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AN ANALYSIS OF THE CARDIOVASCULAR ACTIVITIES OF SELECTED PURINE DERIVATIVES WITH SPECIAL REFERENCE TO THE CONSTITUENT PARTS OF AMINOPHYLLINE

by

Harold Francis Hardman

A dissertation submitted in partial fulfillment

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An Analysis of the Cardiovascular Activities of Selected Purine Derivatives with Special Reference to the Constituent Parts of Aminophylline

by Harold F. Hardman

The purpose of this problem was to examine the cardiovascular properties of selected purine derivatives as related to their chemical constitution. Special reference was made to the constituent parts of aminophylline (theophylline and ethylenediamine).

The isolated cat papillary muscle and dog heart-lung preparations were employed as test objects for evaluating the cardiovascular activities of the compounds examined.

The results obtained in Part I of this study demonstrate that modifications of the basic purine nucleus by appropriate substitutions produces compounds exhibiting a wide range of cardiovascular activities. Positive inotropic and positive chronotropic activity can be separated and modified individually in the purine molecule by selective alteration of its chemical structure.

Several examples are presented in which the pharmacological activity of chemicals commonly employed as solubilizing agents for purine derivatives greatly influence the activity of the solute.

An investigation of the reported cardiac activity of a recently introduced theophylline derivative, 7-diethylaminoethyltheophylline (R-3588), produced negative results. Neither the compound per se nor some of its potential products of hydrolysis exhibited significant cardiac activity as measured by my criteria.

A method has been described employing competence index to predict the approximate duration of action of a drug reversing cardiac failure in the dog heart-lung preparation.

In Part II of the study, the discovery that ethylenediamine possessed marked positive inotropic activity suggested a logical explanation for the apparent superiority of aminophylline over other theophylline salts in the treatment of congestive heart failure. Experiments are reported in which ethylenediamine is shown to enhance the action of theophylline in reversing cardiac failure in the dog heart-lung preparation.

The hypotensive response resulting from rapid intravenous injection of aminophylline in the dog is largely due to the ethylenediamine present in this drug. The site of the ethylenediamine hypotensive action has been shown to be cephalad to the anatomical junction of the internal carotid and vertebral arteries. The mechanism of drug action responsible for the fall in blood pressure is apparently related to the chelate structure of ethylenediamine. The nature of the substance being chelated has not been determined, however, calcium ions have been tentatively implicated.

The general conclusions drawn from this study are that the purine molecule provides an excellent tool for further investigation of the basic mechanisms involved in positive inotropic and positive chronotropic activity in mammalian cardiac muscle. The amino substituted purine derivative, 2,6-diaminopurine sulfate, is of great interest and appears to be superior to the classical methylated xanthines as a cardiac stimulant.

Ethylenediamine can no longer be considered as a pharmacologically inactive solubilizing agent for theophylline in the commercial preparation aminophylline. Ethylenediamine contributes to the positive inotropic activity of aminophylline and to its duration of action. The hypotensive activity of

ethylenediamine is apparently related to the chelate structure of the molecule which acts upon a receptor substance located within the cranial boundaries of the dogs nervous system.

ACKNOWLEDGEMENT

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INTRODUCTION

I. The Origin of the Problem

In 1785 William Withering, a physician and botanist of Birmingham, England, published his famous book entitled "An Account of the Foxglove and Some of Its Medicinal Uses". Withering recognized that digitalis or foxglove was effective only in certain forms of dropsy but did not associate this with the cardiac actions of the drug. He did, however, observe that the heart was affected by the drug and expressed the hope that this property might be converted to a useful purpose. John Ferriar in 1799 apparently was the first to suggest that digitalis acted primarily on the heart and relegated the diuretic effect to a secondary role. Within the last thirty years it has become firmly established that the main value of digitalis and closely related compounds is in the therapy of congestive heart failure. No fully acceptable theory for the mechanism of action of these cardiac glycosides has yet to be presented. The limited availability as well as the complex chemical configuration of this class of compounds has greatly hindered further investigation of their mechanism of action on the heart.

Within the last twenty years several other chemical agents have been reported that appear to stimulate the mammalian myocardium in a manner similar to the action of the cardiac glycosides. The purine derivative theophylline has received extensive laboratory and clinical evaluation and ranks second only to the cardiac glycosides in the treatment of congestive heart failure.

My primary interest was directed toward a better understanding of the mechanisms of drug-induced positive inotropic activity in the

hypodynamic mammalian myocardium. In view of the difficulties involved in obtaining adequate samples of cardiac glycoside derivatives my attention was directed to the purine class of compounds. Many purine derivatives are now commercially available thus permitting a relatively extensive survey of their chemical constitution as related to their biological activity. I, therefore, utilized these compounds to study the factors determining cardiac positive inotropic activity in the purine molecule.

At the present time theophylline is regarded as the purine compound possessing the greatest cardiac activity. It was of interest to note, however, that of all the commercially available theophylline preparations aminophylline (theophylline ethylenediamine) appeared to be the drug of choice as a cardiac stimulant. This apparent superiority of aminophylline over the other complex salts of theophylline has resulted in its inclusion as the only theophylline preparation in the United States Pharmacopeia XIV. Grüter introduced ethylenediamine as a solubilizing agent for theophylline in 1909. In 1930 Schmitt (29) proposed the name aminophylline for the combination of theophylline and ethylenediamine. For many years the pharmacological activity of theophylline and aminophylline have been thought to be identical and investigators have used them interchangeably. In view of the apparent superiority of aminophylline over the other complex salts of theophylline I investigated the pharmacological properties of the component parts of aminophylline. The major emphasis has been placed upon the results obtained with ethylenediamine since it has previously been regarded as a pharmacologically inactive solubilizing agent for theophylline.

EXPERIMENTAL METHODS

I. The Cat Papillary Muscle Preparation

The papillary muscles of the cat's right ventricle have proved to be suitable preparations for the study of pharmacological factors affecting the force of contraction of cardiac muscle. They can be isolated easily and under suitable conditions (33) contract vigorously upon stimulation for as long as twenty-four hours. A carefully chosen muscle consists of about fifty parallel cells of mammalian ventricular muscle from which recordings of contractile activity may be readily obtained (9).

The cat's heart was excised under ether anesthesia and an incision made along the border of the right ventricle. The outer wall of the right ventricle was then turned down as a flap and suitable muscles removed with the chordae tendinae and part of the ventricular wall still attached to the base. The papillary muscle itself was not cut or injured in any way.

The muscle was mounted in a plastic holder and glass chamber according to the method outlined by Garb and Chenoweth (9). Briefly, this consists of tying the base of the muscle to a fixed plastic hook with silk thread and attaching the chordae tendinae to an isometric muscle lever by means of a fine silk thread. The plate shaft of a mechano-electronic transducer vacuum tube (RCA #5734) was utilized as the isometric muscle lever. The transducer tube provides a method for translating mechanical vibrations into electrical current variations which can be observed and measured on the phosphorescent screen of a cathode ray oscillograph as millimeters of deflection. The glass

chamber surrounding the muscle holder was then filled with a modified Locke's solution (10) maintained at 37 - 38 degrees C. by a constant temperature bath. The base of the muscle was in contact with two platinum electrodes attached to a Grass stimulator which stimulated the muscle at a rate of one per second. The voltage and duration of the pulse was maintained at the threshold level, usually 1 - 5 volts for a one millisecond square wave pulse. A mixture of 5 per cent carbon dioxide and 95 per cent oxygen was forced vigorously through the bottom of the muscle chamber where a sintered glass plate increased the dispersion. The optimum rate of gas flow was found to be very rapid.

The muscles were observed for one hour under these conditions and the maximal amplitude of contraction was recorded. Sodium pentobarbital was then added to the muscle bath until the amplitude of contraction of the muscle was reduced to about 50 per cent of the observed maximal contraction. The muscle was then considered to be in a state of failure and the experimental drug was added to the muscle bath at this time. Periodic observations were recorded in order to determine whether or not the experimental drug could reverse the failure state, and the extent of this reversal was evaluated. This was usually measured as the per cent increase in contractile force above the failure level.

II. The Dog Heart-Lung Preparation

A modification of the Krayer-Mendez (20) denervated heart-lung preparation (H.L.P.) was employed in dogs anesthetized with sodium pentobarbital. The left auricular cannula, venous inflow pump, Henderson cardiometer, and coronary sinus cannula were omitted. The Stolnikow stromuhr was substituted for the Weese instrument.

The arterial outflow via the brachiocephalic artery was directed through a peripheral resistance of 80 mm. Hg to the stromuhr and then to the venous reservoir. The blood from the reservoir was returned to the superior vena cava at a temperature of 36 -38 degrees C. to complete the circuit. All other vessels to and from the heart, except the pulmonary and coronary vessels, were ligated. The total amount of blood in the system was approximately one liter. The right auricular pressure (R.A.P.) and arterial pressure (A.P.) were recorded continuously by kymograph tracings. Cardiac output (C.O.) in ml./min. (left ventricular output minus the coronary artery flow), heart rate per minute (H.R.), and competence index (C.I.) were measured periodically.

The competence index as described by Wollenberger (34) was utilized to evaluate the degree of cardiac failure. A C.I. of 0.9 to 1.0 indicates a relative state of non-failure, whereas 0.0 to 0.2 indicates a state of marked failure.

Control readings were taken as soon as the H.R. and C.O. stabilized. The myocardium was failed to a C.I. of 0.0 to 0.2 either spontaneously or by the addition of sodium pentobarbital. The drug to be tested was administered at this time by injection into the superior vena cava cannula and readings were made periodically until the C.I. returned to the failure level. This interval of time was recorded in the tables as the duration of change (Δ) in C.I. and is expressed in minutes. The per cent increase in C.O. was determined five minutes after administration of the drug as compared with the cardiac output during failure. The per cent increase in H.R. is calculated in the same manner. Total delta (Δ) C.I. represents the change in C.I. as a result of administering the drug. In general there is a fairly

good correlation between this value and the duration of action of the drug tested. Those drugs giving a delta C.I. value of 0.9 had a long duration of action while those with a smaller delta C.I. value were shorter acting. No correlation was observed between the per cent increase in C.O. which measures the intensity of cardiac stimulation and the duration of delta C.I. which provides some measure of the duration of cardiac stimulation.

All the drugs were administered as molar equivalents of 165 mgm. of theophylline monohydrate and injected into a blood volume of approximately one liter.

The Influence of Heart Rate upon Cardiac Output

Several of the purine derivatives examined were observed to have significant positive chronotropic activity. In these cases the per cent increase in C.O. may be the result of several independent factors. In the less complicated circumstance where there is no increase in heart rate following drug administration, an increase in C.O. is due to an increase in stroke volume since the peripheral resistance is set at a constant value. On the other hand, in those cases where a positive chronotropic activity was observed, an increase in C.O. could have resulted from a positive inotropic action, a positive chronotropic action, or a combination of both of these mechanisms.

There is no doubt that an increase in heart rate alone can increase C.O., provided that the potential venous return to the heart is not a limiting factor. In all the following experiments the venous return was adjusted in such a manner that it could increase by several hundred milliliters per minute above the resting level if the heart rate or force of contraction was increased following drug administration.

Since it was not possible to quantitate the individual effects of positive chronotropic and positive inotropic action upon C.O. in the heart-lung preparation, experiments were run on the isolated cat papillary muscle in order to observe the per cent increase in inotropic activity of several of the drugs that had exhibited marked positive chronotropic activity in the heart-lung preparation. The results of these experiments are listed in Table 1.

The increased C.O. resulting from administration of 8-chlorotheophylline indicates that an increase in heart rate alone is capable of producing an increase in C.O. since the small per cent increase in contractile force is negligible and within the limits of error. This is substantiated by the results obtained with imidazole.

A comparison of the responses obtained with 8-chlorotheophylline and xanthine indicates that the effects of an increased heart rate and increased force of contraction are additive since 8-chlorotheophylline produces a slightly greater increase in C.O., even though its per cent increase in contractile force is less than that observed with xanthine. All of the drugs that produced a positive chronotropic response will have to be compared in this way before an absolute evaluation of the factors that increase C.O. can be made.

Competence Index

The next point to be considered is the use of total delta ($\triangle$) C.I. as a means of evaluating cardiac activity. The change in C.I. from a failure value of 0.0 - 0.2 to a higher value represents an increased capacity of the heart to respond to an increased load. In other words, the heart is now better able to handle an increased venous return by increasing its output. This can be accomplished by an

increase in heart rate, an increase in force of contraction or a combination of these mechanisms. When the venous return exceeds the capacity of the right ventricle to remove the blood as rapidly as it receives it, as in the case of right congestive heart failure, then the right ventricle begins to dilate and accumulate blood. This accumulation of blood in the right ventricle causes in turn an increase in right auricular blood volume and thereby produces an increase in right auricular pressure. The same chain of events can be initiated in the left ventricle with identical results. The primary defect in both cases is that the contractile force of the ventricles is decreased to a point where they can no longer eject blood at the same rate it is received. Any mechanism that can increase ventricular output will at the same time increase the ventricular input and thereby raise the C.I. value. An increase in contractile force of the ventricles would seemingly be a more efficient means of raising the C.I. value rather than inducing a tachycardia which has relatively limited ability to increase ventricular output. When the rate of contraction exceeds 200 per minute, the succeeding contraction impinges upon the diastolic period of rapid filling of the preceding contraction. From this point on further increases in heart rate cannot increase the cardiac output since the venous input is proportionally decreased.

Table 2 demonstrates that there is a good degree of correlation between the magnitude of the change in competence index (total Δ C.I.) and the duration of a competence index greater than that observed at failure (duration of Δ C.I.). In general one could estimate the approximate duration of an increase in C.I. above the failure level by measuring the magnitude of the change in C.I. ten minutes after administering the drug to a failed preparation.

Table 1

An Evaluation of the Positive Inotropic and Positive Chronotropic Action Producing an Increased Cardiac Output in the Failed Dog Heart-Lung Preparation

Drug *	% Inc. in C.O.	Total ΔC.I.	Duration of ΔC.I.(min.)	% Inc. in H.R.	% Inc. in Contractile Force **
Theophylline	62	0.9	58	20	37
6-Desoxytheophylline	23	0.8	41	17	17
Caffeine	43	0.8	>40	0	22
8-Chlorotheophylline	18	0.0	0	12	1
Xanthine	10	0.6	>23	0	3
8-Bromotheophylline	12	0.0	0	0	5
Imidazole	0	0.0	0	0	1

* All drugs were tested at doses equimolar to 8.3×10^{-4} molar theophylline monohydrate.

** Per cent increase in contractile force was determined on the basis of the increased amplitude of contraction resulting from administration of the drug to isolated cat papillary muscles that had been failed to fifty per cent of their initial maximal contraction. (See Appendix).

Table 2

The Relationship of the Magnitude of Delta C.I. to the Duration of Delta C.I.

DRUG	TOTAL C.I.	DURATION OF C.I.(min.)
7-Chloroethyltheophylline	0.9	75
Theophylline	0.9	60
Theobromine	0.9	54
7-Ethyltheophylline	0.9	51
Caffeine	0.8	48
Adenine Sulfate	0.8	42
6-Desoxytheophylline	0.8	41
2,6-Diaminopurine Sulfate	0.8	20 *
1,3,7-Triethylxanthine	0.7	36
Xanthine	0.6	> 23
1,3-Dimethyluric Acid	0.4	30
Hypoxanthine	0.2	8
Benzimidazole	0.1	10
Adenosine	0.1	10

* This number represents the average of two experiments. The individual values for these experiments were 32 and 9 minutes respectively. In view of the high degree of variability, a larger series of experiments is necessary before a valid average can be determined.

