the end of 3 minutes. Over the period of 3 minutes, the rate of contraction increased.
4. A dose of 0.75 mg of the compound reduced the amplitude of contraction by approximately 50 per cent within 1 minute, with no apparent change in rate.

5. When methyl 3-acetoxy-2-o-tolylcyclohexylacetate had reduced the amplitude of contraction by 50 per cent it was found that it required 4 successive doses of 4.0 mg of calcium chloride each to restore the amplitude of contraction to a normal level. When 16 mg was administered at one time there was complete recovery within 3 minutes.

TABLE XII

Effects of methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) on the isolated rat atria.

Dose (mg)	Number of Trials	% Depression ± S.D.	% Change in Rate (Mean & Range)
1.0	3	25 ± 8	7 (4-10)
1.5	3	31 ± 2	5 (1-11)
2.0	3	56 ± 12	7 (4-10)
3.0	3	31 ± 6	8 (6-10)
4.0	3	46 ± 9	12 (5-22)

TABLE XIII

Effects of methyl 3-acetoxy-2-o-tolylcyclohexyl acetate (SH-26) on the isolated rat atria.

Dose (mg)	Number of Trials	% Depression ± S.D.	% Change in rate (Mean & Range)
0.25	3	15 ± 2	11 (9-14)
0.50	2	31 ± 11	13 (12-14)
0.75	2	51 ± 11	8 (4-11)
1.00	3	55 ± 5	26 (23-29)
2.00	3	44 ± 11	13 (10-15)

TABLE XIV

Effects of 2-o-tolylcyclohexen-2-one (SH-14) on the isolated rat atria.

Dose (mg)	Number of Trials	% Depression ± S.D.	% Change in rate (Mean & Range)
1.0	3	25 ± 15	6 (3-10)
1.5	3	34 ± 8	15 (7-22)
3.0	5	46 ± 6	22 (19-24)
4.5	2	72 ± 2	37 (33-41)

TABLE XV

Effects of the ketal of glycerol and 2-o-tolylcyclohexanone (#5) on the isolated rat atria.

Dose (mg)	Number of Trials	% Depression ± S.D.	Change in Rate (Mean and Range)
0.73	3	23 ± 13	13 (9-16)
1.45	3	49 ± 6	---
2.92	2	69 ± 1	---
5.80	3	75.3 ± 1	---

TABLE XVI

Effects of trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) on the isolated rat atria.

Dose (mg)	Number of Trials	% Stimulation ± S.D.	% Change in Rate
0.5	3	14 ± 2	2 (0-2)
1.0	2	35 ± 1	5 (2-8)
2.0	2	14 ± 6	7 (0-7)

TABLE XVII

A comparison of the ED_{50} of the cyclohexanol derivatives tested on the isolated rat atria.[a]

Compound	Number of Trials	Calculated ED_{50}[b] (mg)
methyl 3-hydroxy-2-o-tolyl-cyclohexylacetate (SH-24)	15	5.85
methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26)	14	1.38
ketal of glycerol and 2-o-tolylcyclohexanone	11	1.75
2-o-tolylcyclohexen-2-one (SH-14)	14	2.56

[a]See Figure 4.

[b]Derived from the regression line formula obtained from the analysis of the results on the IBM 1410 Computer.

