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The Comparative Cardio-Inhibitory Effects of Substituted Cyclohexanols

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**THE COMPARATIVE CARDIO-INHIBITORY
EFFECTS OF SUBSTITUTED CYCLOHEXANOLS**

BY

HAROLD H. SMOOKLER

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

UNIVERSITY OF RHODE ISLAND

1961

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EFFECTS OF SUBSTITUTED CYCLOHEXANOLS

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ABSTRACT

Cyclohexanol and its derivatives 2-g-tolyl, 2-m-tolyl, 2-p-tolyl, 2-g-chlorophenyl, 2-m-chlorophenyl and 2-p-chlorophenyl were tested for cardioplegic effects on intact anesthetized rats, isolated guinea pig hearts, isolated rabbit atria and on dogs undergoing cardio-pulmonary bypass. Most of the compounds produced inhibitory effects varying from a slight depression to complete arrest. Evaluation of potency was based on the ability to produce arrest and the duration of arrest. In both series of substitutions it appears that the para-2-g-derivatives are most potent with the para-2-g-derivatives least potent.

The 2-m and 2-p-chlorophenyl compounds appear to be toxic to the myocardium as the hearts do not return to their normal level of activity.

Mechanism studies indicate an antagonistic effect between these compounds and calcium.

The compound produced cardiac arrest in the dog, however, there appeared to be no direct dose-response relationship.

MASTER OF SCIENCE THESIS

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1961

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I. INTRODUCTION

The introduction of techniques within the past decade which permit extracorporeal maintenance of systemic circulation has made the surgical correction of intracardiac lesions an actuality. As a result of this unprecedented technical development, many sufferers from congenital and acquired cardiac abnormalities, who were previously doomed to a life of incapacitation or early death, are now leading relatively normal lives.

During cardio-pulmonary bypass procedures there has developed the necessity to utilize adjunctive measures for the temporary control of cardiac activity. Such controls result in a motionless heart in which sutures can be placed accurately and deliberately, a bloodless field in which to work and a considerable lessening of the risk of developing dangerous air emboli. These are valuable assets to the cardiac surgeon and have stimulated the search for agents which could induce a controlled and purposeful cardiac arrest. A variety of agents, both of a chemical and physical nature, have been employed with varying degrees of success. To date, potassium citrate is probably the most commonly employed cardioplegic agent, although various cholinergics and drug mixtures are gaining in popularity. Total body hypothermia and more recently selective cardiac hypothermia have also been shown to be of value as methods for inducing cardiac arrest. Because of the many difficulties encountered with their usage, opinions on the employment of present day methods for inducing cardiac arrest have varied from skepticism to acceptance for routine use.

Cyclohexanol has been employed extensively in the pharmaceutical and chemical industry as a solvent and as a raw material in the manu-

fecture of celluloid and insecticides. Although cardioplegic properties have never been ascribed to this compound, it is evident from various reports that certain structural substitutions bring about such an effect.

Nelson and his associates (1958), in several preliminary experiments, reported that trans-2-g-tolyleyclohexanol was able to arrest the heart of a dog but recovery was unsuccessful.

Huitric et al. (1958) published a note concerning the production of mechanical cardiac arrest with trans-2-g-tolyleyclohexanol. During the course of his work, Huitric synthesized a series of substituted cyclohexanols which are listed in Fig. 1.

It was the purpose of this investigation to assess the comparative effects of the various substituents on their ability to produce cardiac arrest and to attempt to elucidate the mechanism through which such an effect is brought about.

II. REVIEW OF THE LITERATURE

Although elective cardiac arrest has only recently become a clinical reality, the concept of temporarily abolishing cardiac activity has been known since the latter part of the nineteenth century. Ringer (1883), in his classical work, drew attention to the fact that an excess of potassium ion in the coronary circulation was capable of stopping the heart in diastole and that an excess of calcium ion would do so in systole.

Rehde (1910) determined that in the isolated arrested mammalian heart, the oxygen requirement was significantly less than that of the beating non-working heart.

Attempts to resuscitate the heart during ventricular fibrillation led d'Hallium (1904) to inject potassium chloride into the jugular vein of dogs concomitantly with cardiac massage to abrogate this arrhythmia. Hooker (1929) successfully abolished ventricular fibrillation by injecting potassium salts into the carotid artery, and Wiggers (1930) injected potassium chloride intraventricularly. More recently Swan and Zeavin (1954) successfully employed the potassium ion to stop ventricular fibrillation during hypothermia.

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

Ryan and White (1953) reported the experimental induction of mechanical and electrical cardiac arrest in forty of eighty dogs with a combination of ether anesthesia and intravenous injections of procaine

hydrochloride at concentrations of 10 to 20 mg/kg. In twenty of the dogs, electrical activity persisted and in the remaining twenty, incomplete arrest or ventricular fibrillation occurred. Of the dogs manifesting cardiac arrest (electrical, mechanical, or both) all were resuscitated.

Lam and his co-workers (1955) attempted to induce cardiac arrest in dogs with a 5 per cent potassium chloride solution injected intraventricularly. The method was subsequently abandoned because of the high incidence of ventricular fibrillation during the recovery phase.

The original work of Malrose and his colleagues (1955) introduced potassium citrate as a cardioplegic agent for open-heart surgery. The technique devised by Malrose consists of cross-clamping the ascending aorta during total body perfusion and injecting into the base of the aorta a mixture of blood and 2.5 per cent potassium citrate. To determine the effective and safe dose of potassium citrate, Bentall and Malrose (1957) perfused isolated hearts with Locke's solution containing various concentrations of the salt. The minimal concentration which arrested the heart was 1 mg/ml. Post-arrest recovery was prompt even after periods of arrest of one half hour. High concentrations, e.g. 20 mg/ml, resulted in a slow recovery and depressed cardiac function.

Elective cardiac arrest with potassium citrate employing the Malrose technique has been applied clinically by Effler and his associates (1956, 1957, 1958) and has subsequently received extensive employment in cardiac surgery by Allen and Lillehei (1957), Kolff et al., (1956, 1957) and by Gerbode and Malrose (1958).

Despite the fact that potassium citrate is accepted for use routinely by a number of cardiac surgeons, experimental evidence has

been accumulating relative to its deleterious effect on the normal physiological pattern of myocardial activity. Kaplan and his group (1958) reported that half of their animals died following potassium arrest of the heart, the common cause being failure of the heart to resume normal rhythmicity after cardioplegia.

In a series of experiments Helmsworth and his co-workers (1958, 1958a, 1959) found that no animals died following thirty minutes of total cardio-pulmonary bypass, whereas, animals in which potassium citrate arrest was induced for thirty minutes had a mortality of nearly 50 per cent, with histological evidence of myocardial degeneration. It was also noted that when the left side of the heart was drained of all blood by a cannula inserted in the atrium and ventricle, only one of seven animals died.

Rigor of the left ventricle has been observed by Helmsworth et al. (1959) following potassium arrest of the heart of dogs. It is of interest to note that abnormally high calcium concentrations were found in the left ventricle of such a heart byoulder's group (1958).

Biochemical studies have indicated that potassium citrate arrest can cause alterations in myocardial metabolism. Hall et al. (1959) and Gatt (1959) have shown that during a standard potassium citrate arrest of one half hour duration, the levels of adenosine triphosphate, phosphocreatine and glycogen fell markedly. There was also a concomitant rise in tissue lactic acid (which has been used as an index of myocardial anoxia during arrest by Bentall and Helrose (1957)). The latter finding has also been confirmed by Greenberg and his associates (1960). The depletion of adenosine triphosphate had led Dodrill et al. (1960) to combine adenosine triphosphate with potassium citrate as a cardioplegic agent. Excellent recoveries were obtained for periods of arrest up to

one hour.

Post-arrest cardiac contractility and work capacity have also been shown to be adversely affected by potassium citrate. Bremwald et al. (1959) demonstrated that left ventricular function was severely depressed in twelve of fourteen dogs under potassium citrate arrest. This finding has also been confirmed by Weirich and his associates (1959).

Willman et al. (1959) noted that following cardiac arrest for thirty minutes with potassium citrate there was a profound decrease in cardiac output, stroke work and total work. A twenty minute arrest period produced less profound though similar changes in function. When digitalis was given just prior to cardiac arrest, there was an increase in post-arrest work capacity as compared with non-digitalized hearts (Willman et al., 1959a). According to Darby and his co-workers (1958), however, the heart was shown to be still capable of doing the same amount of work following recovery from potassium citrate arrest and ventriculotomy in fourteen dogs and ten patients.

A high incidence of ventricular fibrillation following potassium citrate has been reported by McKain (1961), Mann et al. (1959) and Berne et al. (1958, 1958a).

The well established fact that acetylcholine and various synthetic parasympathomimetic agents inhibit cardiac contractions prompted Lee and his associates (1957, 1957a, 1958) and Sergeant et al., (1957) to employ acetylcholine cardioplegia experimentally and in numerous clinical trials. Using the technique suggested by Halross (1955), a dose of approximately 10 mg/kg, or about 150 per cent of that required to stop the heart, was administered. The arrest period was characterized by an occasional beating of the heart and during the recovery phase bizarre electrocardiographic patterns and occasional ventricular fibrillation were observed.

It has been suggested by the authors that there is a need for avoidance of myocardial stretching during the arrest period.

Lam et al. (1958) reported the induction of acetylcholine arrest in eighty-eight clinical cases with rapid resuscitation and a low incidence of ventricular fibrillation.