EXPERIMENTAL RESULTS

I. The Cardiac Pharmacology of Selected Purine Derivatives as Related to their Chemical Constitution

An investigation of the cardiac activity of a series of purine derivatives was made in order to determine the structural requirements essential to positive inotropic activity. It soon became evident, however, that marked changes in chronotropic activity and duration of drug action also resulted from modification of the purine nucleus. These observations are recorded in the text and summarized in the discussion.

My interpretation of the value of a structure activity relationship study was based upon the concept that the chemical properties of the active moieties of a pharmacologically active molecule were of primary importance. A reconstruction of the theophylline molecule with atomic models suggested that the potential chelating configuration* between positions 6 and 7 might be of importance in determining positive inotropic activity. This correlation was based upon the report by Hardman et al. that certain heavy metal chelating agents could produce a positive inotropic action in the hypodynamic cat papillary muscle (15). An earlier report by Chenoweth et al. had implicated the intracardiac chelation of copper as a basic mechanism in the positive inotropic action of the cardiac glycoside ouabain (4).

A. Modifications of the Purine Nucleus in Positions 1,2,3,6,7,8, and 9

Positions 1,3, and 7

* Adrian Albert has recently verified the possibility that certain purines may function as active chelating agents (1).

Replacement of the 1 and 3 methyl groups of theophylline with hydrogen atoms to form xanthine decreases the ability of the drug to increase cardiac output (Table 3). This substitution completely abolishes the positive chronotropic activity of the molecule. In addition the positive inotropic activity of the molecule is greatly reduced (Table 1). The residual activity with respect to increasing cardiac output must be entirely due to the slight positive inotropic action of the molecule. Even though the intensity of this positive inotropic action is slight, it has a moderate duration of action.

A comparison of theobromine and caffeine leaves little to choose between them. This would lead one to believe that the 1 methyl group is relatively less important for positive inotropic activity than the 3 methyl group. The caffeine molecule, however, has less positive inotropic activity than theophylline (Table 1) and no positive chronotropic activity. This comparison clearly indicates that the presence of a methyl group in position 7 significantly reduces the positive inotropic activity of the theophylline molecule and completely eliminates the positive chronotropic activity. The replacement of the 7 methyl group of caffeine by an ethyl group to form 7-ethyltheophylline has no significant effect upon the cardiac activity of the molecule. It is of interest, however, that a complete substitution of ethyl for methyl groups in the caffeine molecule restores positive chronotropic activity without greatly altering positive inotropic action.

In summary the 1 and/or 3 methyl groups contribute to the positive inotropic and chronotropic activity of the theophylline molecule. The addition of a methyl or ethyl group to the 7 position of this molecule completely inhibits positive chronotropic activity and also

significantly reduces positive inotropic activity. The 1,3,7 triethylxanthine molecule in contrast to 1,3,7 trimethylxanthine (caffeine) exhibits as much positive chronotropic activity as the theophylline molecule, however, it has significantly less inotropic activity.

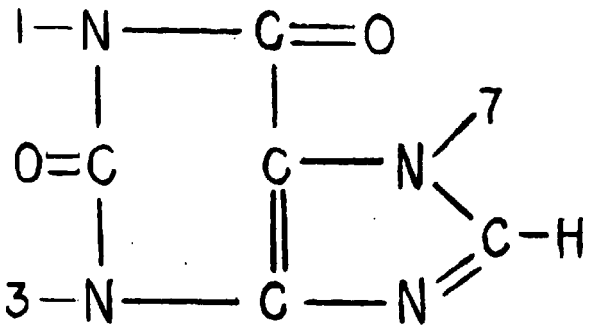
The effects produced by substitutions in the 7 position of the theophylline molecule were of sufficient interest to warrant further study. Table 4 contains the results obtained with several 7 substituted theophylline derivatives. Replacement of the 7 hydrogen atom of theophylline with a methyl group to form caffeine produced a decrease in positive inotropic activity (Table 1) as well as abolishing positive chronotropic activity. The substitution of an ethyl group in position 7 produced about the same effect as the methyl substitution described above. The addition of a chloroethyl group to position 7 of the theophylline molecule produced a marked increase in the positive chronotropic activity of the molecule (See Appendix). Presumably the inotropic activity has been reduced since the per cent increase in cardiac output is significantly less than that obtained with an equimolar dose of theophylline monohydrate. It is of interest to note that the 7-chloroethyl substitution produced the greatest per cent increase in heart rate of any of the purine derivatives tested in this study.

The substitution of a diethylaminoethyl group for the 7 hydrogen atom of theophylline resulted in a compound that had no demonstrable positive inotropic or chronotropic activity. If anything, the drug appeared to have a slight negative inotropic action as indicated by the negative change in total delta C.I. (Some additional studies on this compound are reported in the Appendix.)

In summary none of the 7 substituted theophylline derivatives

Table 3

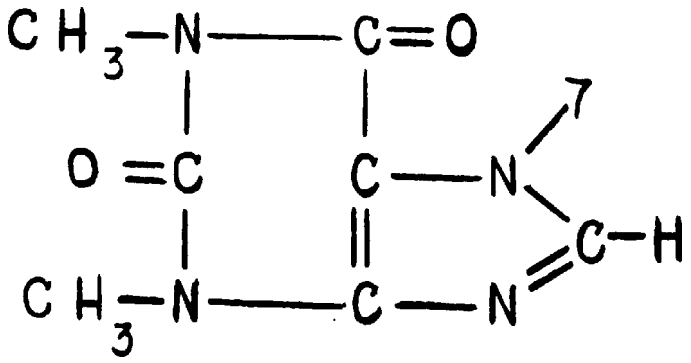
Substitutions in Positions 1, 3, and 7 of the Purine Nucleus



DRUG	DOSE MGM./L.	SUBSTITUTION			% INCREASE IN C.O.	TOTAL △ C.I.	% INCREASE IN H.R.	(min.) DURATION OF △ C.I.	NO. OF EXP.
		#1	#3	#7					
THEOPHYLLINE	165	CH_3	CH_3	H	73	0.9	20	60	5
XANTHINE	139	H	H	H	12	0.6	0	>23	3
THEOBROMINE	150	H	CH_3	CH_3	34	0.9	0	54	4
CAFFEINE	161	CH_3	CH_3	CH_3	31	0.8	0	48	4
7-ETHYLTHEOPHYLLINE	173	CH_3	CH_3	C_2H_5	26	0.9	0	51	3
1,3,7-TRIETHYL XANTHINE	196	C_2H_5	C_2H_5	C_2H_5	40	0.7	20	36	2

Table 4

Substitutions in Position 7 of the Purine Nucleus



DRUG	DOSE MGM./L.	SUBSTITUTION	INCREASE IN C.O.	TOTAL Δ C.I.	INCREASE IN H.R.	(min.) DURATION OF Δ C.I.	NO. OF EXP.
THEOPHYLLINE	165	H	62	0.9	20	58	7
CAFFEINE	161	CH_3	43	0.8	0	>40	4
7-ETHYLTHEOPHYLLINE	173	C_2H_5	40	0.9	0	51	3
7-CHLOROETHYL-THEOPHYLLINE	201	C_2H_4Cl	15	0.9	29	>75	3
7-DIETHYLAMINO-ETHYLTHEOPHYLLINE	289	$(C_2H_5)_2N-C_2H_4$	0	-0.2	0	0	3
*1,3,7 TRIETHYL-XANTHINE	196	C_2H_5	37	0.7	21	36	2

* This compound contains ethyl rather than methyl groups in positions 1 and 3.

studied appeared to be superior to theophylline in their ability to increase cardiac output. From this we must assume that none of them were superior to theophylline in their positive inotropic activity. 7-Chloroethyltheophylline, however, exhibited greater positive chronotropic activity than theophylline. This must be related to the presence of the chlorine atom in the molecule since 7-ethyltheophylline had no positive chronotropic activity. Table 22 contains further information on the activity of 7-chloroethyltheophylline.

Position 2

The results obtained with substitutions in position 2 of the purine nucleus are presented in Table 5. Replacement of the 2 oxygen atom of xanthine by a hydrogen atom to form hypoxanthine appears to increase the intensity of cardiac stimulation slightly, however, the delta C.I. value is reduced and the duration of delta C.I. is considerably less than that obtained with xanthine.

A comparison of adenine sulfate with 2,6-diaminopurine sulfate indicates that replacement of the 2 hydrogen atom with an amino group greatly increases the intensity of cardiac stimulation. 2,6-Diaminopurine sulfate is a compound of great interest. It ranks second only to theophylline, in the series of purines tested, in ability to increase cardiac output in the failed dog heart. Further experiments may well prove it to have a greater positive inotropic action than equimolar doses of theophylline. The structure of 2,6-diaminopurine sulfate reminded me of the simpler molecule, guanidine, which has the same configuration as present in the 1,2, and 3 positions of the 2,6-diaminopurine sulfate molecule. Guanidine hydrochloride was subsequently evaluated in the dog heart-lung preparation and was found to have

considerable positive inotropic and chronotropic activity, although the inotropic activity was significantly less than that obtained with 2,6-diaminopurine sulfate. (See Appendix)

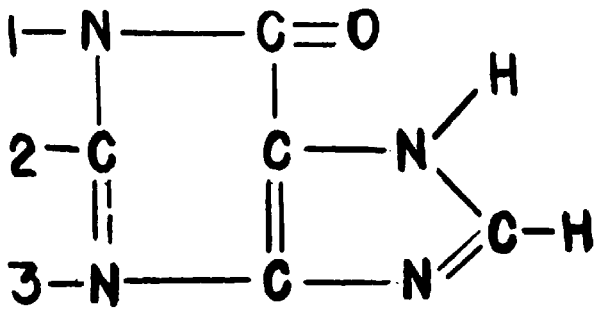
In summary variation of the substituent group in position 2 of the purine nucleus can influence the intensity and duration of cardiac stimulation. None of the four compounds studied exhibited any positive chronotropic activity, therefore, the per cent increases in C.O. are manifestations of a positive inotropic response.

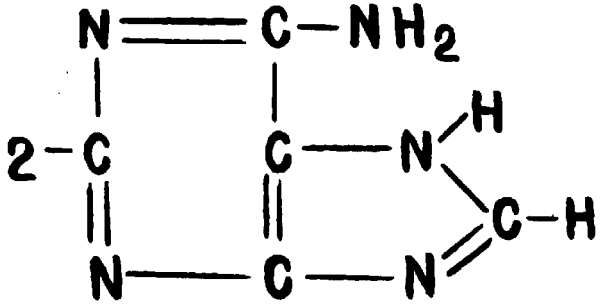
Position 6

The results obtained with substitutions in position 6 of the purine nucleus are presented in Table 6. Substitution of two hydrogen atoms for oxygen atom in the 6 position of theophylline to form desoxy-theophylline produces a marked reduction in the intensity of cardiac stimulation with a decrease in the duration of action. This change does not appear to alter the positive chronotropic activity of the molecule. A comparison of hypoxanthine and adenine sulfate indicates that replacement of the 6 oxygen atom of hypoxanthine by an amino group slightly increases the intensity of cardiac stimulation; however, the duration of this stimulation is greatly prolonged. It should be pointed out that there is another change in the molecular configuration of adenine as compared with hypoxanthine. The 1 hydrogen atom has been eliminated with the resulting formation of a double bond between positions 1 and 6. The extent to which this change may modify the activity of the molecule has not been determined. It is interesting to note that adenine sulfate possesses the same order of activity (intensity and duration) as desoxytheophylline while lacking the positive chrono-tropic action of desoxytheophylline. This type of observation offers

Table 5

Substitutions in Position 2 of the Purine Nucleus





DRUG	DOSE MGM./L.	SUBSTITUTION #1	#2	#3	% INCREASE IN C.O.	TOTAL Δ C.I.	% INCREASE IN H.R.	(min.) DURATION OF Δ C.I.	NO. OF EXP.
XANTHINE	139	H	O	H	10	0.6	0	>23	3
HYPOXANTHINE	113	H	H	-	20	0.2	0	9	2
ADENINE SULFATE	336	-	H	-	27	0.8	0	42	3
2,6-DIAMINOPURINE SO_4	205*	-	NH_2	-	51	0.8	0	21	2

* Dose should have been 319 mgm. in order to be equivalent to 165 mgm. of theophylline monohydrate.

hope for the idea that a highly active purine derivative may be found which lacks the undesirable positive chronotropic activity as a result of modifying the basic purine structure.

Position 8

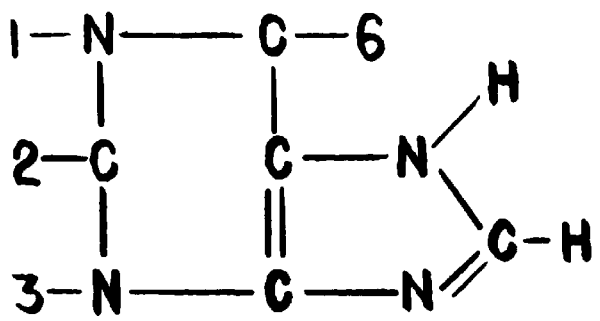
Replacement of the 8 hydrogen atom of caffeine by a chlorine atom to form 8-chlorocaffeine (Table 6) greatly decreases the intensity of cardiac stimulation as well as the duration of this action. The per cent increase in C.O. observed with this drug may be due entirely to the nicotinamide employed as a solvent (Table 22). My impression is that 8-chlorocaffeine is actually a cardiac depressant since it reverses the direction of delta C.I. and the per cent increase in C.O. as well as the change in C.I. is less for the combination of 8-chlorocaffeine plus nicotinamide than for nicotinamide alone.

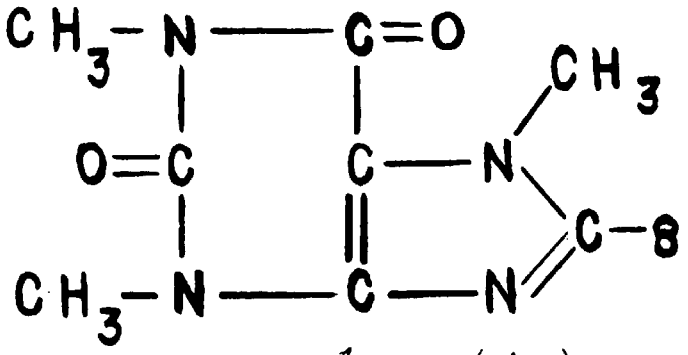
Table 7 presents the results obtained with the substitution of other groups in the 8 position of the purine nucleus. A comparison of theophylline and 8-chlorotheophylline points out the marked decrease in activity obtained upon replacement of the 8 hydrogen atom of theophylline by a chlorine atom. The residual activity observed in the dog heart-lung preparation with 8-chlorotheophylline appears to be due almost, if not entirely, to the positive chronotropic activity of the molecule. This is supported by the results presented in Table 1. 8-Bromotheophylline has even less activity than the 8-chloro derivative*. The difference observed between these two compounds results from the complete abolition of positive chronotropic activity in the molecule even though the 8-bromo-

*All efforts to synthesize 8-Fluorotheophylline have been unsuccessful (28). When this compound becomes available for testing, a firmer basis for speculation on the role of the chlorine atom in modifying positive chronotropic activity will be possible.