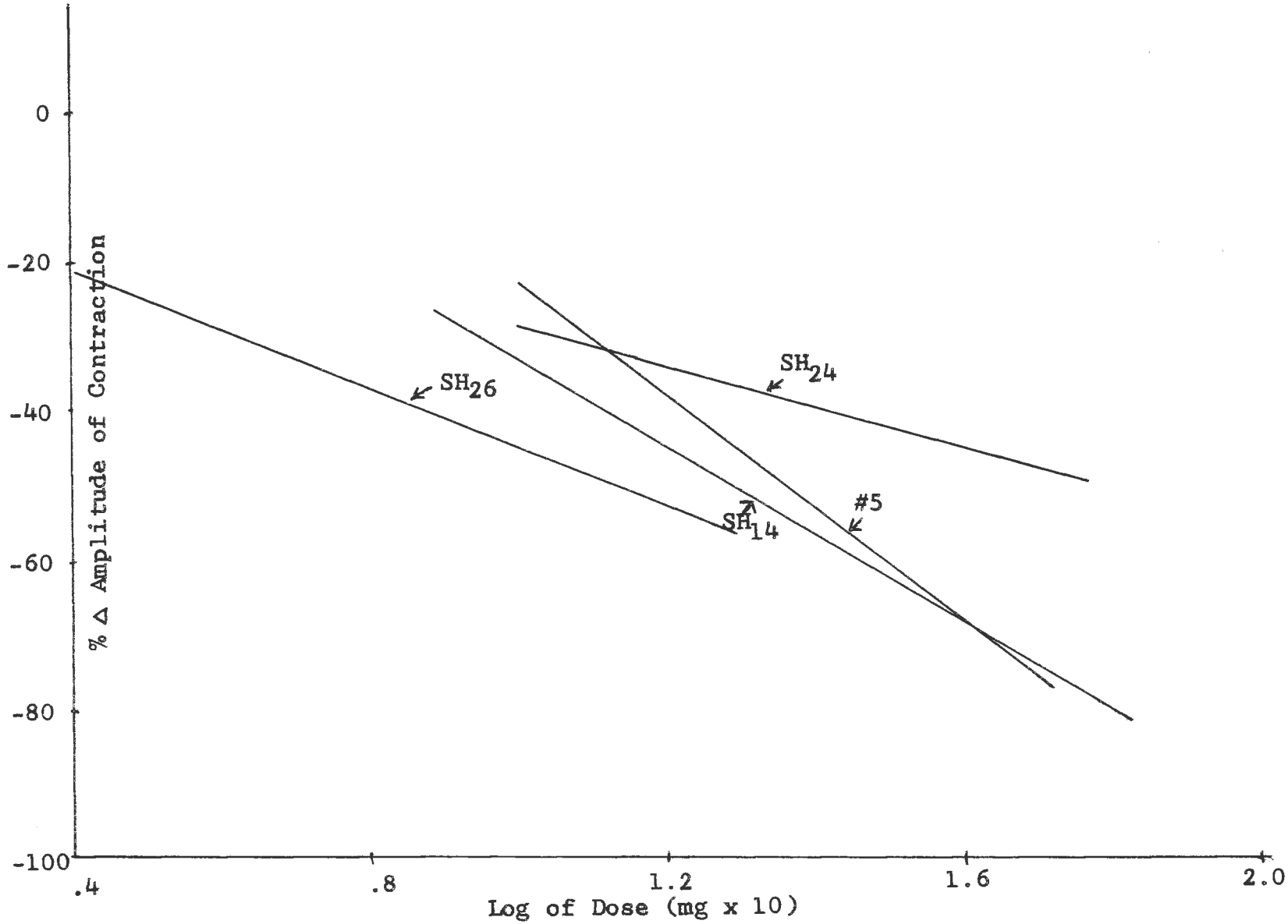


Figure 4. A comparison of the effects produced by the various cyclohexanol derivatives tested on the isolated rat atria.

4. The Effects of Various Cyclohexanol Derivatives on the Isolated Rabbit Ileum

a. Experimental Procedures

In order to determine the effects on smooth muscle some of the compounds were tested on rabbit small intestine, utilizing the Magnus bath. Rabbits of either sex were deprived of food for a period of 24 hours prior to the experiment. The animals were sacrificed by cervical dislocation. The small intestine was then removed from the body and washed with warm Tyrode's solution to remove all fecal matter. A small strip of the tissue (ileum), about 2 cm long, was suspended in a 70 ml Magnus bath containing Tyrode's solution.

A mixture of 95% oxygen and 5% carbon dioxide was continuously bubbled through the solution in the bath. The temperature of the solution was maintained at 38°C by means of a constant temperature unit.

The spontaneous contractions of the ileum were recorded on a slowly moving kymograph drum. After placing the segment of ileum in the bath a period of about one half hour was allowed before any drugs were injected. These were added directly to the muscle chamber with a syringe. A fresh piece of ileum was employed for each drug. The mixing of the drugs in the bath was facilitated by the bubbles of gas by which the bath was oxygenated. Following each dose, fresh solution was added to the bath. If the tissue

recovered sufficiently, it was used for three trials before replacing with a new strip.

The response of the preparation was measured as follows:

1. The change in the amplitude of contraction of the tissue.

2. The change in the rate of contraction of the preparation.

3. The change in the tone of the tissue.

b. Results

The results include the following:

1. The effects of various cyclohexanol derivatives on the isolated rabbit ileum (Tables XVIII to XXII).
2. A dose of 0.70 mcg of acetylcholine established sub-maximal contractions of the intestinal strip within 30 seconds. When the ketal of glycerol and 2-o-tolylcyclo-hexanone had reduced the amplitude of the intestinal strip by approximately 50% at the end of 3 minutes, it was found that the compound did not antagonize the action of acetylcholine.