Several investigators have attained less success with acetylcholine. Schramel 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.

McKain (1961), not being able to produce cardiac arrest with acetylcholine, referred to this drug as a "cardioparetic" agent rather than a cardioplegic agent. Failure to produce cardiac arrest with acetylcholine, along with a high incidence of ventricular fibrillation, has been reported by Berne et al. (1958a) and Kenyon et al. (1960).

Acetylcholine-induced arrest has also been shown to depress myocardial function. According to ventricular function curves, Bramwald et al. (1959) reported severe depression of left ventricular activity following recovery from acetylcholine induced arrest in twelve of fourteen dogs.

Willman et al. (1959) demonstrated impaired ability of the dog heart to work following acetylcholine arrest and Weirich and his associates (1959) have shown deterioration of ventricular function following fifteen to twenty minutes of acetylcholine arrest.

The use of total body hypothermia as an aid to correct intracardiac lesions has largely been the result of the pioneering work of Bigelow et al. (1950, 1950a), Lewis and Trautic (1953) and Svan and Zeavin (1954). The fact that hypothermia has been shown by these

investigators to protect the heart from prolonged periods of oxygen deprivation by decreasing its metabolic requirements has stimulated its use as a method for inducing elective cardiac arrest.

Collan and Nelson (1957) have studied the anoxic tolerance of the hypothermic heart at temperatures ranging from 14°C to 35°C . The beating heart could be deprived of coronary flow for fifteen minutes at 28°C and thirty-three minutes at 20°C without evidence of myocardial damage.

Mechanical and electrical arrest have been reported to occur at temperatures below 16°C by Urschel et al. (1959) and by Urschel and Greenberg (1959), at 13°C by Golan (1954) and at 11.5°C by Miasl and Lewis (1954).

Recently several investigators have suggested a new approach to the induction of hypothermic cardiac arrest. Urschel et al. (1959), Urschel and Greenberg (1959), Shumway and Lower (1959) and Cross et al. (1957) have elaborated upon various methods of selectively cooling the heart to low temperatures (deep hypothermia) while keeping the peripheral circulation under mild hypothermia. Shumway and Lower (1959) placed cold saline solutions into the pericardial cradle to induce selective cardiac cooling. Urschel et al. (1959) and Urschel and Greenberg (1959) have induced selective cooling of the heart by perfusing the coronary artery circulation with Ringer's lactate solution at 2°C while simultaneously applying a 2°C normal saline solution externally to the heart.

Collan et al. (1955) developed a method of cooling the blood in the extracorporeal unit below 10°C , and Cross et al. (1957) have employed infusions of cold blood into the coronaries to induce hypothermic cardiac arrest.

It is of interest to note, however, that Kenyon and his co-workers

(1960) recently reported the use of cold infusions into the coronary arteries as being an unpredictable method of inducing cardiac arrest in terms of complete abolition of cardiac, mechanical and electrical activity.

According to biochemical, histological and cardiac function studies, hypothermia has been shown to be superior to arrest induced with chemical agents. Ellison et al. (1960) pointed out that hypothermia preserves a greater amount of cardiac glycogen stores as compared to chemically induced arrest. Gott's (1959) findings have indicated that with potassium citrate arrest, the adenosine triphosphate, phosphocreatine and glycogen levels fell much more rapidly than during selective cardiac hypothermia. Hall and his associates (1959), investigating myocardial metabolism during hypothermic arrest by analysis of multiple cardiac biopsies, have confirmed the findings of Gott (1959) and Ellison et al. (1960).

The major complication, and consequently the major deterrent, to the use of hypothermic cardiac arrest has been the occurrence of ventricular fibrillation during the cooling and rewarming phase as indicated by Swan et al. (1953), Swan and Zeevin (1954), Bigelow et al. (1950a) and Bigelow (1954).

Various combinations of chemically induced arrest in conjunction with hypothermia have been reported in the literature. Cross et al. (1957), evaluating potassium citrate with local cardiac hypothermia, demonstrated that if the coronary arteries were perfused with cold blood prior to the injection of potassium citrate or if potassium citrate arrest were followed by perfusion of warm blood, a high incidence of ventricular fibrillation and poor post-arrest recoveries occurred. If,

however, the coronaries were infused with cold blood following potassium citrate arrest, the rate of occurrence of ventricular fibrillation was diminished and the number of adequate post-arrest recoveries significantly increased. These findings were in accordance with those of Berne et al. (1958a).

Cooper et al. (1959), utilizing potassium citrate with hypothermia, concluded that hypothermia affords a partial protection to potassium-induced arrest, according to ventricular function curves. These results were not in accord with Morehead and his group (1957) whose findings indicated that it took more potassium citrate to induce cardiac arrest under hypothermia than under normothermic conditions. It was also shown that cardiac arrest occurred with a shorter latent period followed by a more rapid recovery under normothermia than under hypothermia.

Brockman and Fencalsrud (1958) mentioned a unique method of employing a carotid artery perfusion technique combined with hypothermia and methacholine arrest. In effect, a portion of the arterial blood supply was re-routed into the carotid artery and thence to the brain. They reported that nineteen of twenty dogs survived thirty-two to fifty minutes of cardiac arrest. The heart, however, was not completely devoid of irritability. When potassium citrate was used in place of methacholine, fourteen to twenty-one minutes of arrest produced ventricular fibrillation in four of ten dogs.

Moulder et al. (1956) combined acetylcholine arrest with hypothermia and observed a high incidence of ventricular fibrillation during the recovery phase. McKain (1961) and Berne (1958a) could not maintain cardioplegia with acetylcholine even under hypothermic conditions.

Although Merritt et al. (1958) have reported that magnesium by

itself was an unsatisfactory cardioplegic drug, Young et al. (1956), Sealy et al. (1958) and Merritt et al. (1958) have utilized a combination of magnesium sulfate (2.41 per cent), potassium citrate (0.81 per cent) and neostigmine methylsulfate (0.001 per cent) for the induction of cardioplegia.

Young et al. (1956) mentioned that the above combination induces a rapid cardioplegia which is apparently superior to potassium citrate alone in terms of post-arrest recoveries. The addition of magnesium sulfate allows a decrease in the concentration of potassium citrate used and the neostigmine methylsulfate tends to decrease the incidence of post-arrest ventricular fibrillation. Diphenhydramine and tripeleminamine have been shown to exert the same effect as neostigmine.

Sealy et al. (1958) combined potassium, magnesium and neostigmine with hypothermia and found that cardioplegia was well tolerated in dogs up to fifty-two minutes.

Merritt et al. (1958), evaluating potassium, magnesium and neostigmine on the isolated cat heart, pointed out that the potassium and magnesium ions appear to act synergistically.

Hilnes et al. (1958), in their experiments, eliminated the neostigmine and used a mixture of potassium citrate and magnesium sulfate buffered to physiological pH with sodium bicarbonate. Thirty-five minutes of induced asystole was shown to be well tolerated with none of the hearts fibrillating and no electrocardiographic evidence of myocardial damage. Recovery also occurred after thirty-nine to forty-four minutes of arrest but with temporary myocardial injury, and fifty to sixty minutes of arrest ended fatally for all animals.

Several other chemical agents and methods for inducing cardioplegia have been reported in the literature.

Nelson and his associates (1958) have conducted a series of preliminary experiments on a number of compounds for cardioplegic effects. Included were several cholinergic drugs, ions, muscle relaxants, antihistamines and local anesthetics, and some miscellaneous agents. Of the cholinergic drugs, acetylcholine, methacholine and carbamylcholine produced cardiac arrest. The methacholine arrest, however, lasted for less than one minute. Potassium citrate, potassium chloride, lithium citrate and sodium citrate produced cardiac arrest. It is of interest to note that, according to the results obtained from their studies with various citrate salts, the conclusion was drawn that the potassium arrest produced by the doses of potassium citrate generally employed in clinical practice is, in reality, a citrate arrest with potassium exerting a salutary effect on recovery to normal sinus rhythm. Of the antihistamines and local anesthetics tested, diphenhydramine and procaine hydrochloride produced cardiac arrest, and among the miscellaneous category trans-2-g-tolylcyclohexanol and ethylenediamine tetra-acetic acid produced elective arrest. No arrest was obtained with any of the muscle relaxants listed, which included curare, succinylcholine and quinidine. Of the salts tested, magnesium sulfate, sodium chloride and sodium lactate were unsuccessful cardioplegic agents while chlorphenamine among the antihistamines and Hyspaqua among the miscellaneous agents did not produce arrest.

Riberi and Shumacker (1958) have tested sodium citrate and potassium lactate for cardioplegic effects under mild body hypothermia. They demonstrated that sodium citrate produced mechanical arrest in twenty-three dogs with electrical arrest persisting in eleven of twenty-three animals, whereas potassium lactate produced mechanical and electrical arrest in all animals tested. Resuscitation was brought about more

easily under potassium lactate than under sodium citrate arrest.

Clark and his associates (1959) devised a rather ingenious method of inducing cardiac arrest by passing blood through a selectively charged magnesium ion exchange resin. The blood calcium was exchanged for the magnesium while the normal balance of blood sodium and potassium was not altered. The hypocalcemic blood was then perfused into the coronary system producing cardiac arrest.

Hendini et al. (1957) demonstrated that isolated guinea pig hearts were arrested in diastole for three to five minutes with a combination of 100 gamma/ml acetylcholine and 200 gamma/ml of procaine hydrochloride. The effective concentration in dogs was 500 gamma/ml acetylcholine and 200 gamma/ml of procaine.