Table 6

Substitutions in Position 6 and 8 of the Purine Nucleus





DRUG	DOSE MGM./L.	SUBSTITUTION	% INCREASE IN C.O.	TOTAL ΔC.I.	% INCREASE IN H.R.	(min.) DURATION OF ΔC.I.	NO. OF EXP.
		#1 #2 #3 #6	POSITION 6				
THEOPHYLLINE	163	CH_3 O CH_3 O	62	0.9	20	58	7
6-DESOXYTHEOPHYLLINE	135	CH_3 O CH_3 H_2	23	0.8	17	41	3
HYPOXANTHINE	113	H H - O	20	0.2	0	9	2
ADENINE SULFATE	336	- H - NH_2	27	0.8	0	42	3
		#8	POSITION 8				
CAFFEINE	161	H	43	0.8	0	>40	4
8-CHLOROCAFFEINE	190	Cl	2	-0.1	0	0	2

derivative has a slightly greater positive inotropic action (Table 1).

A comparison of theophylline and 1,3-dimethyl uric acid presents a vivid illustration of the importance of the 8 position in determining cardiac activity. Replacement of the 8 hydrogen atom of theophylline by an oxygen atom completely eliminates positive chronotropic activity as well as inhibiting positive inotropic activity. In view of these marked changes it is rather surprising that 1,3-dimethyluric acid can still appreciably alter the competence index. When 1,3-dimethyluric acid is compared with uric acid, it becomes evident that the 1 and 3 methyl groups are important determinants of the duration of drug action. A comparison of theophylline and xanthine versus theophylline and 1,3-dimethyluric acid suggests that alteration of the basic theophylline molecule in the 8 position with an oxygen atom produces a greater decrease in positive inotropic action than removal of the 1 and 3 methyl groups.

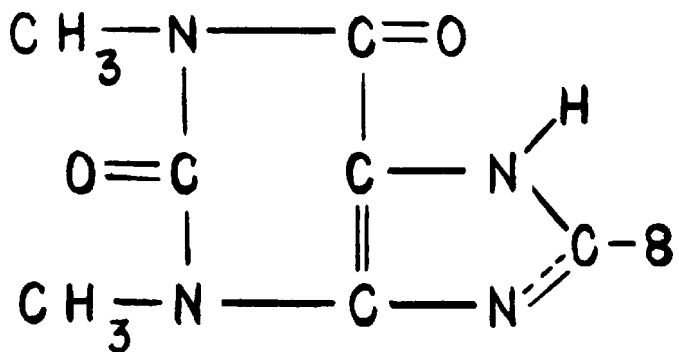
In summary alteration of the basic theophylline or caffeine structure in position 8 profoundly decreases the cardiac activity of the molecule. The positive inotropic and positive chronotropic activity, as well as the duration of drug action, may be greatly reduced or completely eliminated by these alterations.

Position 9

Several substitutions in the 9 position of the purine nucleus were also investigated. A comparison of adenosine and adenine sulfate indicates that the addition of a ribose molecule to the 9 position of the purine nucleus almost completely eliminates the cardiac activity inherent in the adenine molecule (Table 8). In the same manner the presence of a methyl group in the 9 position of the theophylline molecule to form iso-caffeine greatly reduces the overall activity of

Table 7

Substitutions in Position 8 of the Purine Nucleus

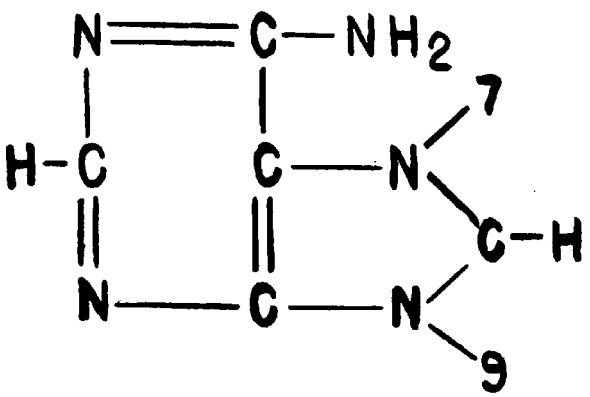


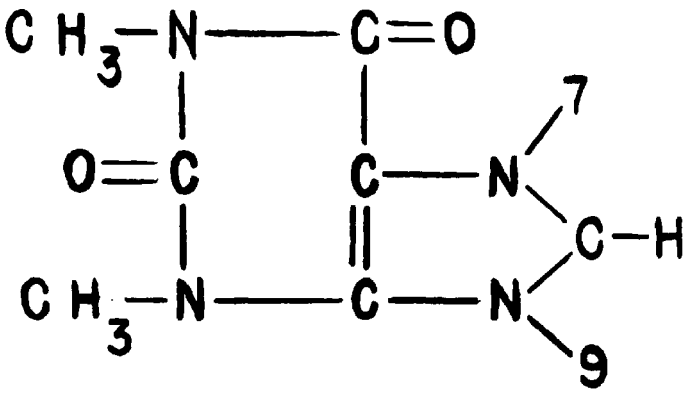
DRUG	DOSE MGM./L.	SUBSTITUTION	% INCREASE IN C.O.	TOTAL Δ C.I.	% INCREASE IN H.R.	(min.) DURATION OF Δ C.I.	NO. OF EXP.
THEOPHYLLINE	165	H	62	0.9	20	58	7
8-CHLOROTHEOPHYLLINE	193	Cl	18	0.07	12	0	3
8-BROMOTHEOPHYLLINE	231	Br	12	0.07	0	0	3
1,3-DIMETHYLURIC ACID	163	O	0	0.1	0	17	2
*XANTHINE	139	H	10	0.6	0	>23	2
*URIC ACID	154	O	0	0.05	0	0	2

* H replaces CH_3 in positions 1 and 3

Table 8

Substitutions in Position 9 of the Purine Nucleus





DRUG	DOSE MGM./L.	SUBSTITUTION		% INCREASE IN C.O.	TOTAL Δ C.I.	% INCREASE IN H.R.	(min.) DURATION OF Δ C.I.	NO. OF EXP.
		#7	#9					
ADENOSINE	222	-	RIBOSE	0	0.1	0	10	3
ADENINE SULFATE	336	H	--	27	0.8	0	42	3
THEOPHYLLINE	165	H	--	62	0.9	20	58	7
CAFFEINE	161	CH_3	--	43	0.8	0	>40	4
ISOCAFFEINE	161	-	CH_3	49	0.0	0	0	2

the molecule. The percent increase in cardiac output produced by isocaffeine is undoubtedly the result of an intense positive inotropic action of very brief duration. A comparison of caffeine and isocaffeine points up the fact that a methyl substitution in either the 7 or 9 position of theophylline reduces the positive inotropic activity of the molecule as well as the duration of this action and completely eliminates the positive chronotropic effect. The 9 methyl substitution has a lesser effect upon reducing the intensity of the positive inotropic action but a greater effect on reducing the duration of this activity than the 7 methyl substitution.

In summary the addition of a ribose molecule to the 9 position of adenine, forming adenosine, greatly reduces positive inotropic activity. A similar change is observed when a methyl group is added to the 9 position of the theophylline molecule.

B. Pyrimidine Derivatives

The data thus far strongly suggests that alterations confined to the pyrimidine portion of the theophylline molecule profoundly alter its cardiac activity. When the 1,3-dimethyl grouping of theophylline is replaced by hydrogen atoms to form xanthine, there is a marked reduction in positive inotropic activity, a decrease in duration of drug action, and a complete loss of positive chronotropic activity (Table 3). If the 6 oxygen group of theophylline is replaced by two hydrogen atoms to form desoxytheophylline, there is a significant reduction in positive inotropic activity with no apparent significant change in positive chronotropic activity or duration of drug action (Table 6). On the basis of these and other similar observations I decided to investigate several available pyrimidine derivatives in

order to determine whether or not they possessed cardiac activity.

Three pyrimidine derivatives were examined by the prescribed method and the results are presented in Table 9. Uracil appeared to be completely inactive. The 4-carboxylic acid derivative of uracil (orotic acid) as well as 1,3-dimethyl 5,6-diaminouracil produced a slight increase in cardiac output in the absence of any positive chronotropic activity. The absolute values of 6 and 7 per cent, however, are probably within the limits of error for the method of measurement. It is possible, however, that a larger series of experiments may demonstrate that these compounds possess a significant positive inotropic action.

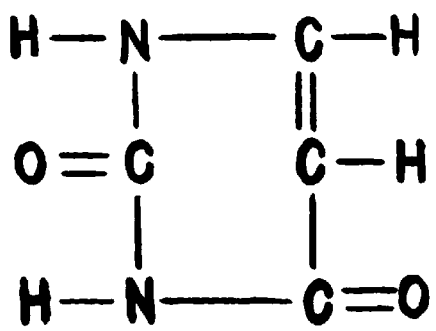
C. Imidazole Structure

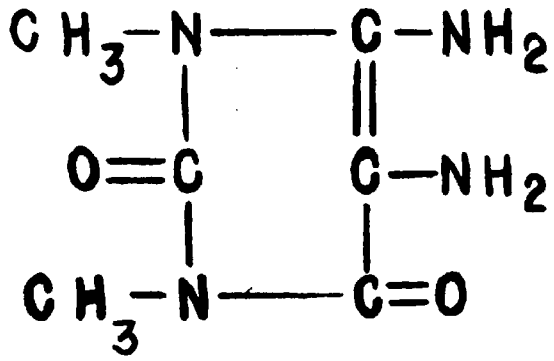
In the same manner that alterations confined to the pyrimidine ring of theophylline can alter cardiac activity, so can alterations confined to the imidazole ring of this molecule. Substitutions on the imidazole ring of the theophylline molecule appear especially important in determining positive chronotropic activity. All the theophylline derivatives with substitutions on the imidazole ring lacked positive chronotropic activity except 8-chlorotheophylline and 7-chloroethyl-theophylline. Evidently the presence of the chlorine atom in some way promotes positive chronotropic activity. The cardiac activity of the imidazole structure was then examined as a separate entity.

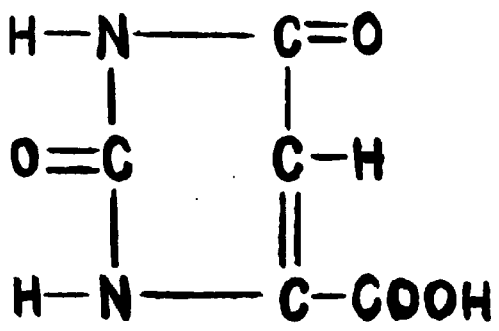
The drug imidazole was evaluated at two different concentrations in the heart-lung preparation and several experiments were also run on the hypodynamic cat papillary muscle. The doses utilized were equivalent to 165 and 330 mgm. of theophylline monohydrate. The results obtained from the heart-lung preparation indicated that the drug had no cardiac stimulating properties (Table 10). The results of the

Table 9

Pyrimidine Derivatives



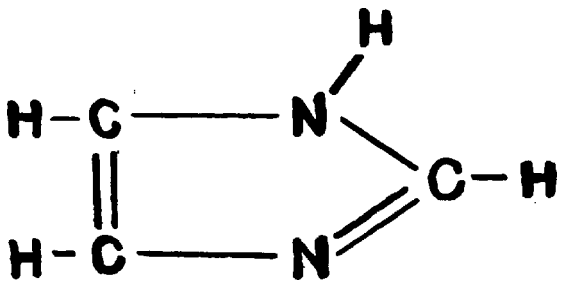


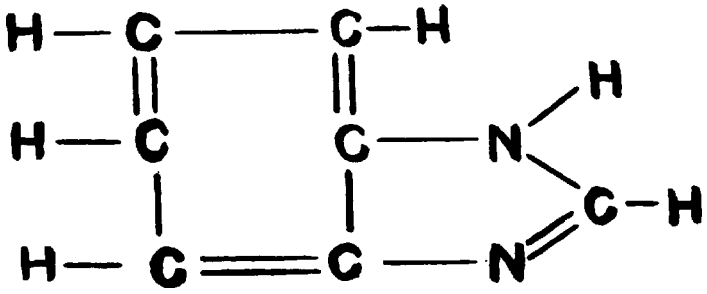


DRUG	DOSE MGM./L.	% INCREASE IN C.O.	TOTAL △C.I.	% INCREASE IN H.R.	(min.) DURATION OF △C.I.	NO. OF EXP.
A. URACIL	93	0	0.0	0	0	1
B. 1,3-DIMETHYL 5,6-DIAMINO URACIL	142	7	0.0	0	0	2
C. OROTIC ACID	130	6	0.0	0	0	2

Table 10

Imidazole Structure





DRUG	DOSE MGM./L.	% INCREASE IN C.O.	TOTAL ΔC.I.	% INCREASE IN H.R.	(min.) DURATION OF ΔC.I.	NO. OF EXP.
IMIDAZOLE	57	0	0.0	0	0	2
A. IMIDAZOLE	113	0	0.0	0	0	1
B. BENZIMIDAZOLE	98	1	0.1	0	10	2

papillary muscle experiments suggested a very slight positive inotropic action which was within the limits of error for the method. Since the imidazole portion of the purine nucleus has been shown to play an important role in determining cardiac activity in the purine derivatives studied, it was decided to evaluate this structure in combination with some six membered ring other than a pyrimidine. Benzimidazole offered a convenient combination of these properties, and it was tested at a dose equivalent to 165 mgm. of theophylline monohydrate. The results obtained suggest that the drug has a very slight positive inotropic action (Table 10).

D. Summary of Section I

The purpose of this summary is to present the broadest generalizations possible concerning the relationship of structural modifications of the purine nucleus to positive inotropic action, positive chronotropic action, and the duration of drug action. A detailed interpretation of the significance of these generalizations is contained in the discussion.

The purine nucleus may be visualized as consisting of a pyrimidine and imidazole ring. Neither the pyrimidine derivatives examined (uracil, orotic acid, and 1,3-dimethyl 5,6-diaminouracil) nor imidazole exhibited significant ($P > 0.05$) positive inotropic or positive chronotropic activity. Apparently these component parts must be joined to form the purine nucleus before highly active compounds can be obtained.

Theophylline is generally considered to be the purine derivative possessing the greatest cardiac activity. For this reason I compared theophylline with several pyrimidine and imidazole substituted purine derivatives in order to determine whether or not there was a significant

difference in a specific cardiac action (positive chronotropic activity, duration of drug action) between random substitutions on the two constituent rings of the theophylline molecule. Each group of substituted derivatives differed from theophylline in having substitutions on either the pyrimidine or imidazole ring only. A drug such as theobromine or 1,3,7-triethylxanthine could not be included in either group since it differs from theophylline in having substitutions on both the pyrimidine and imidazole rings.

The following compounds were considered to be pyrimidine substituted theophylline derivatives: xanthine, hypoxanthine, adenine sulfate, 2,6-diaminopurine sulfate, and desoxytheophylline.

The following compounds were considered to be imidazole substituted theophylline derivatives: caffeine, 7-ethyltheophylline, 7-chloroethyltheophylline, 7-diethylaminoethyltheophylline, 8-chlorocaffeine, 8-chlorotheophylline, 8-bromotheophylline, and 1,3-dimethyluric acid.