TABLE XVIII

Effects of methyl 3-hydroxy-2-o-tolylcyclohexyl-acetate (SH-24) on the isolated rabbit ileum.

Dose (mg)	% Change in Amplitude[a]	% Change in Rate[a]	% Change in Tone[a]
1.00	13	7	0
	8	7	0
1.48	18	14	0
	20	7	0
2.00	26	0	0
	41	7	0

[a]All values represent a decrease from normal values expressed as 100 per cent.

TABLE XIX

Effects of methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26) on the isolated rabbit ileum.

Dose (mg)	% Change in Amplitude[a]	% Change in Rate[a]	% Change in Tone[a]
0.30	0	0	8
	9	0	12
0.50	8	0	11
	4	0	9
0.87	17	0	3
	32	0	16
	22	0	16
1.74	51	0	20

[a]All values represent a decrease from normal values expressed as 100 per cent.

TABLE XX

Effects of 3-hydroxy-2-o-tolylcyclohexylacetic acid (SH-23) on the isolated rabbit ileum.

Dose (mg)	% Change in Amplitude[a]	% Change in Rate[a]	% Change in Tone[a]
2.0	+ 9	+12	-3
	0	0	-3
3.0	+10	0	-7
	+10	0	-6
4.0	+ 4	+ 7	-1
	+ 4	0	-3

[a]Values represent an increase (+) or decrease (-) as compared to normal values expressed as 100 per cent.

TABLE XXI

Effects of 2-o-tolylcyclohexen-2-one (SH-14) on the isolated rabbit ileum.

Dose (mg)	% Change in Amplitude[a]	% Change in Rate[a]	% Change in Tone[a]
3.0	9	0	70
	15	6	48
6.0	13	0	22
	16	7	45
9.0	30	6	47

[a]All values represent a decrease from normal values expressed as 100 per cent.

TABLE XXII

Effects of the ketal of glycerol and 2-o-tolylcyclohexanone on the isolated rabbit ileum.

Dose (mg)	% Change in Amplitude[a]	% Change in Rate[a]	% Change in Tone[a]
0.48	17.8	1.8	6.4
	18.1	0.0	4.0
	38.4	7.0	12.5
	25.0	0.0	10.0
0.89	27.2	6.0	0.0
	32.0	0.0	0.0
1.78	68.0	0.0	10.0
	74.0	17.0	13.0
	71.0	14.0	8.0
2.24	74.0	0.0	7.0
	77.0	0.0	13.0
2.68	77.0	0.0	2.0
	82.0	0.0	2.0
2.90	100.0	-	100.0

[a]All values represent a decrease from normal values expressed as 100 per cent.

IV. DISCUSSION

Previous reports in the literature have indicated that several cyclohexanol derivatives can produce a hypotensive effect and possess cardio-inhibitory properties.

A preliminary investigation by Huitric et al. (1959) demonstrated that an intravenous injection of trans-2-o-tolylcyclohexanol produced a rapid hypotensive effect on the intact dog. A reversible cardiac arrest was produced without affecting myocardial properties when this compound was perfused through isolated cat hearts. Pharmacological evaluation studies by Smookler and DeFeo (1962), Smith (1963), and more recently by Kosegarten (1966) showed the degree of cardio-inhibitory activity to be related to the functional groups and stereochemistry of these compounds. In this investigation, a number of substituted cyclohexanol compounds, synthesized by Huitric, were investigated for their effects on the cardiovascular system in an effort to further determine their possible cardio-inhibitory activity as related to molecular structure.

To determine the gross effects produced by these compounds on the cardiovascular system, studies were undertaken utilizing the blood pressure of rats. Only two of the compounds could be tested for hypotensive activity. The other compounds were not soluble in water and the solvent system employed for these derivatives was found to possess an effect of its own. The compounds investigated were:

methyl 3-hydroxy-2-o-tolylcyclohexylacetate and trans-3-oxo-2-o-tolylcyclohexylacetic acid. The results demonstrate that both compounds produce a rapid hypotensive activity upon intravenous injection. The duration of action varied from a few minutes to over 30 minutes. The magnitude of the effect appears to be somewhat proportional to the dose employed for each compound. It was observed that these compounds usually produced some degree of respiratory depression which paralleled the hypotensive response. These findings confirm those previously described by Smookler and DeFeo (1962), Smith (1963), and Kosegarten (1966).