Yacher et al. (1960) have induced cardioplegia with sodium phytate, also known as sodium inositol hexaphosphate, which exerts its effect by chelating calcium.

Ventricular fibrillation itself has been used as a method for preventing air emboli and producing a quiet heart by Glenn and Sewell (1953). Recently Teale et al. (1960) have devised an electrode to maintain a state of ventricular fibrillation during operative procedures.

Recent advances in the correction of stenotic and insufficient aortic valves have prompted the use of a retrograde perfusion technique of the coronary sinus in order to facilitate direct vision of the diseased valve. Gott et al. (1957) have studied potassium citrate arrest and retroperfusion, alone and in combination. Of the ten dogs submitted to thirty minutes of retroperfusion alone, four died from cardiac causes. When potassium citrate arrest was combined with retroperfusion, two of ten dogs died, whereas ten dogs submitted to fifteen to thirty minutes of potassium citrate arrest without retroperfusion exhibited a 90 per cent

mortality.

Lillehei and his co-workers (1958, 1958a) have employed a short term acetylcholine cardioplegia during coronary retroperfusion. Their findings indicated that the above combination was safer than acetylcholine arrest alone, especially if there was an associated myocardial hypertrophy. Several clinical successes have been achieved using retroperfusion and acetylcholine arrest for aortic valvular disease. (Lillehei 1958a).

From the literature just cited there is obviously little uniformity of opinion concerning the employment of present-day cardioplegics. Because of this, there appears to be justification in assessing the comparative cardioplegic effects of cyclohexanol and nine of its 2-aryl substitution products for possible use in cardiac surgery.

III. THE INVESTIGATION

A. OBJECTIVES

Cyclohexanol and certain of its 2-aryl substitution products represent a new and rather unusual series of compounds unlike any of the cardioplegic agents described in the literature.

Although preliminary experiments by other investigators have shown that trans-2-*p*-tolylcyclohexanol exerts cardioplegic effects, no other reports have been found in the literature concerning the actions of any of the other substitution products.

An investigation was undertaken to determine the comparative ability of cyclohexanol and nine of its substitution products to induce elective cardiac arrest.

The objectives of this project were as follows:

1. To screen pharmacologically the various compounds for cardioplegic effects with special emphasis on chemical structure and spatial configuration.
2. To attempt to determine the mechanism through which this effect is brought about.

B. THE COMPOUNDS

The substituted cyclohexanols employed in this investigation were synthesized by Dr. Alain C. Ruitric of the Department of Pharmaceutical Chemistry, University of Washington, College of Pharmacy, Seattle, Washington. These compounds have a very low aqueous solubility. Since the water solubility of trans-2-*p*-tolylcyclohexanol was the only one reported by Dr. Ruitric, it was necessary to determine the solubility of the remaining liquid 2-aryl cyclohexanols in the series. This was performed according to the

Fig. 1 - CYCLOHEXANOL AND ITS DERIVATIVES

CYCLOHEXANOL

2-o-TOLYL
cis-trans 2-o-CHLOROPHENYL
cis-trans 2-m-TOLYL
trans 2-m-CHLOROPHENYL
trans 2-p-TOLYL
trans 2-p-CHLOROPHENYL
cis-trans

procedure outlined below. Table I lists the physical state and aqueous solubility of the compounds. The water solubility of the solids was not determined because they were dissolved in a mixture of propylene glycol and water prior to testing.

Cyclohexanol, which is prepared by the hydrogenation of phenol, contains traces of the precursor as an impurity. Prior to its employment, the material was extracted in a separatory funnel with three 25 ml portions of a 10 per cent solution of sodium hydroxide and washed with distilled water until neutral to litmus.

C. DETERMINATION OF SOLUBILITY OF THE LIQUID 2-ARYLCYCLOHEXANOLS

The solubilities of the liquid 2-arylcyclohexanols were determined according to the following procedure:

A 10 ml test tube was placed in a 25 ml erlenmeyer flask and both flask and test tube were weighed on a semi-micro balance. Approximately 5 lambdas of the compound was carefully placed in the bottom of the test tube and the test tube and flask were weighed once more. The weight of the compound was determined by difference. In none of the determinations did the difference in weight exceed 4 mg. The test tube was then removed from the flask and the solubility determined in the following manner:

An ultrasonic generator^a was employed to facilitate the solution of the material. The generator water bath was maintained at a temperature of 25°C to simulate laboratory temperatures, and a 60 to 80 pasc current reading was maintained throughout the determination. Upon the addition of a small quantity of water (0.1 to 0.4 ml) from a 10 ml microburette the compound immediately assumed a globular

^aModel G-100, National Ultrasonic Corporation, Irvington, N.J.

Table I Physical state and water solubility of cyclohexanol and its
2-aryl substitution products

COMPOUND	PHYSICAL STATE	WATER SOLUBILITY (mg/ml)
cyclohexanol	liquid	5.67 at 15°C ^a
<u>trans</u> -2- <u>o</u> -tolyl cyclohexanol	liquid ✓	0.8 ^b
<u>cis</u> -2- <u>o</u> -tolyl cyclohexanol	liquid ✓	0.5
<u>trans</u> -2- <u>m</u> -tolyl cyclohexanol	liquid ✓	1.0
<u>trans</u> -2- <u>p</u> -tolyl cyclohexanol	solid	-
<u>trans</u> -2- <u>o</u> -chlorophenyl cyclohexanol	solid	-
<u>cis</u> -2- <u>o</u> -chlorophenyl cyclohexanol	liquid ✓	0.2
<u>trans</u> -2- <u>m</u> -chlorophenyl cyclohexanol	solid	-
<u>trans</u> -2- <u>p</u> -chlorophenyl cyclohexanol	solid	-
<u>cis</u> -2- <u>p</u> -chlorophenyl cyclohexanol	solid	-

^aHandbook of Chemistry and Physics 35th Ed. (1953-1954)

^bValue reported by Dr. Huitric

shape which remained during the entire determination. After each addition of water the test tube and its contents were immersed into the vibrator bath for approximately one minute. When the globule was barely visible the contents of the test tube were carefully examined under a strong beam of light after each addition of water in order to determine more precisely the point of disappearance of the globule. The whole procedure was repeated three times for each compound, and the average of the three determinations was taken as the solubility. The solubility of trans-2-g-tolylcyclohexanol was redetermined by this method and was found to be 0.76 mg/ml, a deviation of -0.04 mg from that originally reported.

D. PREPARATION OF THE COMPOUNDS FOR TESTING

The compounds were prepared for testing according to the following procedures:

1. A saturated solution of the liquid members of the series, excluding cyclohexanol, was prepared by dispersing several drops of the substance in a 30 ml vial of distilled water. The mixture was allowed to stand at room temperature for at least twenty-four hours with intermittent shaking. The supernatant fluid was decanted and filtered through Whatman #1 filter paper prior to administration.

2. Cyclohexanol was prepared for testing by dissolving a calculated volume in distilled water. The concentration of the final solution was expressed in mg/ml by calculating the weight of the volume of compound dissolved. Concentrated solutions of cyclohexanol approaching the maximum solubility value (see Table I) were refrigerated to facilitate solution. The cooled solution remained clear for several hours at room temperature after which time turbidity

began to develop. Rescuing the solution promptly dissolved the precipitate.

3. The solid 2-aryl cyclohexanols were prepared for testing by weighing a quantity of each substance on a Mettler Analytical balance and dissolving it in a mixture of equal volumes of propylene glycol and water. It was found necessary in all cases to employ heat to effect the solution. This was accomplished by placing the mixture in an oven maintained at 50°C until it dissolved. Once the compound had dissolved, it remained in solution for at least several hours or indefinitely depending on the concentration and/or the compound employed.

It was found that with several of the compounds aliquots could be made, using distilled water as the diluent without the formation of a precipitate. This was desirable in that the total amount of propylene glycol in the solvent system could be reduced thus minimizing the effect of the solvent on the heart. Consequently, the percent of propylene glycol varied with the dose and compound employed, the limits ranging from 10 per cent to 50 per cent for all compounds tested. A control dose containing a corresponding percent of propylene glycol per volume of solution was administered at the beginning of each experiment.

E. ANIMALS USED

Rats - Albino rats of the Wistar (DM-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, bred in our laboratories.

Dogs - Mongrel dogs.

V. EXPERIMENTAL PROCEDURES AND RESULTS

This investigation has been divided into four major sections:

- 1- The effects of cyclohexanol and its liquid 2-aryl substitution products on the blood pressure of rats.**
- 2- The effects of cyclohexanol and its 2-aryl substitution products on the isolated guinea pig heart.**
- 3- The effects of trans-2-g-tolylcyclohexanol on isolated rabbit atria.**
- 4- The effects of cyclohexanol and its trans-2-g-tolyl and trans-2-g-chlorophenyl substituted derivatives on the heart of dogs undergoing cardio-pulmonary bypass.**

1. Effects of Cyclohexanol and its Liquid 2-Aryl Substitution Products on the Blood Pressure of Rats

a. Experimental Procedures

The effects on blood pressure were recorded by direct cannulation of the right carotid artery of albino rats weighing between 140 and 313 g. The solid members of the series could not be tested since the hypotensive effect of the propylene glycol interfered with the results.