Other general comparisons between the position and character of the various substitutions are also reported.

1. Positive Chronotropic Activity

a. There is no statistically significant difference ($P > 0.5$) between the positive chronotropic activity of the imidazole and pyrimidine substituted theophylline derivatives studied when treated as separate groups.

b. There is a statistically significant difference ($P < 0.01$) between the positive chronotropic activity of theophylline and the pyrimidine substituted theophylline derivatives treated as a group, excluding desoxytheophylline.

c. There is a statistically significant difference ($P < 0.01$) between the positive chronotropic activity of theophylline and the imidazole substituted theophylline derivatives treated as a group, excluding 8-chlorotheophylline and 7-chloroethyltheophylline.

2. Duration of Drug Action (Duration Δ C.I.)

a. There is a statistically significant difference ($P < 0.02$) between the duration of action of theophylline and the pyrimidine substituted theophylline derivatives treated as a group.

b. There is a statistically significant difference ($P < 0.01$) between the duration of action of theophylline and the imidazole substituted theophylline derivatives treated as a group.

c. There is no statistically significant difference ($P > 0.3$) between the duration of action of the pyrimidine and imidazole substituted theophylline derivatives when treated as separate groups.

3. Positive Inotropic Action

Only those purine derivatives lacking positive chronotropic activity could be compared for their positive inotropic action in the dog heart-lung preparation. The reason for this requirement is discussed in the section on experimental methods.

Several of the drugs possessing positive inotropic activity as well as some with positive chronotropic and inotropic activity were evaluated with the hypodynamic cat papillary muscle preparation. The small number of experiments generally did not permit adequate statistical treatment of the results. A subjective interpretation of the data is contained in the discussion.

Desoxytheophylline was excluded from the pyrimidine substituted theophylline derivatives in the following statistical evaluation.

8-Chlorotheophylline and 7-chloroethyltheophylline were excluded from the imidazole substituted theophylline derivatives since they also possess definite positive chronotropic activity.

a. There is no statistically significant difference ($P > 0.4$) between the positive inotropic activity of the imidazole and pyrimidine substituted theophylline derivatives lacking positive chronotropic activity as determined in the dog heart-lung preparation.

b. Theophylline has a statistically significant ($P < 0.05$) greater positive inotropic activity than xanthine, 8-bromotheophylline, and 8-chlorotheophylline at a concentration of 8.3×10^{-4} M as determined in the cat papillary muscle preparation.

c. No qualitative correlation can be established between the presence of the essential structure for chelation and positive inotropic activity in the series of purine derivatives studied. This conclusion is based upon the observations that desoxytheophylline, caffeine, theobromine, and other compounds lacking the configuration essential to chelation exhibited considerable positive inotropic activity.

II. The Cardiovascular Pharmacology of Ethylenediamine

A literature survey failed to reveal any adequate studies on the cardiovascular pharmacology of ethylenediamine. Howarth in 1947 reported on the results obtained when ethylenediamine (100 mgm.) was administered to several normal patients and to patients in heart failure (18). She concluded that ethylenediamine did not have any appreciable effect on the normal or failed heart; however, figure 5 on page 130 of her article indicates that ethylenediamine did produce a slight increase in cardiac output. Her results suggested the possibility that higher doses of ethylenediamine might exhibit a definite positive inotropic activity.

A. The Positive Inotropic Action of Ethylenediamine

Ethylenediamine (10^{-3} M) was examined on the hypodynamic cat papillary muscle and the results are listed in Table 11. The increase in the amplitude of contraction observed (positive inotropic action) was statistically significant at the 5 per cent level in this small series of experiments.

I decided to test this drug in the dog heart lung preparation where the factors responsible for changes in cardiac output can be evaluated individually. The results of this study are recorded in Table 12 Two doses of the drug were employed, utilizing both the base and the dihydrochloride salt. At the lower dose (66 mgm.) of the dihydrochloride salt (equivalent to 30 mgm. of the base) a positive inotropic action was observed in two of seven experiments. At the higher dose level (100 mgm. of the base or 218 mgm. of the dihydrochloride salt) a positive inotropic response was obtained in every experiment. In four out of the five experiments the inotropic response was of suffi-

Table 11

The Action of Ethylenediamine (10^{-3} Molar) on the Hypodynamic Cat Papillary Muscle

EXP. NO.	MAXIMAL OBSERVED CONTRACTION (%)	CONTRACTION (%) AT FAILURE	CONTRACTION (%) AFTER DRUG	% DIFFERENCE
1	100	48	60	12
2	100	31	70	39
3	100	76	95	24
4	100	51	51	0
5	100	55	96	41
6	100	64	64	0
7	100	47	66	19

$\bar{x} = 19$

$P < 0.05$

Table 12

The Effect of Ethylenediamine on the Failed Dog Heart-Lung Preparation

EXP. NO.	DOSE (mgm./l.)	CONTROL C.O.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.	DURATION (min.)
15	30	546	120	0.9	380	120	0.1	485	120	-	10
21	30	440	120	0.9	291	124	0.1	250	124	0.1	0
22	30	500	148	0.9	325	132	0.1	400	132	0.5	30
23	30	508	144	0.9	250	144	0.1	250	144	0.1	0
24	30	527	140	0.9	435	144	0.0	395	144	0.0	0
25	30	652	132	0.8	460	132	0.0	395	132	0.0	0
26	30	536	148	0.9	295	148	0.0	250	148	0.0	0
27	100	732	142	0.5	333	126	0.1	750	126	0.8	120
28	100	625	140	0.9	357	136	0.1	730	136	0.9	45
29	100	938	150	0.9	323	120	0.2	652	120	0.9	20
30	100	938	152	0.9	517	138	0.4	938	138	1.0	20
32	100	517	136	0.9	385	136	0.1	577	136	0.9	108

The doses are expressed as ethylenediamine base.

cient magnitude to increase the cardiac output from the failure level to a point where it equaled or exceeded the control value. Figure 1 illustrates a typical experiment. The increase in cardiac output was due to an increased stroke volume because there was no change in heart rate after the drug.

B. The Additive Effect of the Ethylenediamine Component of Aminophylline on the Duration of Reversal of Cardiac Failure in the Dog Heart-Lung Preparation

Once the positive inotropic action of ethylenediamine had been established, its practical implications were considered. This drug has been combined with theophylline in the U.S.P. preparation aminophylline, which has enjoyed considerable popularity in the treatment of angina pectoris and acute congestive heart failure. The reason for the apparent superiority of aminophylline over other theophylline preparations has never been clearly defined. The general opinion has been that ethylenediamine is present in aminophylline solely for its ability to make the theophylline more soluble. In view of the observed positive inotropic action of ethylenediamine it was decided to investigate the role of the component parts of aminophylline in reversing failure in the dog heart-lung preparation. This was done by measuring the duration of reversal of failure produced by a given dose of aminophylline and then evaluating the component parts of this drug individually under the same conditions. The procedure employed with the heart-lung preparation has been described in detail in the section on experimental methods.

Drugs:

Group I. Aminophylline (Merck) was tested at a dose level of 200

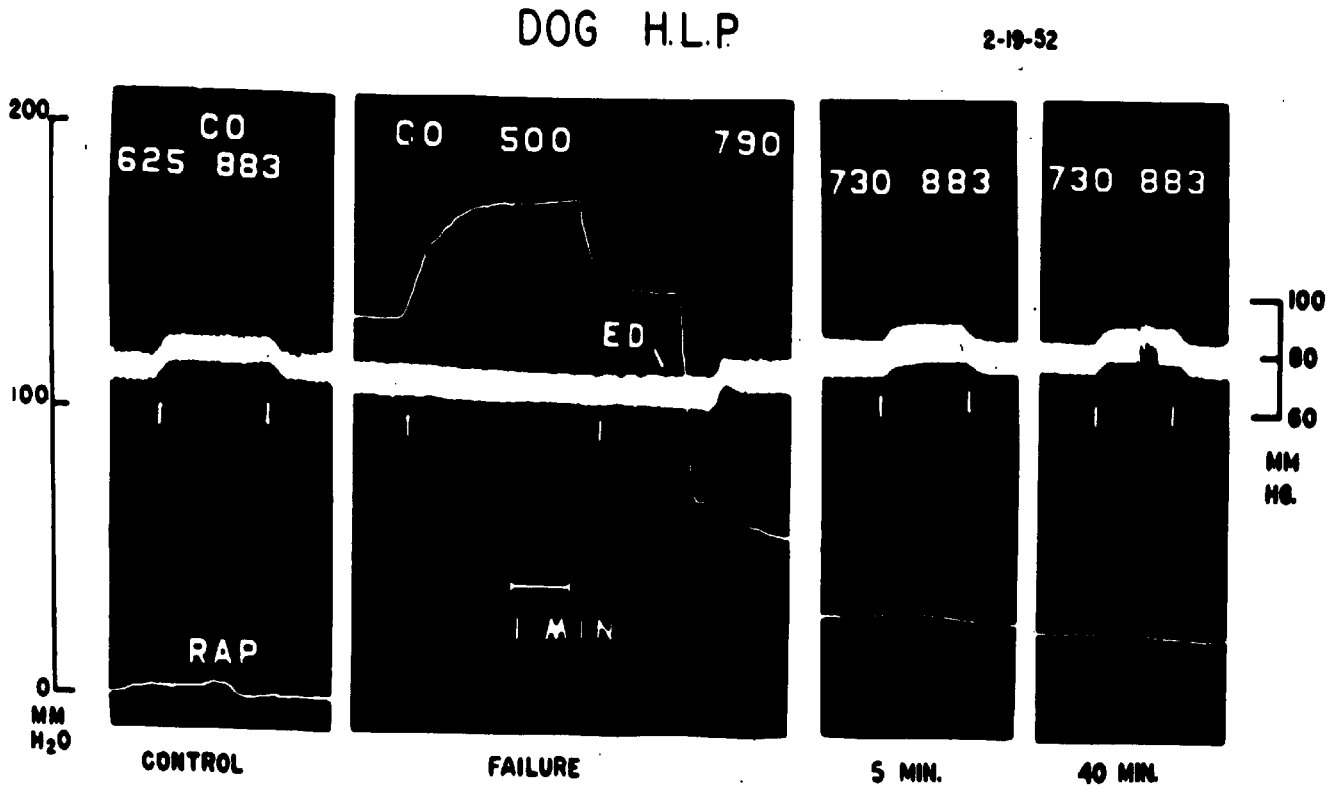


FIG. 1. From above downward: cardiac output in ml./min., arterial pressure in mm. of Hg, and right auricular pressure in mm. of H_2O. The temperature of the circulating blood was maintained at 38°C., and the heart rate did not vary throughout the course of the experiment. Ethylenediamine base was administered at "E.D." (100 mgm./l.), while the heart was in a state of failure (C.I. = 0.1). The upward shift of the arterial pressure record between frames 2 and 3 is artefact. The arrows below the blood pressure record indicate raising and lowering of the venous reservoir 5 cm.

Fig. 1. The action of ethylenediamine on the failed dog heart-lung preparation.

mgm. per liter of circulating blood. According to the U.S.P. XIV this dose of aminophylline contains 78 - 82 per cent (150 - 164 mgm.) of anhydrous theophylline. It also contains 12.3 - 13.8 per cent (24.6 - 27.6 mgm.) of ethylenediamine base. The drug was injected slowly into the venous return cannula in 20 ml. of distilled water warmed to 37 degrees C.

Group II. Theophylline monohydrate was tested at a dose of 165 mgm. per liter of circulating blood. The dose chosen is the molar equivalent of that required as the minimum concentration of anhydrous theophylline (150 mgm.) present in 200 mgm. of aminophylline according to the U.S.P. XIV. This drug was also injected slowly into the venous return cannula in 20 ml. of distilled water warmed to 37 degrees C.

Group III. The dose of ethylenediamine employed was slightly in excess of that present in a 200 mgm. dose of aminophylline according to the U.S.P. XIV. The upper limit allowable is 27.6 mgm. A dose of the dihydrochloride salt equivalent on a molar basis to 30 mgm. of the ethylenediamine base was used. This amounted to 66 mgm. of ethylenediamine dihydrochloride administered in a volume of 10 ml. of warm distilled water. When no response to this drug was observed, a subsequent dose of theophylline monohydrate (165 mgm.) was administered ten minutes later.

Results:

Group I. Aminophylline, 200 mgm. per liter of blood, exhibited an average duration of action of 94.6 minutes in a series of six experiments (Table 13)

Group II. Theophylline monohydrate, 165 mgm. per liter of blood, exhibited an average duration of action of 57.7 minutes in a series of

Table 13

The Effect of Aminophylline, Theophylline, and Ethylenediamine plus Theophylline on the Duration of Reversal of Cardiac Failure in the Dog Heart-Lung Preparation

AMINOPHYLLINE

EXP. NO.	DOSE (mgm./l.)	Control C.O.	Control H.R.	Control C.I.	Failure C.O.	Failure H.R.	Failure C.I.	After Drug C.O.	After Drug H.R.	After Drug C.I.	DURATION (min.)
5	200	430	148	0.7	250	148	0.1	500	200	1.0	90
8	200	365	136	1.0	308	140	0.1	350	200	1.0	120
9	200	430	132	0.9	250	136	0.1	430	222	1.0	80
10	200	477	132	1.0	250	120	0.0	577	184	1.0	83
18	200	447	132	0.9	300	132	0.0	470	180	1.0	105
19	200	600	128	0.7	385	132	0.0	652	188	1.0	90
										Avg.	94.6

THEOPHYLLINE (MONOHYDRATE)

6	165	470	144	1.0	250	144	0.0	492	144	1.0	56
7	165	485	160	1.0	316	160	0.2	357	184	1.0	63
12	165	440	164	0.9	308	152	0.1	588	200	1.0	40
13	165	500	168	1.0	308	164	0.2	517	212	1.0	75
14	165	500	140	1.0	250	140	0.1	500	168	1.0	90
16	165	770	136	0.9	477	136	0.0	750	160	1.0	45
20	165	566	120	0.7	308	120	0.0	455	152	0.9	35
										Avg.	57.7

Table 13
(cont.)