The comparison of the ED_{50} (hypotensive) indicates that methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) and trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) were of approximately equal potency. The actual ED_{50} dose values of these compounds differed by only 0.20 mg with the former exerting a slightly greater effect (Table IV). Smookler and DeFeo have reported that substitution of a tolyl or chlorophenyl moiety in the number two position of the cyclohexanol ring increases its hypotensive activity from 6 to 40 times. It is evident that the free acid (SH-16) and the methyl ester (SH-24) show little difference in activity. This slight difference could be attributed to the free movement of the hydroxy group in the number 3 position in compound SH-24 in comparison to the restriction on SH-16 due to the presence of a double bond. Because of the non-availability

of the *cis* and *trans* isomers of the first compound and the *cis* isomer of the second compound, no correlation could be made between spatial configuration and hypotensive effect.

All the compounds were tested for their effects on isolated guinea pig hearts. Control injections using the solvent system employed for some of the compounds (Polyethylene glycol) showed the solvent to produce a transient positive inotropic effect. From Tables V to X, it can be seen that, at the dose levels employed on the isolated guinea pig hearts, all the compounds tested depressed the amplitude of contractions of the isolated hearts and produced complete arrest, with the exception of 3-hydroxy-2-*o*-tolylcyclohexylacetic acid. With this compound only a 30 per cent decrease in the amplitude of contractions of the isolated heart was obtained in response to doses up to 8.0 mg.

Smookler and DeFeo (1962) reported that 8 of the 10 compounds tested on the isolated guinea pig hearts produced cardiac arrest. All the compounds tested by Smith (1963) produced cardiac arrest. Furthermore, all the compounds tested by Kosegarten (1966) produced mechanical arrest, except *trans*-2-*o*-tolyl-*cis*-4-hydroxy-*trans*-5-hydroxycyclohexanol. Thus, our findings are in accord with those of previous investigators.

In this investigation, on the basis of the minimum calculated effective dose to produce cardiac arrest, it appears that methyl 3-hydroxy-2-*o*-tolylcyclohexylacetate

(SH-24) and trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) were equally effective and the most potent (Table XI). The ED_{95} (cardio-inhibitory) of these compounds differed by 0.30 mg with the former exerting a slightly greater effect. However the duration of the arrest and the recovery time were more prolonged with SH-24 than with the other compounds tested. It will be recalled from the blood pressure studies, that these same compounds were also equally potent in producing a hypotensive response. The ketal of glycerol and 2-o-tolylcyclohexanone was the least potent in its cardio-inhibitory effects. From these findings it can be deduced that the presence of either a free hydroxy group or other substituent in the number 3 position is necessary to elicit the desired pharmacological effect. This has been observed by previous workers in this field. The receptor site, therefore, must possess some area to fit with this substituent moiety. It could also be said that the larger group at the 3 position, the ketal of glycerol, must be producing a steric hindrance to the receptor sites, hence this compound was the least active of those tested. The longer duration of action of the methyl ester (SH-24) is obviously due to the blocking of the free carboxylic acid grouping necessary for conjugation.

In these experiments, in general, with regard to the duration of cardiac arrest and to the per cent depression in the amplitude of contraction, a dose-related response to

the compounds was found. None of the compounds showed signs of cardiac toxicity, as indicated by the recovery of the hearts from the effects of the compounds following either cardiac arrest or depression.

Studies on the isolated rat atria showed that the compounds tested decreased the amplitude of contraction, except trans-3-oxo-2-o-tolylcyclohexylacetic acid (SH-16) which produced a positive inotropic effect. Due to an insufficient amount of this compound, only a 35 per cent increase in the amplitude of the atrial contractions was recorded in response to 1.0 mg of the compound. The solvent employed for some of the compounds (polyethylene glycol) was found to cause a transient inotropic effect that lasted for approximately 60 seconds. Upon administration of the compounds, this same effect occurred before and separate from the action of the compound. Based on the calculated ED_{50}, methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26) was the most potent in decreasing the amplitude of the isolated atria. The ketal of glycerol and 2-o-tolylcyclohexanone was second in comparative effectiveness, followed by 2-o-tolylcyclohexen-2-one. Methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) was the least potent. The mechanism of action of these compounds on the isolated atria is slightly different from that of compounds in previous tests. From the findings it can be said that there is a great need for the presence of a bulky substituent at the 3 position of

the cyclohexanol ring. It is better to say that the o-tolyl substituent should have a big group in an adjacent position. Both methyl 3-acetoxy-2-o-tolylcyclohexylacetate and the ketal of glycerol and 2-o-tolylcyclohexanone were the most potent. It is known that the bulky groups always try to occupy equatorial positions. The o-tolyl and the acetoxy in the first compound and the ketal of glycerol on the second compound may be occupying an equatorial position to give the compound the best spatial configuration for its maximum action.