The system consisted of a mercury manometer connected to a cannula via a fluid bridge of 7.5 per cent sodium citrate solution. The blood pressure fluctuations were recorded on a slowly moving smoked kymograph. To prevent clotting, the rats received an intravenous injection of 0.01 ml of a 1:5,000 solution of heparin sodium prior to cannulation and 0.01 ml of heparin sodium was added to the cannula. The animals were anesthetized with 1.0 g/kg of urethan administered intraperitoneally. Injections were made intravenously via a needle permanently secured in either femoral vein.

Several injections of the same dose were administered during each experiment with the total number of injections not exceeding five per experiment. These compounds produced an immediate drop in blood pressure followed by a rapid return to normal, the latter depending on the dose employed. In any case the return to normotensive levels took place within a ten minute period. Each succeeding dose was administered at not less than a twenty minute interval to insure complete recovery from the previous dose.

At the conclusion of the majority of experiments the urine was removed from the bladder with a syringe and examined for blood. In a few of the experiments simultaneous electrocardiograms were obtained.

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

1. The percent drop in blood pressure produced by the various compounds.
2. The duration of hypotensive effect.

b. Results

The results include the following:

- (1) Effects of cyclohexanol and its liquid 2-aryl substitution products on the blood pressure of rats (Tables II to VI)
- (2) A comparison of cyclohexanol and its 2-aryl substitution products on the blood pressure of rats (Table VII; Fig. 2).

Table II Effects of cyclohexanol on the blood pressure of rats

ANIMAL NUMBER	DOSE (mg/kg)	NUMBER OF TRIALS	MEAN $\pm$ DROP IN BLOOD PRESSURE $\pm$ S.D.	DURATION OF DROP (Mean and Range) (Sec.)
1	40	5	59 $\pm$ 5	208(108-270)
2	40	5	62 $\pm$ 7	223(180-264)
3	60	2	70 $\pm$ 0	218(196-240)
4	60	4	53 $\pm$ 13	182(104-224)
5	80	5	64 $\pm$ 12	285(224-400)
6	80	2 ^a	86	184
7	100	4	67 $\pm$ 5	637(360-780)
8	100	3 ^b	91 $\pm$ 1	288(270-320)

^aThe animal died on second dose.^bThe animal died on third dose.

Table III Effects of cis-2-p-tolyleyclohexanol on the blood pressure of rats

ANIMAL NUMBER	DOSE (mg/kg)	NUMBER OF TRIALS	MEAN % DROP IN BLOOD PRESSURE ± S.D.	DURATION OF DROP (Mean and Range) (Sec.)
1	1	3	21 ± 12	36(8-75)
2	1	3	38 ± 12	10(8-15)
3	1	1	16	8
4	2	5	56 ± 25	24(8-262)
5	2	5	63 ± 11	103(30-225)
6	2	5	71 ± 24	142(11-225)
7	3	4	70 ± 28	103(8-175)
8	3	2 ^a	76	135
9	3	4	73 ± 25	132(15-233)
10	3	5	77 ± 7	92(26-128)

^aThe animal died on second dose.

Table IV Effects of trans-2-g-tolyloxylohexanol on the blood pressure of rats

ANIMAL NUMBER	DOSE (mg/kg)	NUMBER OF TRIALS	MEAN % DROP IN BLOOD PRESSURE ± S.D.	DURATION OF DROP (Mean and Range) (Sec.)
1	0.5	2	38 ± 15	13(10-15)
	1.0	2	54 ± 6	15(15)
2	0.5	1	19	15
	1.0	3	51 ± 6	45(7-106)
3	2.0	5	73 ± 11	100(30-192)
4	2.0	5	69 ± 16	80(50-112)
5	2.0	4	85 ± 5	121(90-210)
6	2.0	3 ^a	54 ± 19	82(50-115)
7	4.0	3 ^b	83 ± 4	69(60-78)

^aThe animal died on third dose.

^bThe animal died on third dose.

Table V Effects of trans-2-n-tolylcyclohexanol on the blood pressure of rats

ANIMAL NUMBER	DOSE (mg/kg)	NUMBER OF TRIALS	MEAN $\pm$ DROP IN BLOOD PRESSURE $\pm$ S.D.	DURATION OF DROP (Mean and Range) (Sec.)
1	1	2	18 $\pm$ 4	18(15-20)
	2	3	25 $\pm$ 3	12(11-15)
2	1	2	10 $\pm$ 4	8(8)
	2	3	25 $\pm$ 10	10(8-15)
3	1	3	19 $\pm$ 6	12(11-15)
4	1	2	16 $\pm$ 8	13(11-15)
5	2	2	13 $\pm$ 7	16(11-20)
6	2	2	29 $\pm$ 6	60(30-90)
7	4	4	36 $\pm$ 13	92(11-225)
8	4	4	23 $\pm$ 6	34(20-60)
9	4	2	57 $\pm$ 2	213(150-277)
10	4	2	44 $\pm$ 17	32(23-40)
11	6	3	69 $\pm$ 14	143(40-225)
12	6	3	76 $\pm$ 22	155(75-243)
13	6	3 ^a	99 $\pm$ 0	169(150-188)

^aThe animal died on third dose.

Table VI Effects of cis-2-g-chlorophenylcyclohexanol on the blood pressure of rats

ANIMAL NUMBER	DOSE (mg/kg)	NUMBER OF TRIALS	MEAN % DROP IN BLOOD PRESSURE ± S.D.	DURATION OF DROP (Mean and Range) (Sec.)
1	0.5	3	48 ± 12	61(38-76)
2	0.5	4	37 ± 22	76(20-137)
3	1.0	4	70 ± 25	151(58-223)
4	1.0	4	65 ± 22	173(87-237)
5	1.5	3	91 ± 3	232(190-271)
6	1.5	2 ^a	84	423

^aThe animal died on second dose.

Fig. 2 A comparison of the hypotensive effects produced by cyclohexanol and its liquid 2-aryl substitution products in rats.^a

A - cis-2-o-chlorophenylcyclohexanol

C - trans-2-o-tolylcyclohexanol

B - cis-2-p-tolylcyclonexanol

D - trans-2-m-tolylcyclohexanol

E - cyclohexanol

^aSee Table VII

Table VII A comparison of the ED₅₀ (hypotensive) of cyclohexanol and its liquid 2-aryl substitution products on the blood pressure of rats ^a

COMPOUND	TOTAL TRIALS	CALCULATED ED ₅₀ ^b (mg/kg)
cyclohexanol	26	25.1
<u>cis</u> -2- <u>p</u> -tolyleyclohexanol	36	0.85
<u>trans</u> -2- <u>p</u> -tolyleyclohexanol	25	0.9
<u>trans</u> -2- <u>m</u> -tolyleyclohexanol	39	4.0
<u>cis</u> -2- <u>p</u> -chlorophenyleyclohexanol	18	0.6

^aSee Fig. 2.

^bderived from the regression line formula which was obtained from the analysis of results on the IBM 1620 Computer

2. Effects of Cyclohexanol and its 2-Aryl Substitution Products on the Isolated Guinea Pig Heart

a. Experimental Procedures

Guinea pigs of either sex weighing between 500 and 965 g were sacrificed with a sharp blow to the base of the head. The thoracic cage was opened by removal of the sternum and the ribs were extended as far dorsally as possible. The ascending aorta was carefully mobilized from its surrounding tissue and a loose ligature was placed under the vessel just proximal to the innominate artery. A cannula was inserted into the aorta via an incision made in the ventral surface of the aorta and tied firmly in place with the previously placed ligature. It was found advantageous at this point to gently force several ml of warm perfusate into the coronary arteries to remove the residual blood from the vessels and to determine whether the tip of the cannula was occluding the coronary openings. The heart was quickly dissected free and transferred to the perfusion chamber.

The Andersen-Craver Heart Perfusion Apparatus^a was employed for the isolated guinea pig heart experiments. This apparatus provides a continuous recycling of the effluent perfusate and also maintains the external environment of the heart at approximately body temperature. The water bath temperature was maintained at 38°C throughout the experiment.

After allowing the apparatus to stabilize, the heart was secured in place. Any remaining extraneous tissue was dissected free and a stainless steel hook was inserted into the ventricular musculature at the apex of the heart. Contractions of the heart were recorded on a

^aMetro Industries, Long Island City, New York.

smoked kymograph by passing a thread from the hook to a recording lever via a pulley system. The heart was allowed to equilibrate on the apparatus for about one half hour at which time any necessary adjustments were made.

The perfusion solution employed for the isolated hearts was basically that recommended by Chenoweth and Koelle (1946). It was slightly modified by replacing the magnesium chloride with an equivalent amount of magnesium sulfate^a in order to reduce the total concentration of chloride ion in the perfusate. The composition of the perfusion solution expressed in grams per liter and in millimoles per liter is as follows:

	<u>g/l</u>	<u>mM/l</u>
NaCl	7.0	119.8
KCl	0.42	5.63
CaCl ₂	0.24	2.16
MgSO ₄	0.52	2.11
dextrose	1.80	10.00
NaHCO ₃	2.1	25.00

A stock solution containing 350 g sodium chloride, 21.0 g potassium chloride, 16.0 g calcium chloride and 26.0 g magnesium sulfate was prepared and stored at room temperature. The sodium bicarbonate and dextrose were added just prior to making the final solution. One liter of perfusion solution was prepared for each experiment and immediately oxygenated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The pH of the final solution was 7.8.