The Effect of Aminophylline, Theophylline, and Ethylenediamine plus Theophylline on the Duration of Reversal of Cardiac Failure in the Dog Heart-Lung Preparation

ETHYLENEDIAMINE DIHYDROCHLORIDE & THEOPHYLLINE (MONOHYDRATE)

EXP. NO.	DOSE (mgm./l.)	Control C.O.	Control H.R.	Control C.I.	Failure C.O.	Failure H.R.	Failure C.I.	After Drug C.O.	After Drug H.R.	After Drug C.I.	DURATION (min.)
21	66, 165	440	120	0.9	291	124	0.1	463	160	1.0	138
23	66, 165	508	144	0.9	250	144	0.1	380	164	1.0	107
24	66, 165	527	140	0.9	435	144	0.0	546	160	1.0	85
25	66, 165	652	132	0.8	460	132	0.0	536	156	1.0	113
26	66, 165	536	148	0.9	295	148	0.0	526	160	1.0	125
15	*30, 165	546	120	0.9	357	120	0.1	652	164	1.0	105
										Avg.	112.1

ETHYLENEDIAMINE DIHYDROCHLORIDE

22	66	500	148	0.9	325	132	0.1	400	132	0.5	30

ETHYLENEDIAMINE BASE

15	30	546	120	0.9	380	120	0.1	485	120	-	10

*In this case ethylenediamine base was substituted for the dihydrochloride salt.

seven experiments (Table 13). This response was significantly less than that observed with aminophylline (Table 14)

Group III. In a series of six experiments following prior administration of ethylenediamine dihydrochloride (66 mgm.), the average duration of action of theophylline as measured from the time of administration of the theophylline monohydrate was 112.1 minutes (Table 13). This response was not significantly different from that observed with aminophylline, however, it was significantly greater than that observed with theophylline monohydrate alone (Table 14). In two experiments an immediate response to the ethylenediamine was observed. The base (30 mgm.) was employed in one case and the dihydrochloride salt (66 mgm.) in the other. The response was manifested in the form of an increased C.O. and C.I. with a concurrent decrease in R.A.P. (See experiments 15 and 22 in Table 13). In the case of experiment 15 the action of the ethylenediamine was very transient and the C.I. returned to the failure level of 0.1 within ten minutes. A new series of failure readings was taken and 165 mgm. of theophylline monohydrate was administered. This experiment was then included in the Group III series.

The data presented indicate that it is the positive inotropic action of the ethylenediamine component of theophylline ethylenediamine that is responsible for the significant difference between the duration of action of theophylline ethylenediamine (aminophylline) and theophylline. Ethylenediamine can no longer be considered as a pharmacologically inactive solubilizing agent when one considers the cardiovascular action of aminophylline.

C. The Role of Ethylenediamine in the Aminophylline Induced Depressor Response in the Intact Animal

Table 14

Statistical Evaluation of Aminophylline
Versus Theophylline and Theophylline Ethylenediamine

			Significance
Aminophylline 200 mg. (anhydrous theophylline 165.9 mg. ethylenediamine base 26.7 mg.)	vs.	Theophylline monohydrate 165 mg.	$P < 0.01$
Theophylline monohydrate 165.0 mg. after Ethylenediamine·2HCl 66.0 mg.	vs.	Theophylline monohydrate 165 mg.	$P < 0.01$
Aminophylline 200 mg.	vs.	Theophylline monohydrate 165 mg. after Ethylenediamine·2HCl 66 mg.	$P > 0.05$

The doses of theophylline monohydrate and ethylenediamine dihydrochloride reported in the above table are molar equivalents of anhydrous theophylline (150 mg.) and ethylenediamine base (30 mg.). The aminophylline was assayed using the method described in U.S.P. XIV. "P" is calculated according to Snedecor (30).

The hypotensive response resulting from rapid intravenous injections of aminophylline is well known, however, the role of ethylenediamine in this response has never been determined. I investigated the possibility that the ethylenediamine content of aminophylline might contribute to this depressor response.

Table 15 contains the results of several typical experiments. Experiment A demonstrates that the depressor response resulting from aminophylline (100, 200 mgm.) is not entirely dependent upon the theophylline content of this drug. Experiments B and C indicate that at least fifty per cent of the observed depressor response is due to the ethylenediamine content of these doses of aminophylline.

Desgrez and Dorleans (7) were the first to report that intravenous ethylenediamine hydrochloride produced a transitory fall of blood pressure in the anesthetized rabbit, cat, and dog. Barbour and Hjort (2) confirmed the above results and extended their study in order to locate the site of action of this drug. Their purpose was to establish whether the depressor action was central or peripheral. This was done by determining the action of ethylenediamine after elimination of the vasomotor center of rabbits and cats by pithing as well as determining the action of the drug in nicotinized rabbits, cats, and dogs. They were unable to obtain a depressor response from ethylenediamine after the above procedures and concluded that the central nervous system played at least a large part in the blood pressure reducing effect of this drug. The authors make note of the fact that the pithing type experiment is unsatisfactory because of the marked circulatory depression produced by the procedure itself.

It was decided to investigate the site of action of ethylene-

Table 15

The Depressor Response of Intravenous Aminophylline and its Constituents in the Anesthetized Dog

A. 6-3-53 15.6 Kg. male

Anesthesia: Morphine Sulfate (75 mgm.) S.C., Chloralose (900 mgm.)I.P.

TIME		DOSE	DEPRESSOR RESPONSE (mm. Hg.)
1530	Aminophylline	100 mgm.*	42
1545	Theophylline Monohydrate	86 mgm.*	18
1550	Saline Control	10 ml.	None
1558	Aminophylline	100 mgm.	42
1624	Theophylline Monohydrate	172 mgm.	28
1629	Aminophylline	200 mgm.	70
1633	Saline Control	20 ml.	None

B. 6-4-53 7.4 Kg. female

Anesthesia: Sodium Pentobarbital 35 mgm./Kg.

1107	Aminophylline	200 mgm.	70
1120	Theophylline Monohydrate	172 mgm.	44
1137	Ethylenediamine Dihydrochloride	58 mgm.	46

C. 10-15-53 14.4 Kg. female

Anesthesia: Sodium Pentobarbital 35 mgm./Kg.

1612	Ethylenediamine Dihydrochloride	58 mgm.	52
1625	Aminophylline	200 mgm.	96
1655	Theophylline Monohydrate	172 mgm.	40
1710	Bilateral Cervical Vagotomy		
1725	Ethylenediamine Dihydrochloride	58 mgm.	50
1736	Aminophylline	200 mgm.	74
1758	Theophylline Monohydrate	172 mgm.	40

* All drugs injected rapidly into a femoral vein in a volume of 20 ml. of saline except these two drugs which were injected in a 10 ml. volume.

diamine dihydrochloride further, giving special attention to the carotid chemoreceptors described by Heymans et al. (16), the aortic chemoreceptors described by Comroe and others(5,16), and the myocardial chemoreceptors described by Dawes (6). These possible sites of action had not been eliminated by previous workers. Initially the work of Barbour and Hjort (2) was reinvestigated in some phases and then expanded in order to localize the site of drug action more precisely.

1. The Site of the Hypotensive Action of Ethylenediamine

Ethylenediamine dihydrochloride will produce a definite depressor response in anesthetized dogs with doses as low as 0.25 mgm./Kg. The threshold dose varies from animal to animal but generally a response can be obtained with 0.5 mgm./Kg.; however, larger doses produce a sharper response and were often employed for this purpose.

Figure 2 illustrated a typical depressor response to several doses of the drug before and after vagotomy. Whereas bilateral cervical vagotomy may often reduce the depressor response considerably, it generally does not eliminate it. The rapid onset and short duration of action of the fall in blood pressure suggested the involvement of some reflex mechanism.

In view of the observation that only part of the depressor response could be blocked by vagotomy, it was decided to test the effect of a ganglionic blocking agent upon the ethylenediamine-induced depressor response. Tetraethylammonium bromide (5 mgm./Kg.) was administered intravenously to an anesthetized dog (pentobarbital 35 mgm./Kg.) prior to ethylenediamine (5 mgm./Kg.) and a depressor response similar to the ethylenediamine control response, but quantitatively less, was observed. The ganglionic blockade produced by the above dose of TEA was not

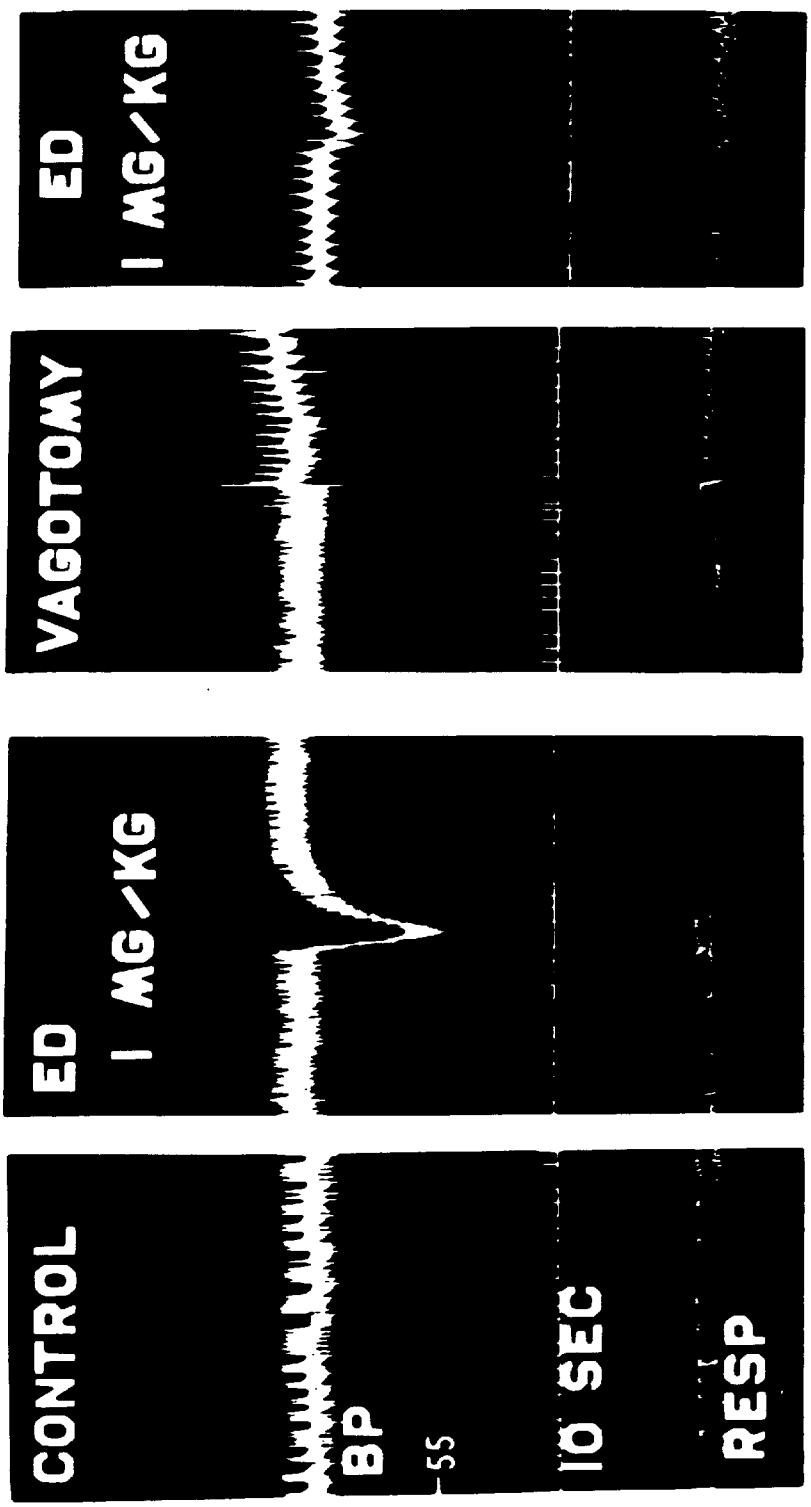


Fig. 2. The ethylenediamine depressor response in the anesthetized dog before and after bilateral cervical vagotomy.

completely satisfactory due to its short duration of action, therefore, the longer acting hexamethonium bromide was employed in subsequent experiments. A typical experiment of this type is recorded in Table 16. The results illustrate that ganglionic blockade can greatly reduce or abolish the depressor response to suprathreshold doses of ethylenediamine.

In view of these observations a study of the effect of this drug on the nictitating membrane was made in order to determine whether or not ethylenediamine possessed a transient ganglionic blocking action. The results indicated that no such activity resulted from doses of 5 - 50 mgm./Kg., however, 100 mgm./Kg. produced a definite but slight blocking action. Such high doses of the drug required for activity excludes ganglionic blockade as a mechanism for the depressor response from 5 mgm./Kg. of intravenous ethylenediamine dihydrochloride in the dog.

Direct cardiac depression was ruled out as a possible source of the hypotensive response in accordance with the observed positive inotropic action of ethylenediamine in the dog heart-lung preparation and isolated cat papillary muscle. This interpretation was substantiated by experiments to be described subsequently.

It was observed that the threshold dose of ethylenediamine required to produce the typical depressor response upon injection into the superior vena cava was always ineffective when injected into the descending aorta. A typical experiment demonstrating this fact is recorded in Table 17. These results indicate that the area most sensitive to the action of ethylenediamine is located either in the heart, lungs, or cerebral circulation.

Table 16

Blockade of Ethylenediamine Depressor Response
by Hexamethonium Bromide

5-26-53 9.0 Kg. Male Dog

Anesthesia: Sodium Pentobarbital 35 mgm./Kg. I.V.

All injections made into the left femoral vein.

TIME	(mm. of Hg) BLOOD PRESSURE	HEART RATE PER MINUTE
		128
1130	130	128
1134	Ethylenediamine Dihydrochloride 5 mgm./Kg.	
1134	46	96
1136	130	128
1140	Hexamethonium Bromide 2 mgm./Kg.	
1145	70	96
1146	Ethylenediamine Dihydrochloride 5 mgm./Kg.	
1146	62	88
1217	74	98
1240	90	100
1345	84	108
1505	94	104
1508	Ethylenediamine Dihydrochloride 5 mgm./Kg.	
1508	52	88
1510	86	92
1512	Bilateral Cervical Vagotomy	
1513	94	104
1534	Ethylenediamine Dihydrochloride 5 mgm./Kg.	
1534	64	100
1536	88	100
1548	Hexamethonium Bromide 5 mgm./Kg.	
1549	68	100
1553	58	96
1556	Ethylenediamine Dihydrochloride 5 mgm./Kg.	
1556	58	96
1558	58	96

Table 17

Localization of the Ethylenediamine Depressor Response

6-2-53 8.4 Kg. Male Dog

Anesthesia: Sodium Thiopental 25 mgm./Kg. I.V.
Sodium Barbital 250 mgm./Kg. I.P.

TIME	(mm. of Hg) BLOOD PRESSURE	HEART RATE PER MINUTE
1240	108	164
1300	Ethylenediamine Dihydrochloride 0.5 mgm./Kg. (femoral vein)	
1300	58	133
1305	112	140
1330	Ethylenediamine Dihydrochloride 0.25 mgm./Kg. (superior vena cava)	
1330	90	136
1332	112	140
1345	Ethylenediamine Dihydrochloride 0.25 mgm./Kg. (descending aorta)	
1345	112	140
1400	Ethylenediamine Dihydrochloride 0.5 mgm./Kg. (descending aorta)	
1401	90	-
1415	96	140
1417	Ethylenediamine Dihydrochloride 0.5 mgm./Kg. (superior vena cava)	
1417	56	132
1420	102	132

The next step was to identify more precisely the exact area involved in the depressor response. This was accomplished by recording the time intervals from completion of injection of the drug into the different areas of the heart, lungs, and cerebral vessels to the beginning of the fall in blood pressure in open chest dogs. It could then be assumed that the point of injection having the shortest elapsed time interval before a fall in blood pressure occurred would represent the shortest route to the site of action of the drug.