It is of interest to mention that Smith (1963) reported that 2-o-tolylcyclohexen-2-one produced a positive inotropic effect on the isolated atria in the dosage range tested. This is in contrast to the findings in this investigation. However, most of the doses employed by Smith were lower than those employed in this investigation.

Studies with methyl 3-acetoxy-2-o-tolylcyclohexylacetate (SH-26) demonstrated that, following the depressant action of the compound on the isolated atria, the addition of calcium to the bath restored the amplitude of contraction of the atria to normal. These findings are similar to those reported by Smookler and DeFeo (1962).

In order to determine the effect of the compounds on smooth muscle, isolated intestinal strips were utilized. The solvent employed for some of the compounds (polyethylene glycol) had a transient stimulating effect on the intestine.

Neither the tone nor the rate of contraction was affected by the solvent. In these experiments, all the compounds decreased the amplitude of contractions of the isolated intestine, except 3-hydroxy-2-o-tolylcyclohexylacetic acid (SH-23), which caused an opposite effect in the dose range tested (Table XX). The effects on the rate were variable and inconsistent. All the compounds lowered the tone in varying degrees except methyl 3-hydroxy-2-o-tolylcyclohexylacetate (SH-24) which did not affect the tone (Table XVIII).

The results in Tables XVIII to XXII show that the ketal of glycerol and 2-o-tolylcyclohexanone was the most potent in depressing the amplitude of contractions and lowering the tone of the isolated rabbit ileum. 2-o-Tolylcyclohexen-2-one was the least potent. Thus, substitution at the number 1 position in the cyclohexanol ring does not favor the intestinal contraction. However, once again, a bulky group at the 3 position is best suited for depressing the amplitude of the isolated intestine. The receptor site may have either a cavity or a flat surface to accommodate this bulky group.

Several experiments were carried out in an effort to determine the mechanism of action of these compounds on the smooth muscle tested. When acetylcholine was added to the bath after the depressant action of the ketal of glycerol and 2-o-tolylcyclohexanone had occurred, it was found that

the subsequent response of acetylcholine was not diminished; therefore it appears that the compound is not acting at the terminal synapses of the parasympathetic nerves.

In other experiments when barium chloride was added to the bath after the depressant action of the ketal of glycerol and 2-o-tolylcyclohexanone, the response remained unchanged.

V. SUMMARY AND CONCLUSIONS

1. Several derivatives of cyclohexanol, recently synthesized, were tested for various pharmacological actions. The tests were carried out on intact animals, isolated cardiac tissue, and rabbit small intestine. These compounds were found to actively affect the cardiovascular system and the intestine smooth muscle.
2. Due to the limited water solubility of some of the compounds, only two derivatives were tested on the blood pressure. The results indicate that these compounds produce a rapid hypotensive response on the intact anesthetized rat. The magnitude of the effect varied with the dose administered. Both compounds, methyl 3-hydroxy-2-o-tolylcyclohexylacetate and trans-3-oxo-2-o-tolylcyclohexylacetic acid, had approximately equal potency.
3. Experiments conducted on isolated guinea pig hearts indicate that decreased myocardial contractility is responsible for the cardioplegia produced by these compounds. Both methyl 3-hydroxy-2-o-tolylcyclohexylacetate and trans-3-oxo-2-o-tolylcyclohexylacetic acid exerted the greater cardio-inhibitory activity, and were equally effective, while the ketal of glycerol and 2-o-tolylcyclohexanone was the least active among the compounds studied. None of the compounds appeared to be cardio-toxic.

4. In the isolated atrial studies, the usual response was a decrease in the amplitude of contraction with no significant change in the rate of contraction. Trans-3-oxo-2-o-tolylcyclohexylacetic acid differed from the other compounds tested since it produced a positive inotropic effect on the atrial preparation. Methyl 3-acetoxy-2-o-tolylcyclohexylacetate was the most potent. Methyl 3-hydroxy-2-o-tolylcyclohexylacetate possessed the least activity. The negative inotropic effect of methyl 3-acetoxy-2-o-tolylcyclohexylacetate was antagonized by calcium.

5. All of the compounds were tested for their action on isolated intestinal muscle. All produced depression of the intestinal preparation, except 3-hydroxy-2-o-tolylcyclohexylacetic acid which caused an opposite effect. The ketal of glycerol and 2-o-tolylcyclohexanone was the most potent, while 2-o-tolylcyclohexen-2-one was the least active on the isolated intestine. Several experiments with the ketal of glycerol and 2-o-tolylcyclohexanone indicate that this compound does not antagonize the action of acetylcholine.

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