Injectons were made via an injection spiral connected to the perfusion canal. Since the calculated volume of the spiral was 1.5 ml, this placed a limit on the upper level of dosage for the liquid compounds because of their poor water solubility. As a result the final volume to

^aThe heptahydrate salt was used throughout this work.

to be injected did not exceed 1 ml. It was observed that volumes exceeding this value could not be assured of completely gaining access to the perfusion canal.

The normal cardiac contractions were recorded for approximately one minute following which the compound was administered and displaced from the spiral with a sufficient amount of perfusate. During this time a 30 ml portion of the effluent perfusate was collected and discarded in order to prevent re-cycling of the drug and contamination of the pure perfusion fluid. It should be noted that a normal heart will contract from two to three hours in the apparatus before signs of failure appear. In all of the experiments the compounds acted within one minute.

Each administration, with the exception of the control dose, was considered a trial. The number of trials performed depended on the ability of the heart to recover from the previous one, and, in any case, did not exceed five. Succeeding doses were administered not less than ten minutes from the time of recovery of the previous dose. Recovery was allowed to take place spontaneously in order to compare the rates of recovery from the effects of the various compounds. In order to standardize the results, a depression of 95 per cent or more from the normal was considered to be cardiac arrest.

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

1. The incidence of cardiac arrest at a particular dose, and the duration of arrest at that dose.
2. The incidence and percent of cardiac depression if no arrest occurred.
3. Time of return to 50 per cent of the normal contraction.

b. Results

The results include the following:

- (1) Effects of cyclohexanol and its 2-aryl substitution products on the isolated guinea pig heart (Tables VIII to XVII; Figs. 3 to 7).
- (2) The comparative effects of cyclohexanol and its 2-aryl substitution products on the isolated guinea pig heart (Table XVIII).

Table VIII. Effects of cyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	Z P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION $\pm$ S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	4.0	0/0.5	0/5	-	82 $\pm$ 13	13(9-17)
2	8.0	0/0.75	5/5	22(9-56)	-	26(8-56)
3	8.0 12.0	0/0.75 0/0.5	2/2 3/3	10(9-10) 20(17-22)	- -	10(8-13) 21(13-29)
4	12.0 24.0	0/0.5 0/1.0	2/2 3/3	21(8-35) 71(68-75)	- -	24(21-27) 40(40)
5	12.0 24.0	0/0.5 0/1.0	2/2 2/2	14(12-15) 24(20-27)	- -	4(3-5) 5(3-6)
6	24.0	0/1.0	2/2	21(19-23)	-	10(0-21) ^b
7	24.0	0/1.0	2/2	39(37-60)	-	41(40-43)

^a per cent propylene glycol^b The 0 indicates that the first contractions following arrest were greater than 50 per cent.

Table IX Effects of dlf-2-g-tolycyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	2 P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.25	0/0.5	0/3	-	35 ± 21--34 ^b ↑	-
2	0.5	0/1.0	0/4	-	67 ± 11	65(16-115)
3	0.5	0/1.0	0/4	-	46 ± 6	-
4	0.5	0/1.0	0/3	-	86 ± 7	152(97-180)
5	0.5	0/1.0	0/5	-	55 ± 17	58(26-78)
6	0.5	0/1.0	0/5	-	68 ± 16	58(17-116)
7	0.5	0/1.0	0/4	-	60 ± 9	65(21-145)

^aper cent propylene glycol

^bindicates per cent stimulation produced by one dose

Fig. 3 THE EFFECT OF trans-2-o-TOLYLCYCLOHEXANOL (0.6 mg) ON THE ISOLATED GUINEA PIG HEART.

Fig. 4 THE EFFECT OF trans-2-o-CHLOROPHENYLCYCLOHEXANOL (1.0 mg) ON THE ISOLATED GUINEA PIG HEART.

Table X Effects of trans-2-g-tolylcyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TWIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.4	0/0.5	3/4	11(7-14)	85	140(117-151)
2	0.4	0/0.5	2/2	12(10-14)	-	131(123-139)
	0.6	0/0.75	2/2	31(13-42)	-	45(34-76)
3	0.6	0/0.75	4/4	54(35-75)	-	312(81-430)
4	0.6	0/0.75	3/3	24(18-30)	-	115(63-207)
5	0.8	0/1.0	4/4	135(73-242)	-	239(143-394)
6	0.8	0/1.0	5/5	65(32-92)	-	79(62-91)
7	0.8	0/1.0	4/4	53(37-63)	-	132(114-155)
8	0.8	0/1.0	2/2	110(74-145)	-	201(180-222)

^a per cent propylene glycol

Table XI Effects of trans-2,2-tolylcyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% F.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN $\pm$ S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.5	0/0.5	0/5	-	79 $\pm$ 10	77(16-101)
2	0.5	0/0.5	0/3	-	55 $\pm$ 10	66(32-55)
3	1.0	0/1.0	0/3	-	83 $\pm$ 15	127(81-202)
4	1.0	0/1.0	2/3	50(47-68)	96	139(87-186)
5	1.0	0/1.0	0/2	-	89 $\pm$ 1	162(140-146)
6	1.0	0/1.0	0/2	-	86 $\pm$ 5	161(131-191)
7	1.0	0/1.0	3/3	22(11-29)	-	121(63-172)
8	1.0	0/1.0	0/5	-	84 $\pm$ 4	98(48-172)

^a per cent propylene glycol

Table XII Effects of *11000-2-2-p-tolylpropylchamomel* on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.5	10/0.5	0/3	-	60 ± 9	53(40-74)
2	0.75	10/0.75	0/3	-	69 ± 14	67(39-103)
3	1.0	10/1.0	0/2	-	73 ± 16	217(213-219)
4	1.0	10/1.0	0/4	-	81 ± 7	180(71-304)
5	1.0	10/1.0	0/4	-	68 ± 7	84(25-176)
6	1.5	15/1.0	3/3	101(37-182)	-	140(101-165)
7	1.5	15/1.0	2/3	164(54-275)	80	163(78-250)
8	1.5	15/1.0	0/5	-	89 ± 2	98(31-148)
9	1.5	15/1.0	2/5	109(46-171)	88 ± 8	205(64-315)
10	2.0	50/0.5	4/5	77(37-118)	89	146(85-215)
11	2.0	50/0.5	3/3	90(69-121)	-	81(70-87)

^aper cent propylene glycol

Table XIII Effects of cis-2,6-chlorophenylcyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.1	0/0.5	0/4	-	18--80 [†] 91 [†] 29 [†] b	-
2	0.1	0/0.5	0/4	-	31 ± 9	-
3	0.1	0/0.5	0/4	-	22 ± 4--100 [†] 36 [†] b	-
4	0.2	0/1.0	0/4	-	27 ± 15--30 [†] b	-
5	0.2	0/1.0	0/4	-	31 ± 16--126 [†] 83 [†] b	-
6	0.2	0/1.0	0/4	-	21 ± 10--131 [†] b	-

^aper cent propylene glycol^bindicates per cent stimulation produced by one dose

Fig. 5 THE POSITIVE INOTROPIC EFFECT OF cis-2-o-CHLOROPHENYLCYCLOHEXANOL (0.2 mg) ON THE ISOLATED GUINEA PIG HEART.

Fig. 6 THE EFFECT OF 0.5 ml OF A 50 PER CENT SOLUTION OF PROPYLENE GLYCOL IN WATER ON THE ISOLATED GUINEA PIG HEART.

Table XIV Effects of **1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethanol** on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	1 P.G./ ² DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	0.5	10/0.5	0/3	-	77 ± 19	157(79-262)
2	0.75	10/0.75	0/3	-	79 ± 5	170(114-234)
3	1.0	10/1.0	3/3	89(69-127)	-	173(129-255)
4	1.0	10/1.0	4/4	91(29-167)	-	232(181-260)
5	1.0	10/1.0	2/4	81(32-129)	93 ± 1	233(216-246)

²per cent propylene glycol

Table XV Effects of 11mg-3-*p*-chlorophenylcyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% P.C. ^a DOSE VOL., (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF SOLE RETURN (Mean and Range) (Sec.)
1	0.5	10/0.5	0/5	-	63 ± 19--45 ^b	129(113-142)
2	0.75	10/0.75	1/5	12	70 ± 6	175(40-191)
3	0.75	10/0.75	0/2	-	76 ± 4	238(234-242)
4	1.0	30/1.0	3/4	196(62-284)	91	166(64-232)
5	1.0	30/1.0	0/4	-	95 ± 13	124(51-153)
6	2.0	50/0.5	3/5	132(91-162)	78	179(124-263)
7	3.0	50/0.5	1/4	273(257-342)	91 ± 14	322(302-342)

^a per cent propylene glycol^b indicates per cent stimulation produced by one dose

Table XVI Effects of 2,2-chlorophenylcyclohexanol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	Z P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	1.0	50/0.5	0/2	-	70 ± 4	127(111-142)
2	1.0	50/0.5	0/4	-	70 ± 14--91 ^b	173(131-238)
3	2.0	50/0.5	0/1	-	85	1900(308) ^c
4	2.0	50/0.5	2/2	319(314-324)	-	181(133-220)
5	2.0	50/0.5	1/3	148	69 ± 10--100 ^d	308(308)
6	3.0	50/0.5	1/1	1002	-	N.R. ^d
7	3.0	50/0.5	1/1	1008	-	N.R. ^d
8	3.0	50/0.5	1/1	1860	-	N.R. ^d

^a per cent propylene glycol^b indicates per cent stimulation produced by one dose^c Contractions returned to 30 per cent of the normal level at the end of 1980 seconds.^d no recovery

Fig. 7 THE CARDIO-TOXIC EFFECT OF trans-2-p-CHLOROPHENYLCYCLOHEXANOL (2.0 mg) ON THE ISOLATED GUINEA PIG HEART.