The drug was injected in a volume of 0.1 ml. and the time interval to the depressor response was recorded visually with a stop watch. Blood pressure was recorded with a mercury manometer which resulted in a considerable lag in the time intervals recorded, therefore, the relative time values rather than the absolute values will be of primary interest. Table 18 contains one of several experiments which indicate that the area involved in the depressor response is cephalad to the heart and lungs. It also suggests that ethylenediamine is not acting on the aortic or carotid chemoreceptors since the depressor response can still be obtained after vagotomy or when both carotid arteries are ligated. Further experiments were performed in which the sinus nerve of the functional common carotid artery was sectioned (the other common carotid was cannulated caudal to the cranial thyreoid branch of the carotid in order to record blood pressure) and this procedure did not alter the magnitude or time interval for the depressor response to any appreciable extent. The shortened time interval was thought to be due to the increased arterial pressure resulting in an increased blood velocity and thus decreasing the circulation time. The experiment in Table 18 illustrates that the carotid arteries are not

Table 18

Further Localization of the Ethylenediamine Depressor Response

10-26-53 15.0 Kg. Male Dog

Anesthesia: Sodium Pentobarbital 30 mgm./Kg. I.V.

Drug: Ethylenediamine dihydrochloride (0.5 mgm./Kg.) administered in a volume of 0.1 ml. at fifteen minute intervals

Blood pressure recorded from left subclavian artery

AREA OF INJECTION	TIME (SECONDS) TO DEPRESSOR RESPONSE	(mm. of Hg) MAGNITUDE OF DEPRESSOR RESPONSE
Sup. Vena Cava	22.1	14
Pulmonary Artery	18.4	10
Descending Aorta	None	None
Rt. Common Carotid (artery then ligated)	6.5	16
Ascending Aorta	13.3	18
Left Ventricle	14.7	20
Left Common Carotid	6.0	16
Sup. Vena Cava	18.4	16
Left Common Carotid Ligated		
Sup. Vena Cava	16.0	16
Bilateral Cervical Vagotomy		
Sup. Vena Cava	14.2	22

essential to the depressor response. This has been confirmed by direct injection of the drug into the subclavian arteries which resulted in the typical depressor response observed after intracarotid injections.

These results suggest that the area involved in the hypotensive response to ethylenediamine dihydrochloride is located cephalad to the anatomical junction of the vertebral and internal carotid arteries. The efferent pathways from this area include vagal fibers in addition to other fibers. The vagal pathways usually carry the majority of impulses originating from this area as demonstrated by the generally reduced response to ethylenediamine after vagotomy (Figure 2). In addition to the vagal fibers other autonomic pathways must be implicated, since the ganglionic blocking agent hexamethonium bromide is capable of inhibiting the residual depressor response to large doses of ethylenediamine following bilateral cervical vagotomy (Table 16).

2. The Mechanism of the Hypotensive Action of Ethylenediamine

The fundamental question that arises from any study in which a pharmacological response is obtained upon administration of a drug is the mechanism involved in this response. In the case of the ethylenediamine depressor response the chemical properties of the drug offered a logical starting point. My original interest in this drug was based upon its ability to chelate heavy metals. It can also form a chelate complex with calcium ions in which the calcium remains in solution but is highly undissociated. The possibility that ethylenediamine was producing its depressor effect by virtue of sequestering available calcium seemed worthy of investigation since citrate is thought to produce its depressor response by a similar mechanism.

The disodium salt of ethylenediamine tetra-acetic acid

(disodium Versene) appears to share most of the pharmacological properties of its parent compound, ethylenediamine. In equimolar doses it produces somewhat less of a depressor response and the respiratory stimulation and bradycardia is less intense. A calcium chelate salt of disodium Versene was administered intravenously to an anesthetized dog in a dose equimolar to ethylenediamine dihydrochloride (5 mgm./Kg.) and it had no effect upon the blood pressure. The magnesium, manganese, cobalt, cupric, and zinc chelate salts of disodium Versene were then tested under similar conditions in doses equimolar to ethylenediamine dihydrochloride (5 mgm./Kg.) and they had no effect upon the blood pressure. These results suggest that the process of chelation is essential to the mechanism of the depressor response induced by disodium Versene and by inference to that produced by ethylenediamine dihydrochloride. In order to test this point further two compounds known for their ability to chelate heavy metals but having a structural configuration very different from ethylenediamine or disodium Versene were examined in vivo. The compounds tested were 8-hydroxyquinoline sulfate and Cupferon (the ammonium salt of N-nitrosophenyl-hydroxylamine). These drugs were found to produce a depressor response when injected into dogs in doses equimolar to the threshold dose of ethylenediamine employed (Figure 3). The time interval from completion of the injection to the depressor response with these drugs is similar to that recorded for ethylenediamine in the same animal. In addition it has been observed that a suprathreshold dose of disodium Versene, 8-hydroxyquinoline, Cupferron or ethylenediamine will effectively block or reduce the subsequent depressor response to any one of the other drugs in this series within a given time period. This blockade or partial inhibition

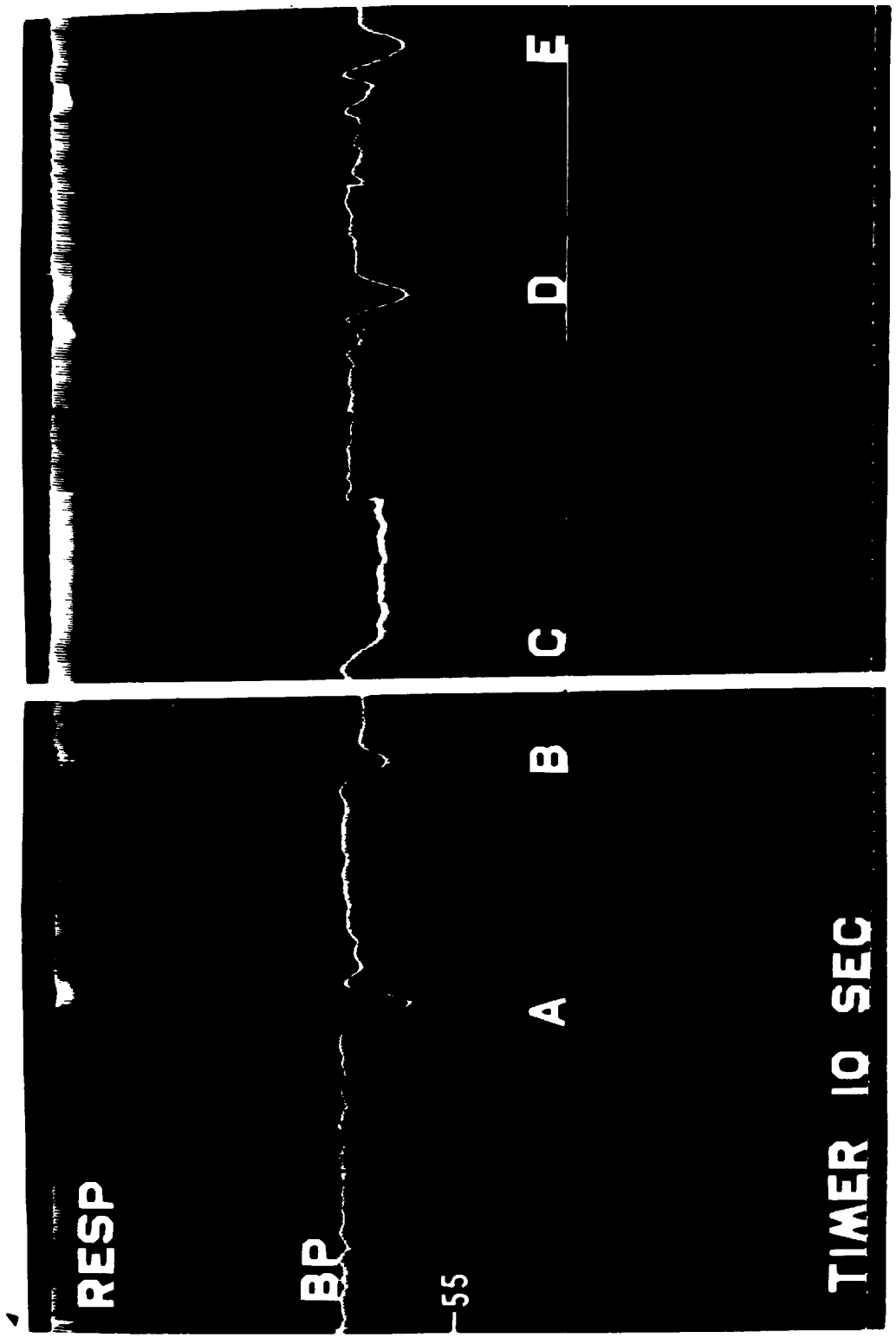


Fig. 3. A comparison of the depressor responses produced by ethylenediamine 2 mgm./Kg. (D and E) and equimolar doses of 8-hydroxyquinoline sulfate (A), disodium Versene (B), and Cupferron (C).

may last from five to ten minutes after the first drug is administered in the above series. Tachyphylaxis to the initial drug is also present during this period.

For a system containing a number of cations which can be chelated by disodium Versene, such as blood and body tissues, two factors will govern the formation of the metal-Versene-chelate structure. The cationic chelate of the greatest stability constant will form with Versene provided that its concentration in the solution is large enough to match that of possible competing ions. For biological systems in general the cation primarily affected by Versene is calcium. The other possibility is that Versene might have pharmacological activity of its own by virtue of its unnatural amino acid structure. Since the calcium chelate of disodium Versene did not produce a depressor response in doses equimolar with effective doses of the calcium free Versene, a direct action appears to be unlikely. The data obtained supports the concept that disodium Versene produces its depressor response by virtue of its chelating ability. It is assumed that ethylenediamine dihydrochloride, disodium Versene, 8-hydroxyquinoline sulfate, and Cupferron produce their depressor response by the same mechanism. Indirect evidence points to calcium as the substance being chelated. Additional work, however, is required to confirm this suspicion.

D. Summary of Section II

It has been demonstrated that ethylenediamine in the doses employed possesses marked positive inotropic activity as determined in the hypodynamic cat papillary muscle and failed dog heart-lung preparation.

The data presented establishes the positive inotropic action

of the ethylenediamine component of theophylline ethylenediamine as the factor responsible for the statistically significant difference between the duration of action of theophylline ethylenediamine (aminophylline) and theophylline alone.

The hypotensive response resulting from rapid intravenous injections of aminophylline into intact anesthetized dogs has been shown to be largely due to the ethylenediamine content of this drug. The area involved in the initiation of the hypotensive response to intravenous ethylenediamine in the dog has been located cephalad to the anatomical junction of the vertebral and internal carotid arteries. Evidence is presented which suggests that the mechanism of the ethylenediamine depressor response is dependent upon the chelating ability of this molecule.

DISCUSSION

I. The Cardiac Pharmacology of Selected Purine Derivatives as Related to their Chemical Constitution

The terms significant, definite, etc. have been used freely in the section on experimental results and will again be used in this section. These terms are to be considered as the subjective interpretation of the data by the author. They are not synonymous with statistical significance and wherever the latter interpretation is desired, it is expressed as such with an accompanying probability value.

Approximately twenty-eight compounds were evaluated for cardiac activity in the section on purine derivatives. No attempt has been made to determine the statistically significant difference between the activities of each of these drugs. Wherever possible the results have been treated as a group in order to obtain the maximal generalizations possible within the limits of statistical interpretation. The original data utilized in calculating the tables presented in section I has been included in the appendix. The interested reader may, therefore, form his own conclusions as to the relative activities of the drugs employed in this study.

Generally the number of experiments performed on any given drug is relatively small and the series should be expanded before a definitive interpretation is made. In my hands both the heart-lung and papillary muscle preparations yielded highly variable results as quantitative procedures. For this reason a larger series of experiments is essential before a final statistical evaluation of all the results is warranted. In many cases the differences between compounds is obvious and the data to support my interpretations is presented in the appendix.

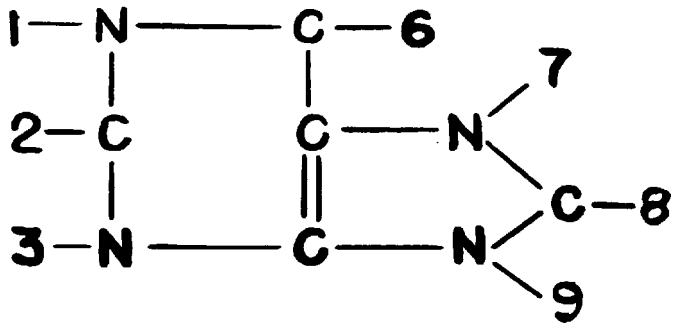
Positive Chronotropic Activity

Table 19 lists those compounds possessing positive chronotropic activity in the series of purine derivatives studied. The presence of a 1,3-dimethyl pyrimidine grouping is common to almost all structures having high chronotropic activity. It therefore seems evident that the 1,3-dimethyl grouping must contribute to this activity. A comparison of xanthine and theophylline substantiates this assumption since xanthine lacks positive chronotropic activity. The 6 position of the pyrimidine ring appears to be another key position for altering chronotropic activity. A comparison of theophylline and desoxytheophylline indicates that replacement of the 6 oxygen atom by two hydrogen atoms does not enhance the chronotropic activity of the molecule.

The imidazole portion of the purine nucleus also contributes to the chronotropic activity of the molecule. With one exception (7-chloroethyltheophylline), many additions or substitutions in the 7 position of the theophylline molecule completely eliminate positive chronotropic activity. A comparison of theophylline with caffeine, 7-ethyltheophylline, or 7-diethylaminoethyltheophylline illustrates this point. Why 7-chloroethyltheophylline forms an exception to this rule is not understood. If the 1,3-dimethyl groups of theophylline are replaced by ethyl groups, then an ethyl group may be added to the 7 position without loss of chronotropic activity. A comparison of this compound (1,3,7-triethylxanthine) with 7-ethyltheophylline indicates that the 1,3-diethyl substitution has not greatly changed the character of the molecule. Positive chronotropic activity has been greatly enhanced, however, positive inotropic activity has been somewhat reduced. The generalization that many additions or substitutions in the 7 position of the

Table 19

Chemical Configuration Associated with Positive Chronotropic Activity



DRUG	#1	#2	#3	#6	#7	#8	#9	% INCREASE IN H.R.
7-Chlorcethyltheophylline	CH_3	O	CH_3	O	C_2H_4Cl	H	-	29
1,3,7-Triethylxanthine	C_2H_5	O	C_2H_5	O	C_2H_5	H	-	21
Theophylline	CH_3	O	CH_3	O	H	H	-	20
6-Desoxytheophylline	CH_3	O	CH_3	H_2	H	H	-	17
8-Chlorotheophylline	CH_3	O	CH_3	O	H	Cl	-	12

theophylline molecule completely eliminates positive chronotropic activity can be carried over to the 8 position. A comparison of theophylline with 8-bromotheophylline or 1,3-dimethyluric acid substantiates this statement. Once again, however, an exception is observed in the case of the chlorine substitution (8-chlorotheophylline). 8-Chlorotheophylline has considerably less chronotropic activity than its parent compound (theophylline), while 8-bromotheophylline has no chronotropic activity at all. Only one 9 substituted theophylline derivative (isocaffeine) has been examined and it has no demonstrable positive chronotropic activity.