Table XVII Effects of trans-2-p-chlorophenylephedranol on the isolated guinea pig heart

ANIMAL NUMBER	DOSE (mg)	% P.G. ^a DOSE VOL. (ml)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)	TIME OF 50% RETURN (Mean and Range) (Sec.)
1	1.0	50/0.5	0/3	-	59 ± 7--100 ↑ ^b	100(92-103)
2	1.0	50/0.5	0/3	-	48 ± 11--29 ↑ ^b 7 ↑ ^b 61 ↑ ^b	103
3	1.0	50/0.5	0/2	-	85 ± 5	219(203-236)
4	1.0	50/0.5	0/3	-	84 ± 4	224(171-296)
5	2.0	50/0.5	1/1	182	-	372(43%) ^c
6	2.0	50/0.5	1/1	340	-	N.R. ^d
7	2.0	50/0.5	1/1	645	-	510(10%) ^e
8	2.0	50/0.5	1/1	600	-	600(12%) ^f

^a per cent propylene glycol

^b indicates per cent stimulation produced by one dose

^c Contractions returned to 43 per cent of the normal level at the end of 372 seconds.

^d no recovery

^e Contractions returned to 10 per cent of the normal level at the end of 510 seconds.

^f Contractions returned to 12 per cent of the normal level at the end of 600 seconds.

Table XVIII Comparative effects of cyclohexanol and its derivatives on the isolated guinea pig heart

COMPOUND	MINDEN ^a EFFECTIVE DOSE (mg)	CARDIAC ARREST ARRESTS/TRIALS	DURATION OF ARREST (Mean and Range) (Sec.)	MEAN % DEPRESSION ± S.D. (if no arrest)
cyclohexanol	8.0	7/7	18(9-58)	-
<u>cis</u> -2- <u>p</u> -tolylcyclohexanol	0.5 ^b	0/25	-	63 ± 17
<u>trans</u> -2- <u>p</u> -tolylcyclohexanol	0.4	5/6	12(7-14)	85
<u>trans</u> -2- <u>p</u> -tolylcyclohexanol	1.0	5/18	33(11-53)	85 ± 7
<u>trans</u> -2- <u>p</u> -tolylcyclohexanol	1.5	7/16	170(37-275)	88 ± 6
<u>cis</u> -2- <u>p</u> -chlorophenylcyclohexanol	0.2 ^b	0/12	-	26 ± 12-93(4) ^c
<u>trans</u> -2- <u>p</u> -chlorophenylcyclohexanol	1.0	9/11	88(29-167)	93 ± 1
<u>trans</u> -2- <u>p</u> -chlorophenylcyclohexanol	1.0	3/8	197(62-284)	62 ± 16
<u>cis</u> -2- <u>p</u> -chlorophenylcyclohexanol	2.0	3/6	262(148-324)	71 ± 18-100(1) ^d
<u>trans</u> -2- <u>p</u> -chlorophenylcyclohexanol	2.0 ^e	4/4	442(182-645)	-

^athe minimum dose which will produce cardiac arrest

^bthe maximum allowable dose injected but not the minimum effective dose

^cStimulation occurred in 4 of 12 doses with a mean of 93 per cent.

^dStimulation occurred in 1 of 6 doses.

^etoxic dose

3. Effects of trans-2-g-Tolylcyclohexanol on the Isolated Rabbit Atria

a. Experimental Procedures

The procedure employed was basically that described by Webb (1950). Albino rabbits weighing between 3 and 4.2 kg were sacrificed with a sharp blow to the base of the head. The thoracic cavity was opened by removal of the sternum and the heart was quickly extirpated and immersed in a beaker of the perfusion solution maintained at a temperature of 32°C. The ventricles were gently compressed to force any residual blood out of the chambers. The atria were dissected, intact, from the ventricular mass at the atrio-ventricular junction. Any extraneous tissue was cut away and care was taken to remove any remaining ventricular tissue attached to the atria.

A stainless steel hook attached to a length of thread was inserted into the right atrial appendage and a second hook was inserted into the left atrial appendage. The latter was attached to a removable glass aeration tube of an isolated tissue bath. The temperature of the tissue bath was maintained at 32°C, as suggested by Webb(1950), in order to reduce the rate and increase the amplitude of contractions. The atria were suspended in the tissue bath and the thread was fastened to an aluminum writing lever. Contractions were recorded on a slowly moving smoked kymograph. The lever was adjusted for maximum magnification of contractions.

The perfusion solution employed in these experiments was the same as that employed on the isolated guinea pig heart. Oxygenation was achieved by bubbling a mixture of 95 per cent oxygen and 5 per cent carbon dioxide into the perfusion bottle and into the tissue bath. The latter was adjusted so that a fine stream of bubbles was produced.

All materials were administered directly into the tissue chamber with a syringe. The volume of the bath was maintained at 70 ml.

trans-2-g-Tolylcyclohexanol was selected for testing on the isolated auricles because of its potency and reproducible consistent effect. A 4.0 per cent solution of calcium chloride was used to determine the possible interactions with the above compound. All substances were allowed to act for exactly three-minute periods before adding other agents or washing.

trans-2-g-Tolylcyclohexanol produced an effect lasting indefinitely when not washed, and calcium chloride produced an effect that lasted for at least thirty minutes if not washed. The experiments were conducted so that the effects of the above compounds were recorded well within the above periods in order to assure that the responses were due to the drugs alone.

b. Results

The results are as follows:

- (1) A dose of 0.6 mg of trans-2-g-tolylcyclohexanol was able to reduce the amplitude of contraction by approximately 50 per cent in three minutes without apparently affecting the rate (Fig. 8).
- (2) A dose of 4 mg of a 4.0 per cent solution of calcium chloride was sufficient to increase the amplitude of contraction by approximately 100 per cent within three minutes (Fig. 9).
- (3) When trans-2-g-tolylcyclohexanol (0.6 mg) had reduced the amplitude of contractions by 50 per cent at the end of three minutes, it was found that it required three successive doses of 8 mg each of calcium chloride, administered at three minute

intervals, to restore the amplitude of contraction stepwise to a normal level (Fig. 10).

- (4) When a total of the above amount of calcium chloride (24 mg) was administered at one time, there was a continuous recovery within three minutes (Fig. 11).
- (5) When trans-2-g-tolyleyclohexanol followed calcium chloride (4 mg), it was observed that the onset of action was delayed several minutes and the inhibitory effect which followed only overcame the effects of the calcium chloride, thus restoring the amplitude of contractions to a normal or slightly below normal level.
- (6) When trans-2-g-tolyleyclohexanol was administered in 0.2 mg increments at three minute intervals following 4 mg of calcium chloride, it was found that the first increment produced no change in amplitude at the end of three minutes and that the second increment produced a rapid reduction of amplitude to normal levels at approximately one and one half minutes after administration (Fig. 12).

Fig. 8 THE EFFECT OF trans-2-o-TOLYL-CYCLOHEXANOL
(0.6 mg) ON ISOLATED RABBIT ATRIA.

Fig. 9 THE EFFECT OF CALCIUM CHLORIDE
(4.0 mg) ON ISOLATED RABBIT
ATRIA.

Fig. 10 THE EFFECT OF CALCIUM CHLORIDE (8.0 mg DOSES) ON ISOLATED RABBIT ATRIA FOLLOWING 0.6 mg OF trans-2-o-TOLYLCYCLOHEXANOL.

Fig. 11 THE EFFECT OF CALCIUM CHLORIDE (24.0 mg) ON ISOLATED RABBIT ATRIA
FOLLOWING 0.6 mg OF trans-2-o-TOLYLCYCLOHEXANOL.

Fig. 12 THE EFFECT OF trans-2-o-TOLYLCYCLOHEXANOL (0.2 mg DOSES) ON ISOLATED RABBIT ATRIA FOLLOWING 4.0 mg OF CALCIUM CHLORIDE.

4. Effects of Cyclohexanol and its trans-2-o-Tolyl and trans-2-o- 61.
Chlorophenyl Substituted Derivatives on the Heart of Dogs Undergoing
Cardio-Pulmonary Bypass

a. Experimental Procedures

Nine mongrel dogs weighing from 16 to 22 kg were anesthetized with 30 mg/kg of pentobarbital sodium administered intravenously. Ventilation was maintained through a large endotracheal tube connected to a mechanical respirator. The chest was opened through a right thoracotomy incision at the level of the fourth intercostal space or by a midsternotomy. Superior and inferior vena cavae were cannulated through the axillary vein and right atrial appendage respectively, and the venous blood was collected by gravity. Oxygenation of the blood was achieved with a mixture of 98 per cent oxygen and 2 per cent carbon dioxide in a modified Kay-Cross oxygenator. The arterial blood was pumped by a DeBakey pump into a femoral artery. Total body perfusion, at a rate of 80 to 100 ml/kg/min. of body weight, was maintained for periods ranging from ten to sixty minutes. The temperature of the blood in the oxygenator was maintained from 35 to 37°C.

Electrocardiograms and arterial blood pressure were monitored on a Polyvisograph^a during the procedures. In several experiments electrocardiograms were recorded on a Sanborn or Birtcher electrocardiograph. After five to ten minutes of total body perfusion, and while maintaining a satisfactory blood pressure, a non-crushing occlusion clamp was placed across the ascending aorta and the compound was injected slowly (#21 needle) into the coronary arteries. After varying periods of cardiac arrest, at which time right and left thoracotomies were made, the aortic

^aElectronics for Medicine Inc., Boston, Mass.

clamp was released and the coronary arteries were perfused with arterialized blood.

In cases which necessitated the use of electrical defibrillation, shocks of 1 to 2 amp. were applied singly or in series.

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

1. The ability of the compound to induce complete arrest (electrical) and mechanical) of the heart.
2. The ability of the heart to recover from that arrest.

b. Results

The results appear on Table XIX.

- (1) trans-2-g-Tolylcyclohexanol, in three of five experiments, produced complete cardiac arrest ranging from 4 to 17 minutes. In one experiment, in which cardiac arrest failed to occur, the ventricular fibrillation which occurred could not be reverted to a normal sinus rhythm. Mechanical arrest occurred in the other attempt, however electrical excitability persisted. Post-arrest ventricular fibrillation occurred with one of the animals but was reverted to normal sinus rhythm by an intraventricular injection of calcium chloride and electrical defibrillation. It is interesting to note that in the experiment in which a 1 per cent v/v solution of trans-2-g-tolylcyclohexanol in 50 per cent propylene glycol in water was administered, the ensuing cardiac arrest occurred only after removal of the aortic clamp. In this instance normal sinus rhythm was observed electrically following arrest, but from gross observation

the myocardial contractions appeared to be feeble and incompetent. In all of the cases where cardiac arrest occurred, the heart did not appear to be anoxic.

- (2) trans-2-g-Chlorophenylcyclohexanol produced complete cardiac arrest, ranging from four to eight minutes, in two of the three experiments attempted. In one case a similar situation occurred with this compound as did with the trans-2-g-tolylcyclohexanol, namely that cardiac arrest occurred following removal of the aortic clamp. It was observed that the post-arrest recovery periods with these compounds were slow, and in one instance there was a persistent auricular arrest with no reversion to normal sinus rhythm. Mechanical arrest occurred in one experiment followed by post-arrest ventricular fibrillation, which responded to a combination of calcium chloride and electrical defibrillation.
- (3) In one experiment employing cyclohexanol, complete cardiac arrest occurred for a period of twenty-five minutes. Post-arrest ventricular fibrillation was overcome with calcium chloride and electrical defibrillation. It was noted that during the arrest period the myocardium seemed irritable and occasional contractions were observed.

Table XIX Effects of cyclohexanol and its trans-2-g-tolyl and trans-2-g-chlorophenyl substituted derivatives on the heart of dogs undergoing cardio-pulmonary bypass

ANIMAL NUMBER	COMPOUND	DOSE (mg/kg)	EFFECT ARREST	DURATION (Min.)	RECOVERY	REMARKS
1	<u>trans</u> -2- <u>g</u> -tolyl cyclohexanol	1.6	partial	7	NSR ^b	-
2	<u>trans</u> -2- <u>g</u> -tolyl cyclohexanol	1.5	complete	4	NSR ^b	-
3	<u>trans</u> -2- <u>g</u> -tolyl cyclohexanol	1.5	none	-	none	fibrillation
4	<u>trans</u> -2- <u>g</u> -tolyl cyclohexanol	10 ml ^a (1% v/v)	complete	6	NSR ^b	arrest occurred after removal of aortic clamp
5	<u>trans</u> -2- <u>g</u> -tolyl cyclohexanol	2.0	complete	17	NSR ^b	fibrillation overcome with calcium and defibrillation
6	<u>trans</u> -2- <u>g</u> -chlorophenyl cyclohexanol	3.3 ^a	complete	4	NSR ^b	arrest occurred after removal of aortic clamp
7	<u>trans</u> -2- <u>g</u> -chlorophenyl cyclohexanol	6.0 ^a	complete	8	none	auricular arrest persisted
8	<u>trans</u> -2- <u>g</u> -chlorophenyl cyclohexanol	5.7 ^a	partial	4	NSR ^b	defibrillation
9	cyclohexanol	33.0	complete	25	NSR ^b	fibrillation overcome with calcium and defibrillation

^adissolved in a 50 per cent solution of propylene glycol in water

^bnormal sinus rhythm

Fig. 13 ELECTROCARDIOGRAMS OF THE EFFECTS OF trans-2-o-TOLYLCYCLOHEXANOL
(2 mg/kg) ON THE HEART OF A DOG DURING CARDIO-PULMONARY BYPASS

A Before Perfusion

B Partial Perfusion

C Total Perfusion

Fig. 13 (Cont.)

I Removal of Aortic Clamp after 17 Minutes of Cardiac Arrest
Note Ventricular Fibrillation

J Injection of Calcium Chloride (7 ml of a 1 per cent solution) and
Electrical Defibrillation Note Tachycardia.

K Normal Sinus Rhythm 2 Minutes after Calcium Chloride and
Defibrillation

IV. DISCUSSION

Since preliminary investigations by Huitric et al. (1958) and Nelson et al. (1958) have demonstrated concurrently that trans-2-o-tolyleyclohexanol can arrest the heart of a dog, an investigation was undertaken to determine whether cyclohexanol and nine of its 2-tolyl and 2-chlorophenyl substitution products possess cardioplegic properties. In this series of compounds cis-trans isomers of the 2-o-tolyl, 2-o-chlorophenyl, and 2-p-chlorophenyl substitutions were available. The trans isomers of these compounds are those in which both the substituted groups and the hydroxy group occupy equatorial positions on the cyclohexane ring. The cis isomers are those where the substituted groups are equatorial and the hydroxy group axial to the cyclohexane ring.

In order to determine the rapidity with which these compounds affect the cardiovascular system and possibly to correlate this effect with chemical structure and spatial configuration, studies were undertaken utilizing the blood pressure of rats. In these experiments only the liquid members of the series were tested, since it was shown that the solvent system employed for the solids exerted similar effects and would thus mask the response to these compounds. The results obtained demonstrate the cyclohexanol and its liquid 2-aryl members produce an immediate drop in blood pressure in response to intravenous injection. The magnitude of this effect appears to be proportional to the dose employed for each compound. From statistical determination of the ED₅₀ (hypotensive) of the compounds tested (Table VII), it appears that substitution of a tolyl or chlorophenyl moiety in the number two position of the cyclohexanol ring increases its hypotensive activity from six to forty times. With respect to relative hypotensive potency among the

substitutions, cis-2-p-chlorophenylcyclohexanol displayed the most potent hypotensive effect followed by cis-2-p-tolylcyclohexanol, trans-2-p-tolylcyclohexanol, and trans-1-p-tolylcyclohexanol. It should be noted (Table VII) that the actual ED_{50} dose values of the trans-2-p-tolylcyclohexanol and the cis-2-p-tolylcyclohexanol differed by only 0.05 mg, with the latter exerting a slightly greater effect. cis-2-p-chlorophenylcyclohexanol, the most effective of the substitutions, was approximately seven times more potent than the trans-2-p-tolylcyclohexanol, which displayed the least potency in terms of substitution. Because only one cis-trans isomer combination was tested for hypotensive activity, and since the difference in hypotensive activity was slight, no correlation could be made between spatial configuration and hypotensive effect.

The fact that these compounds exert their hypotensive effect with such rapidity indicates the possibility of direct myocardial depression. Simultaneous electrocardiograph tracings in several of the experiments demonstrated that the hypotensive response was characterized by alterations in myocardial electrical activity which included bradycardia and depression of amplitude of the QRS complex.

It was interesting to note the manner in which the animals responded to injections of the compounds. Low doses usually elicited a short period of dyspnea. As the dose levels were increased the duration of dyspnea was increased concomitantly. At high dose levels complete cessation of respiration ensued, usually lasting from fifteen seconds to more than one minute, depending upon the magnitude of hypotension.

At the conclusion of the majority of experiments, the urine was aspirated from the bladder. All of the compounds tested produced hematuria as evidenced by the color of the urine. It was also noted

during the course of the investigation that trans-2-p-tolylcyclohexanol when mixed with blood from a dog produce hemolysis.

On the basis of the results obtained on the isolated guinea pig heart experiments, several conclusions can be drawn concerning comparative cardio-inhibitory effects in relation to chemical structure and spatial configuration. At the dose levels employed in these tests it appears that cyclohexanol and its 2-aryl substitution products are capable of depressing the amplitude of contraction of the isolated mammalian heart, and that substitution of tolyl or chlorophenyl groupings on the number two position of cyclohexanol increases the cardio-inhibitory effects from four to twenty times (Table XVIII).

On the basis of the minimum effective dose that will produce cardiac arrest (Table XVIII) it appears that within the tolyl substitution products, trans-2-p-tolylcyclohexanol is apparently the most effective in producing cardiac arrest at the lowest dose followed by a good recovery. trans-2-m-Tolylcyclohexanol is second in comparative cardio-inhibitory effectiveness, followed by trans-2-o-tolylcyclohexanol, as indicated by minimum effective doses of 1.0 mg and 1.5 mg respectively. Although not on a quantitative basis, it can be concluded qualitatively, that transfer of the methyl group on the phenyl moiety from para to meta to ortho positions increases cardioplegic effectiveness of the tolyl substitutions respectively.