My first impression had been that the imidazole ring of the theophylline molecule was the key point for alteration of positive chronotropic activity. It seemed possible that the imidazole substitutions were inhibiting positive chronotropic activity by destroying the tautomeric structure of the imidazole ring. The observation that 7-chloroethyltheophylline possessed definite chronotropic activity did not agree with this hypothesis. The contribution of the chlorine atom to positive chronotropic activity is of unusual interest. 7-Ethyltheophylline has no chronotropic activity, however, the addition of a terminal chlorine atom to form 7-chloroethyltheophylline produced the most active positive chronotropic agent in the series of purines studied. The influence of the chlorine atom on heart rate is not limited to the 7 position as demonstrated by the chronotropic activity of 8-chlorotheophylline. The 8-bromo and oxy substitutions by contrast do not show similar positive chronotropic activity.

It can be said that random substitutions on either the pyrimidine or imidazole ring of theophylline generally reduces the positive

chronotropic activity of the basic molecule. The exceptions to this generalization have been discussed above. The demonstration that there is no statistically significant difference between the positive chronotropic activity of the imidazole and pyrimidine substituted theophylline derivatives studied when treated as separate groups indicate that both ring structures function to determine positive chronotropic activity. Evidently the specific groups are of more importance than the alteration of the double bond structures of these rings in determining positive chronotropic activity.

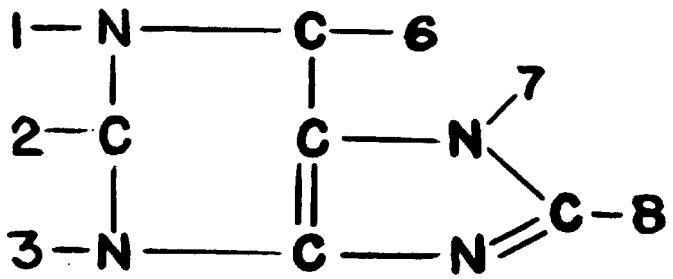
Duration of Drug Action

As stated earlier the duration of change in competence index is directly related to the magnitude of this change. Table 2 illustrates this point. The duration of change in C.I. may be thought of as the duration of action of the drug representing the duration of positive inotropic activity, positive chronotropic activity, or a combination of both factors. It has been demonstrated that positive inotropic and positive chronotropic activity can be effectively separated in the purine molecule. The intensity of these activities can also be separated from their duration of action. Isocaffeine (Table 8) offers a good illustration of this point. This drug increased the C.O. 49 per cent in the absence of positive chronotropic activity, therefore, it possessed only positive inotropic activity. The intensity of this inotropic activity was exceeded only by one or possibly two other compounds in the series of purines examined. Despite the high intensity of inotropic activity its duration was less than 10 minutes. Hypoxanthine, 8-bromotheophylline, and 8-chlorotheophylline represent similar cases of compounds having an appreciable intensity of activity with no significant

duration of action. The conclusion to be drawn from these observations is simply that intensity of activity and duration of activity are not necessarily directly related. Table 20 contains a list of the relatively long acting purine compounds examined. This list includes both methyl and amino substituted purine derivatives while excluding the pyrimidine derivatives. This suggests that the imidazole ring of the purine nucleus contributes in some way to the duration of action as well as to the intensity of positive inotropic or positive chronotropic activity. A comparison of the duration of action of adenine sulfate and adenosine (Table 8) illustrates the marked reduction in duration of action obtained upon modification of the imidazole ring of the purine nucleus. In the same manner a comparison of xanthine or hypoxanthine with theophylline indicates that the pyrimidine portion of the purine nucleus also contributes to the duration of action. These observations seem reasonable since the factors responsible for the total change in C.I. in the heart-lung preparation are dependent upon inotropic and chronotropic activity. Any alteration of a <u>persistent</u> inotropic and/or chronotropic activity resulting from structural modifications of the purine nucleus should be reflected in the total change in competence index. In the same way a positive inotropic response of low intensity but long duration would be expected to alter the C.I. 1,3-Dimethyluric acid (Table 7) illustrates this point. The conclusion drawn from these observations was that only those drugs producing a persistent inotropic or chronotropic response could significantly modify the C.I. regardless of the intensity of the response. In addition there was no statistically significant difference between the duration of action of the pyrimidine and imidazole substituted theophylline derivatives.

Table 20

Chemical Configuration Associated with Long Duration of Action



DRUG	#1	#2	#3	#6	#7	#8	TOTAL △ C.I.
7-Chloroethyltheophylline*	CH_3	O	CH_3	O	C_2H_4Cl	H	0.9
Theophylline	CH_3	O	CH_3	O	H	H	0.9
Theobromine	H	O	CH_3	O	CH_3	H	0.9
7-Ethyltheophylline	CH_3	O	CH_3	O	C_2H_5	H	0.9
Caffeine	CH_3	O	CH_3	O	CH_3	H	0.8
Adenine Sulfate	-	H	-	NH_2	H	H	0.8
6-Desoxytheophylline	CH_3	O	CH_3	H_2	H	H	0.8
2,6-Diaminopurine Sulfate	-	NH_2	-	NH_2	H	H	0.8
1,3,7-Triethylxanthine	C_2H_5	O	C_2H_5	O	C_2H_5	H	0.7

* Part of the action recorded is due to the solvent employed (nicotinamide). See Table 22.

The high degree of correlation between the magnitude of total delta C.I. and the duration of delta C.I. (Table 2) suggests that the measurement of total delta C.I. five minutes after administering the drug to the failed heart-lung preparation provides a simple method for estimating duration of drug action. Naturally a uniform degree of failure must be obtained in all the preparations.

Walker, Lourie, and Burn (32) have suggested that the reduction of C.O. in the dog heart-lung preparation may be a linear function of time. Lanzoni (22) has suggested that the spontaneous rate of failure in this preparation is also a linear function of time. My results may be interpreted as meaning that the reduction in total delta C.I. after drug administration is also a linear function of time. The only serious discrepancy in this interpretation was obtained with 2,6-diamino purine sulfate. It could temporarily be eliminated from the series on the basis of technical difficulties arising in one of the two experiments performed (See Appendix).

My experiments with hypoxanthine and isocaffeine do not support the concept that the reduction in C.O. in the dog heart-lung preparation after drug administration is a linear function of time. The use of C.I. appears to be a more reliable standard for evaluating the functional state of the heart than C.O.

Positive Inotropic Activity

The purines as a class of compounds offer outstanding potentialities for investigating the mechanisms of cardiac physiology and pharmacology. Certain members of this series possess marked positive inotropic activity as well as positive chronotropic activity. My results demonstrate that the two types of activity may be separated

within the same molecule by appropriate substitutions. The elimination of positive chronotropic activity is undoubtedly a desirable characteristic in drugs having marked positive inotropic activity since an increase in heart rate is generally thought to increase the energy demands of the myocardium in excess of the work accomplished. This situation should certainly be avoided when dealing with a myocardium in failure. Any drug, therefore, possessing outstanding positive inotropic activity and lacking positive chronotropic activity should receive careful consideration as a possible therapeutic agent.

My impression has been that theophylline possesses the greatest positive inotropic activity in the series of methylated purines examined. In addition it produces a marked positive chronotropic response which is additive with respect to the observed increase in cardiac output. None of the substituted theophylline derivatives so far examined have proven to be superior to the parent compound from the standpoint of increasing cardiac output. It is quite possible that theophylline possesses the maximal activity obtainable within the series of methylated purines.

The results obtained with 2,6-diaminopurine sulfate are very encouraging and suggest that a departure from the classical methylated xanthines may provide compounds with greater potentialities for the treatment of heart failure. From the standpoint of pure positive inotropic activity 2,6-diaminopurine sulfate is the most potent purine compound examined to date. The physiological role of this compound has not been fully determined, however, Bendich and Brown (3) have suggested that it may act as an intermediate in the conversion of adenine to guanine. In the lower species of microorganisms Hitchings (17) has shown 2,6-diaminopurine to be a metabolic inhibitor of adenine.

Theobromine, theophylline, caffeine, and paraxanthine have also been shown to function as metabolic inhibitors of naturally occurring purine compounds (25). Up to the present time no attempt has been made to relate the cardiac activity of the commonly employed purine derivatives to their purine inhibiting properties.

Recently Greiner has reported that the spontaneous decline in systolic force of isolated cat papillary muscles is due to the fall in concentration of ATP. Evidence is presented to implicate a deficiency of total adenosine nucleotide as the cause of the fall in concentration of ATP. He further suggests the possibility that the effect of digitalis on contractile force is mediated by an action on purine metabolism which restores adenosine nucleotide to normal levels (12). The implication of a defect in normal purine metabolism in the case of spontaneous failure of a papillary muscle offers much room for speculation. Indeed the structural relationship of the barbiturates, commonly used to induce cardiac failure in the papillary muscle or heart-lung preparation, to the pyrimidine nucleus is considerable. Woods has suggested that barbituric acid can serve to displace uracil, a naturally occurring pyrimidine found in nucleic acids (35). An investigation of the possible inhibition of normal uracil metabolism in barbiturate induced cardiac failure might prove fruitful in better understanding the mechanism of drug induced cardiac failure.

Some mention should be made of the recent publication by Green and Stoner (13) concerning the effect of purine derivatives on the cardiovascular system. They have summarized much of the information accumulated since the subject was reviewed by Drury (8) in 1936. Green and Stoner have reported that adenine has only a negligible effect on

the amplitude of contraction of the isolated perfused rabbit heart. My studies indicate that adenine definitely increases the force of contraction of the failed dog heart. This discrepancy can probably be accounted for on the basis of the relatively small doses they employed in their work.

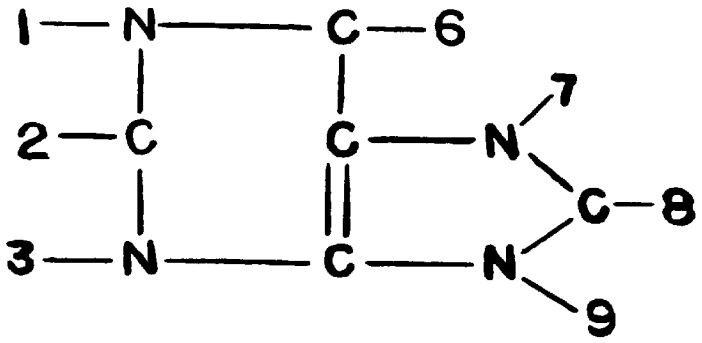
My studies on the purine derivatives have eliminated chelation as a possible basic mechanism in the production of positive inotropic activity in this series of compounds. Neither the pyrimidine nor imidazole ring per se of the theophylline molecule appears to determine positive inotropic activity. Apparently these component parts must be joined to form the purine nucleus before highly active compounds can be obtained. Modifications of either the pyrimidine or imidazole ring of the basic theophylline molecule by the addition of substituent groups, however, readily produce compounds with a wide range of positive inotropic activity. Table 21 lists those compounds exhibiting positive inotropic activity in the absence of positive chronotropic activity.

In conclusion it appears that the nature and position of the substituent groups per se are the primary factors in determining positive inotropic activity of the purine derivatives. No single position or grouping is qualitatively responsible for the positive inotropic activity of the theophylline molecule. The basic xanthine structure possesses some positive inotropic activity which is greatly enhanced by the addition of methyl groups to the 1 and 3 positions to form theophylline. Further alteration of the methylated xanthine nucleus failed to produce any compound with greater positive inotropic activity than the parent theophylline molecule.

The amino substituted purine derivatives, however, may prove

Table 21

Chemical Configuration Associated with Positive Inotropic Activity in the Absence of Positive Chronotropic Activity



DRUG	#1	#2	#3	#6	#7	#8	#9	% INCREASE IN C.O.
2,6-Diaminopurine SO_4	-	NH_2	-	NH_2	H	H	-	51
Isocaffeine	CH_3	O	CH_3	O	-	H	CH_3	49
Caffeine	CH_3	O	CH_3	O	CH_3	H	-	43
7-Ethyltheophylline	CH_3	O	CH_3	O	C_2H_5	H	-	40
Theobromine	H	O	CH_3	O	CH_3	H	-	34
Adenine Sulfate	-	H	-	NH_2	H	H	-	27
Hypoxanthine	H	H	-	O	H	H	-	20
Xanthine	H	O	H	O	H	H	-	10

to be of greater practical importance than the classical methylated xanthines. Apparently 2,6-diaminopurine sulfate has greater positive inotropic activity than equimolar doses of theophylline while lacking the undesirable positive chronotropic activity. The demonstration of high inotropic and chronotropic activity with guanidine is of great interest. It suggests that this simple structure contains the basic units required to stimulate the receptors responsible for increasing heart rate and contractile force. There are several compounds reported in the literature that possess cardiac activity which bear some structural resemblance to guanidine. These include glycocyamine and ethylenediamine. Perhaps even triethylamine, tetraethylammonium bromide, diethylaminoethanol, and hexamethonium bromide may be related to guanidine on the basis of similar chemical properties and thereby provide some common denominator to explain the cardiac activity of this miscellaneous group of compounds. The incorporation of the guanidine structure into the purine molecule provides a means by which the cardiac activities of guanidine may be readily modified. 2,6-Diaminopurine represents an example in which the positive inotropic activity has been enhanced while the undesirable positive chronotropic activity has been eliminated. It is quite possible that other similar compounds may prove to be more active than 2,6-diaminopurine sulfate. A further investigation of the guanidine type purine derivatives is certainly indicated on the basis of the results obtained in the experiments reported in this thesis.

II. The Cardiovascular Pharmacology of Ethylenediamine

A. Positive Inotropic Action

Ethylenediamine (10^{-3} M) did not consistently produce a positive inotropic response when examined in the hypodynamic cat papillary muscle preparation. There appeared to be a critical dose level required to demonstrate stimulation of any given failed muscle with the above drug. Concentrations of ethylenediamine greater than 10^{-3} M always produced depression (negative inotropic effect) of the muscle. It was observed that the critical dose required for stimulation of the failed papillary muscle varied with the supply of distilled water employed in preparation of the Ringers solution. This observation suggested that the concentration of heavy metals present in the distilled water might be a primary factor in determining whether stimulation or depression of the muscle resulted from administration of the drug. In the presence of excessive amounts of the chelating agent calcium ions can be effectively removed from the Ringers solution and thereby depress the activity of the muscle. The addition of calcium ions to the muscle bath can then reverse the depression. The mechanism involved when stimulation is observed with this drug can be explained in several ways. One possibility is that the drug has a direct stimulating effect upon the failed muscle. A second possibility is that the drug indirectly stimulates the muscle by virtue of removing heavy metal contaminants from the solution which depress the activity of the muscle. The magnitude of muscular depression resulting from the presence of traces of heavy metal contaminants in Ringers solution is easily demonstrated. If one substitutes Mallinckrodt reagent grade sodium chloride for Merck

reagent grade sodium chloride for biological work in the preparation of the Ringers solution, a marked depression of muscle activity is noted. The reason for this difference is apparently due to the fact that the former compound is crystallized in silver lined tubs, while the latter compound is crystallized in wooden tubs. The trace amounts of silver carried along in the crystallization process with the Mallinckrodt product is sufficient to produce a considerable depression in activity of the isolated muscle. Theoretically this depression could be removed by the addition of the proper amount of a chelating agent. The observed response would, therefore, represent an indirect stimulating effect. This type of action could explain the inconsistent positive inotropic activity observed with ethylenediamine alone. It offers a reason for the inability to reproduce the results obtained in the above type of experiment with a given dose of drug when different batches of distilled water were utilized. Under these circumstances a readjustment of the drug concentration was necessary before stimulation could be reproduced. It is, therefore, essential that a single batch of distilled water be used in this type of experiment and once the critical dose of chelating agent required for stimulation is established, the results are easily reproduced.