Among the chlorophenyl substituted derivatives, trans-2-o-chlorophenylcyclohexanol appears to possess the most potent cardioplegic activity according to its minimum effective dose (Table XVIII). This is followed by trans-2-m-chlorophenylcyclohexanol and trans-2-p-chlorophenylcyclohexanol respectively. On the basis of the comparative cardio-

inhibitory effects of the chlorophenyl substitutions, transfer of the chlorine atom on the phenyl moiety from para to meta to ortho positions qualitatively increases its cardioplegic effectiveness.

In relation to spatial configuration versus cardio-inhibitory effects, it appears that in the doses employed the cis-g-isomers (Tables IX and XIII) are the least depressant while the corresponding trans-g-isomers (Tables X and XIV) are the most effective in each of their respective series. The cis-p-chlorophenyl isomer (Table XVI) is less effective than the corresponding trans-g-chlorophenyl isomer (Table XVII), although the latter is not the most potent in its respective series. These facts indicate the importance of the spatial configuration of these compounds in producing their cardio-inhibitory actions.

There is evidence of cardio-toxicity with several of the compounds as indicated by the apparent failure of the hearts to recover to 50 per cent of normal amplitude following either cardiac arrest or depression. The results obtained with cis-2-g-chlorophenylcyclohexanol (Table XVI) and trans-2-g-chlorophenylcyclohexanol (Table XVII) demonstrate that at dose levels capable of producing cardiac arrest, the amplitude of post-arrest contractions did not recover to 50 per cent of the pre-arrest levels. In several instances the heart failed to resume contractions following arrest. It is interesting to note that the minimum effective dose of trans-2-g-chlorophenylcyclohexanol (Table XVII) was also the toxic dose, and at that dose the heart failed to return to 50 per cent in all trials attempted. It should also be mentioned that upon gross observation there was an indication that trans-2-m-chlorophenylcyclohexanol exhibited cardio-toxicity at effective dose levels as evidenced by its relatively slow recovery to 50 per cent contraction levels (Table XV).

On the strength of the aforementioned findings and in light of the apparent positive inotropic effects of the solvent system employed it would be difficult to compare exactly the relative cardioplegic effectiveness of tolyl substitutions versus chlorophenyl substitutions. There are, however, several indications of the following relationships:

1. The tolyl substituted derivatives appear to be the more potent cardioplegic agents, and are the least toxic.
2. The substitution of the chloro group for the methyl group on the phenyl ring appears to increase cardio-toxicity without apparently increasing cardioplegic potency.

It is worth noting that the latter finding is in accordance with the well known cardio-toxicity of chlorinated hydrocarbons (Goodman and Gilman, 1956).

The apparent positive inotropic effects demonstrated by several of the compounds (Tables IX, XIII, XV, XVI, XVII; Fig. 5) is rather difficult to reconcile with the overall consistency of cardiac depression exhibited by these compounds. In many cases, however, the cardiac stimulation which occurred was the initial response and was followed by a more persistent depression. It was noted that when stimulation was elicited with a particular compound it occurred in the first trials and never extended beyond the third trial. In many cases the amplitude of stimulation decreased progressively with each succeeding trial. It is conceivable that the propylene glycol and water solvent system may have contributed to the stimulatory effect, however, cis-2-chlorophenyl-cyclohexanol, which was dissolved in an aqueous medium exhibited the most consistent stimulatory effects, as evidenced by the occurrence of stimulation in five of twelve trials at 0.1 mg and four of twelve trials at the 0.2 mg dose (Table XIII). The fact that stimulation occurred

almost exclusively among the chlorophenyl derivatives indicates that this effect may be due to the presence of the chloro substituent.

Studies with trans-2-g-tolylcyclohexanol, alone and in combination with calcium, on isolated atrial tissue have revealed several possibilities concerning the mechanism of action on cardiac muscle. trans-2-g-Tolylcyclohexanol produces a negative inotropic effect on atrial tissues without apparently affecting the rate. When enough calcium was added to the bath following the depressant effect, it was able to restore contractions to a normal level (Fig. 11). Apparently the antagonism between calcium and trans-2-g-tolylcyclohexanol is of a physiological type. The major effect of calcium, however, was to increase the latent period of the compound's effect.

The fact that trans-2-g-tolylcyclohexanol can depress the amplitude of atrial contractions without apparently affecting the rate indicates that this compound does not primarily affect pacemaker activity. This finding can be correlated with gross observations on the isolated arrested heart. In all cases where cardiac arrest was produced with these compounds, it was observed that the atria were contracting at a relatively normal rate while the ventricles were arrested. It seems plausible, therefore, to consider the possibility that these compounds, or at least trans-2-g-tolylcyclohexanol, can arrest the heart by preventing atrial impulses from exciting the ventricles, or in effect, by causing an atrio-ventricular block.

The results obtained with experiments on dogs utilizing cardio-pulmonary bypass indicates that trans-2-g-tolylcyclohexanol, trans-2-g-chlorophenylcyclohexanol and cyclohexanol are capable of inducing elective cardiac arrest (Table XIX). Complete arrest was achieved with all compounds tested. There appeared to be no predictable relationship between

the dosage of the compounds employed and the production of cardiac arrest. Only a minimum amount of compound was necessary in some experiments while in others considerably more was required. Yet recovery was satisfactory even after twenty-five minutes of cardiac arrest. Although the recovery rate after production of cardiac arrest followed by perfusion was not consistent, it was noted that recovery with trans-2-p-tolylcyclohexanol was faster and more predictable than with trans-2-p-chlorophenylcyclohexanol. The major complications during recovery were ventricular fibrillation and incompetent myocardial contractions. In most cases fibrillation was overcome with electrical defibrillation, however, in one case with trans-2-p-tolylcyclohexanol and fibrillation was irreversible and did not respond to any stimuli.

The duration of arrest seemed to have no relationship either to the rate of recovery or to the dose of the compound injected. The production of arrest following removal of the aortic clamp in two of the experiments is difficult to explain. It is conceivable that the propylene glycol and water solvent system employed for these compounds may have been a factor.

V. SUMMARY

A study of the comparative cardio-inhibitory effects has been made on cyclohexanol and nine of its 2-aryl substitution products. Experiments were carried out on intact animals, isolated cardiac tissue, and in-situ cardiac preparations.

The effects of cyclohexanol and its liquid 2-aryl substitution products on the blood pressure of rats indicate that these compounds are capable of producing an immediate hypotensive effect associated with bradycardia. The magnitude of hypotension appears to coincide with the dose employed. OF the compounds tested, cis-2-p-chlorophenylcyclohexanol is the most potent followed by cis-2-p-tolylcyclohexanol, trans-2-o-tolylcyclohexanol, trans-2-p-tolylcyclohexanol and finally cyclohexanol. Substitution of chlorophenyl or tolyl groups in the 2-position of the cyclohexanol moiety increases the hypotensive effectiveness from six to forty times. No correlation could be made between the hypotensive activity and spatial configuration. There was evidence that these compounds are capable of producing hematuria possibly through a hemolytic action.

Experiments conducted on isolated hearts revealed that cyclohexanol and its 2-aryl substitution products are capable of depressing myocardial contractility. The addition of tolyl or chlorophenyl groups in the 2-position of the cyclohexanol ring augments the cardio-inhibitory effect from four to twenty times. Within their respective series the trans-ortho-substituted aryl groups confer the greatest cardio-inhibitory potency followed by the trans-meta and trans-para analogs respectively. At the dose levels employed it appears that the cis-isomers are the weakest in their respective series. This finding indicates the importance

of spatial configuration of the molecule to cardio-inhibitory effectiveness.

There was evidence of cardio-toxicity with several compounds as indicated by failure of the heart to return to normal levels. The toxicity was present only among the chlorophenyl compounds and was attributed to the presence of the chloro substituent. Several of these compounds displayed cardio-excitatory properties. As in the case with cardio-toxicity, this effect was mainly restricted to the chlorophenyl compounds and was thus attributed to the presence of the chloro substituent.

Isolated atrial studies revealed that the depressant effects of trans-2-g-tolyleyclohexanol can be counteracted by various amounts of calcium. This antagonism is apparently of a physiological type. It was also noted that calcium prolonged the latent period of trans-2-g-tolyleyclohexanol. Since it was shown that trans-2-g-tolyleyclohexanol can depress atrial contractions without affecting rate, it was concluded that this compound does not arrest the heart via depression of the pacemaker. This finding, along with gross observation of the isolated arrested guinea pig heart, suggests that these compounds may produce their cardioplegic effects by an atrio-ventricular block.

Experiments employing extracorporeal circulation have demonstrated that cyclohexanol, trans-2-g-tolyleyclohexanol and trans-2-g-chlorophenyl-cyclohexanol are capable of arresting the heart of a dog. There appears to be no predictable relationship between the dose of the compounds and the production of cardiac arrest. Ventricular fibrillation and incompetent myocardial contractions were the major problems following arrest. It was noted that recovery with trans-2-g-tolyleyclohexanol was

more consistent and faster than with trans-2-g-chlorophenylcyclohexanol.

VI. CONCLUSIONS

1. Cyclohexenol and certain of its 2-tolyl and 2-chlorophenyl substitution products are capable of depressing myocardial contractility.
2. The ortho position of both tolyl and chlorophenyl derivatives appears to be the most effective in each series, followed by the meta and para positions respectively.
3. The cis-ortho isomer appears to be the least effective in each of the respective series.
4. The chlorophenyl substituted derivatives appear to exhibit a greater cardio-toxicity than the tolyl substituted derivatives and the production of stimulatory effects in some cases may be attributed to the chloro substituent.
5. All compounds studied appear to exert their cardio-inhibitory effects via an atrio-ventricular block.

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