In my opinion Leitch and Haley (23) were unable to demonstrate a positive inotropic action with ethylenediamine tetra-acetic acid, which I have found to possess positive inotropic activity at about 10^{-4} M in the hypodynamic papillary muscle preparation, because of the large doses employed. It was of interest to observe that a definite stimulation of the perfused guinea pig heart always followed the initial depression after administration of the drug. The duration of this stimulation was

usually longer than the period of depression (see Figure I. in their article) which suggests that the positive inotropic action of the drug appeared when the perfusate diluted the injected drug to the critical concentration required for stimulation. This point could be determined by adding the drug directly to the perfusion fluid rather than injecting into the perfusion cannula. I suspect that a typical bell shaped curve could be obtained with graded doses of this drug or ethylenediamine in this type of preparation. This discussion is not meant to eliminate the possibility that ethylenediamine or its tetra-acetic acid derivative have a direct stimulating effect on heart muscle. The primary purpose is to point out that associated factors are involved in the interpretation of results obtained with these agents in isolated systems. Both compounds produce a marked positive inotropic action in the heart-lung preparation.

Dimercaprol (7.5×10^{-6} M) by contrast exhibits a significant positive inotropic action in the isolated papillary muscle preparation and only a negative inotropic action in the heart-lung preparation at comparable doses. These results suggest that the positive inotropic action in vitro is an indirect effect, while the negative action in vivo is probably a direct effect upon the heart.

The response obtained when these chelating agents are added to an isolated system may, therefore, represent the algebraic sum of the effects of the agent on the tissue directly and on the ionic composition of the perfusion or bath media.

The results obtained in this section demonstrate that ethylenediamine (10^{-3} M) produces a significant increase in the force of contraction of the hypodynamic cat papillary muscle (Table 11). When tested in

vivo (dog heart-lung preparation) ethylenediamine base or the dihydrochloride salt produced a marked increase in the cardiac output of the failed heart. It has been shown that the increase in cardiac output results from a positive inotropic action. At a concentration of approximately 10^{-3} M, ethylenediamine dihydrochloride produced an occassional positive inotropic response. With higher doses (100 mgm., about 1.6 x 10^{-3} M) a consistent marked stimulation of the failed heart in vivo was always observed. Larger doses exhibit a comparable effect with a relatively greater increase in the positive inotropic response and a longer duration of action. It is apparent that the intact animal preparation differs considerably from the in vitro preparation. The primary point of differentiation is that a critical dose level is required to demonstrate stimulation of the failed muscle in vitro and larger doses readily produce depression of the muscle. In vivo, by contrast, the critical dose level for stimulation can be exceeded several times with a corresponding increase in activity. The results obtained in vivo, therefore, are readily duplicated with suprathreshold doses, whereas confirmation of stimulant activity in vitro is difficult. The difference between the behavior of ethylenediamine in vitro and in vivo is probably related in part to the limited availability of calcium ions in the isolated preparation. Other factors may also be involved but they have not been investigated to date.

Evidently this drug acts directly upon cardiac tissue to produce its stimulant effect, however, the basic mechanism involved is unknown. It is quite possible that chelation is involved in this process but this has not been demonstrated directly. The fact that ethylenediamine does possess a positive inotropic action is of considerable

importance. The following section presents one aspect of the practical implications of this finding.

B. The Effect of the Ethylenediamine Component of Aminophylline on the Duration of Reversal of Cardiac Failure in the Dog Heart-Lung Preparation

The data obtained clearly indicate that the ethylenediamine component of theophylline ethylenediamine is responsible for the significant difference between the duration of action of theophylline ethylenediamine (aminophylline) and theophylline. Howarth and coworkers (18) administered ethylenediamine (100 mgm.) to several patients and observed a slight rise in cardiac output that was thought to result from a fall in venous pressure. There was no evidence presented to substantiate this interpretation and in view of my results I would interpret their data differently. This type of work should be repeated using doses of the drug that will give a blood concentration of approximately 100 mgm./liter. The drug is relatively innocuous and larger doses could probably be tolerated. I have administered as much as 460 mgm. per Kg. to an intact unanesthetized dog with no serious consequences. The animal exhibited a marked hyperpnea and a tachycardia but recovered uneventfully. Doses less than 100 mgm. per Kg. do not produce the tachycardia but the hyperpnea persists even with very small doses. The respiratory stimulation observed is of short duration and should not constitute a contraindication for further studies with this compound. It must be emphasized that the drug should be administered slowly, preferably by intravenous drip, in order to avoid the transient central hypotensive action previously described. The results obtained thus far with ethylenediamine should encourage further experi-

mentation and ultimately a clinical trial in cases of congestive heart failure.

C. Hypotensive Action

The area involved in the hypotensive response of anesthetized dogs to small doses (0.5 mgm./Kg.) of ethylenediamine dihydrochloride is located cephalad to the anatomical junction of the vertebral and internal carotid arteries. The carotid and aortic chemoreceptors as well as myocardial chemoreceptors have been eliminated as possible sites of action. The efferent neural pathways from this central site of action responsible for the hypotensive action lie within the autonomic nervous system.

The mechanism involved in central stimulation is apparently related to the chelating property of ethylenediamine. The exact nature of the substance being chelated has not been established, however, indirect evidence suggests that the calcium ion may be involved. If subsequent work confirms the above interpretation of the data obtained, then a better understanding of the mechanism involved in the hypotensive response to a new series of chemical agents will have been established.

The experimental approach utilized in this section was based upon a similarity of chemical properties in a group of compounds having diversified chemical structures. The basic property common to all the drugs tested was the ability to form chelate structures. The results obtained suggest that this type of approach may offer a better starting point for the elucidation of the mechanism of drug action than the classical structure activity relationship studies. The chemical properties of the active moieties of a pharmacologically active agent are undoubtedly of more fundamental importance than the steric orientation

of the molecule since structure must be subservient to function.

The aminophylline depressor response was examined in a series of anesthetized dogs as well as the potential toxicity of this drug upon rapid intravenous administration in unanesthetized dogs.

Several cases of fatalities in humans following intravenous administration of aminophylline have been reported by Merrill (27). As a result of this report it has become standard procedure to administer intravenous aminophylline slowly in order to minimize its potential toxicity. I investigated the effects produced by the rapid intravenous administration of relatively large doses of this drug in unanesthetized dogs. No fatalities occurred in a series of twelve dogs exposed to this treatment and only a transient hyperpnea and tachycardia was observed in these normal animals. The failure to produce any mortalities in normal dogs with higher mgm./Kg. doses of aminophylline than those reported by Merrill in humans suggests that the condition of the cardiovascular system is of primary importance in determining the response to intravenous aminophylline. When one observes the intense positive chronotropic response of the failed dog heart to aminophylline or theophylline, it is not difficult to appreciate the real possibility that these agents might induce ventricular fibrillation in a manner similar to epinephrine as suggested by Merrill. The metabolic demands imposed upon a heart in failure by an agent with intense positive chronotropic activity are certainly undesirable and one could probably demonstrate a significant difference in the LD_{50} values of aminophylline in normal dogs as compared to dogs with congestive heart failure. By implicating the positive chronotropic activity of aminophylline in the production of acute fatalities to intra-

venous administration of this drug, the ethylenediamine component would be of little importance since it lacks positive chronotropic activity in the doses employed. Thompson and Warren (31) found no appreciable difference in the acute intravenous LD_{50} values for theophylline ethylenediamine and theophylline aminoisobutanol in rabbits which suggests that the theophylline component of these complexes is primarily responsible for their acute toxicity. In my opinion the ethylenediamine component of aminophylline is probably not responsible for the acute fatalities resulting from intravenous administration of this compound. Further work, including the elucidation of the nature of the toxic reaction, is necessary before any definite conclusion can be reached regarding the role of ethylenediamine in this response. The investigation of a possible relationship between the intense positive chronotropic activity of aminophylline and acute fatal reactions in dogs with congestive failure might serve as a starting point for this study.

The depressor response resulting from rapid intravenous administration of aminophylline in anesthetized dogs was examined. The results of this study indicate that the ethylenediamine content of the doses of aminophylline employed largely contributes to the depressor response obtained. Approximately fifty per cent of the observed fall in blood pressure can be attributed to the ethylenediamine present in the doses of aminophylline utilized. The mechanism of the ethylenediamine depressor response has been discussed in the previous section. The mechanism of the theophylline depressor response is not known, however, it may be due to a central chelating action as with ethylenediamine. It seems unlikely that the fall in blood pressure is due to

the precipitation of the theophylline alkaloid in the blood as suggested by Goodman and Gilman (11). My disagreement with their hypothesis is based upon the observation that the feebly acidic theophylline alkaloid is not precipitated when added to an alkaline media of pH 7 - 10. It can readily form the soluble sodium or potassium salts when these cations are available as occurs in vivo.

Much confusion has resulted from the use of theophylline complex salts which probably could have been avoided. The rationale for the use of these salts was based upon the reported insolubility of the theophylline alkaloid in aqueous solutions. I have always employed the alkaloid in preference to the complex salts and find it readily soluble in dilute or warm aqueous solutions. A dose of 165 mgm. of theophylline monohydrate is soluble in 20 ml. of warm (37 degrees C.) distilled water. This solution is maintained when diluted to one liter with distilled water at room temperature and within a pH range of 6.0 to 10.0. For practical purposes the complex salts are preferred in clinical practice, however, there is no reason why the alkaloid cannot generally be used in animal laboratory work. The use of theophylline and probably theobromine complex salts in experimental pharmacology has produced results that may require reinvestigation with pure compounds. Several examples of the influence of these complex salts have been presented in this thesis, however, the range of influence of the salts upon the activities of the pure alkaloids has yet to be determined.

SUMMARY

The discovery that ethylenediamine possessed marked positive inotropic activity in vitro and in vivo suggested a logical explanation for the apparent superiority of aminophylline over other theophylline salts in the treatment of congestive heart failure. Experiments are reported in which ethylenediamine is shown to enhance the action of theophylline in reversing cardiac failure in the dog heart-lung preparation.

The hypotensive response resulting from rapid intravenous injections of aminophylline in the dog is largely due to the ethylenediamine present in this drug. The site of the ethylenediamine hypotensive action has been shown to lie cephalad to the anatomical junction of the carotid and vertebral arteries. The mechanism of drug action responsible for the fall in blood pressure is apparently related to the chelate structure of ethylenediamine. The nature of the substance being chelated has not been determined, however, calcium ions have been tentatively implicated.

The realization that theophylline possessed the essential structure for chelation suggested a possible explanation for the positive inotropic activity of this molecule since the chelating agent, ethylenediamine, also exhibited positive inotropic activity. An investigation of the cardiac activities of selected purine derivatives as related to their chemical constitution was performed. The results obtained eliminated chelation as the mechanism responsible for positive inotropic activity in the series of purine derivatives examined.

An analysis of the relationship of chemical structure to positive inotropic activity, positive chronotropic activity, and dura-

tion of drug action in the purine series is also presented. Appropriate substitutions on the purine nucleus can readily modify the individual cardiac activities of this class of compounds.

Theophylline appears to possess the greatest positive inotropic activity in the series of methylated purine derivatives. The amino substituted purine derivative, 2,6-diaminopurine sulfate, is of great interest and may prove to be superior to the classical methylated xanthines as a cardiac stimulant.

The demonstration that guanidine possesses marked positive inotropic and positive chronotropic activity classifies it as the simplest molecule known to combine these activities. Further investigation of the chemical properties of this molecule may provide a logical common denominator for a large series of miscellaneous amine derivatives with cardiac activity.

Several examples are presented in which the pharmacological activity of chemicals commonly employed as solubilizing agents for purine derivatives may greatly influence the activity of the solute.

A method has been described employing competence index to predict the approximate duration of action of a drug reversing cardiac failure in the dog heart-lung preparation.

An investigation of the reported cardiac activity of a recently introduced theophylline derivative, 7-diethylaminoethyltheophylline (R-3588), produced negative results. Neither the compound per se nor its potential products of hydrolysis exhibited significant cardiac activity as measured by my criteria.

APPENDIX

I. An Investigation of the Cardiac Activity of 7-Diethylaminoethyltheophylline (R-3588) and its Potential Products of Hydrolysis

A publication by Merlen and Soubiran (26) in 1949 reported that 7-diethylaminoethyltheophylline possessed all the pharmacodynamic properties of theophylline. No data concerning the species utilized or doses employed was presented in their publication. I included this compound in my structure activity relationship study of the purine molecule (Table 4). The results obtained indicated that the compound could not reverse the failure state in the dog heart-lung preparation. The lack of positive inotropic or positive chronotropic activity suggested that the compound differed greatly from the parent theophylline molecule. Further studies were undertaken in order to resolve the discrepancies between my work and the report by Merlen and Soubiran.

The first point investigated was the possibility that the drug was highly active and the dose of 289 mgm/l. was sufficient to produce toxic effects. A dose of R-3588 was administered (138.2 mgm/l.) which was calculated to be equivalent to 85 mgm./l. of theophylline monohydrate. The results obtained with this dose in the hypodynamic dog heart-lung preparation were qualitatively similar to those observed with the 289 mgm./l. dose. An initial transient decrease in right auricular pressure and a slight increase in C.O. occurred which was less than thirty seconds in duration. These effects were quickly reversed and a state of marked failure followed which was not spontaneously reversed over the course of one hour. The heart was in such poor condition at this time that further observation was discontinued. One experiment was run utilizing a dose of 500 mgm./l. of R-3588 and the

results obtained were almost identical with those reported for the smaller doses.

Several experiments were run utilizing the hypodynamic cat papillary muscle preparation in order to confirm the above observations. Concentrations of 0.43×10^{-3} M and 1.5×10^{-3} M of R-3588 were employed which are equivalent to the 138.2 and 500 mgm./l. doses employed in heart-lung experiments.

The results obtained showed a close correlation with those observed in vivo. Both doses produced the same pattern of transient stimulation followed by depression. With the higher dose (1.5×10^{-3} M) the muscle became refractory to the driving stimulus (20 volts at 1/sec.). Increasing the voltage to 300 could not overcome this effect. The voltage was reset at 20 and after a period of 30 minutes the muscle again began to contract regularly. One hour later it was still contracting regularly but less forcefully than at any time prior to the drug, and it began to fail rapidly. With the smaller dose of R-3588 (0.43×10^{-3} M) a slight stimulation followed by depression of contractile activity was observed. The muscle, however, did not become refractory to the driving stimulus. The depression of contractile activity was readily reversed by administering (0.43×10^{-3}M) aminophylline one hour after the R-3588 had been given.

The failure to demonstrate any appreciable cardiac stimulating activity with R-3588 in the dog heart-lung and cat papillary muscle preparations suggested that the compound might be metabolized in the intact animal to some active derivative. It was quite obvious that R-3588 per se was not a cardiac stimulating agent. The possibility remained, however, that the liver or some other organ which was excluded

from the circulation in the heart-lung preparation could metabolically alter the compound and form an active derivative. The possibility of hydrolytic cleavage of R-3588 at the 7 position was suggested (21). Cleavage at this point could give rise to theophylline and diethylethanolamine or triethylamine. The activity of theophylline is well documented; however, the activity of diethylethanolamine and triethylamine has not been extensively investigated at the doses employed in this work. An evaluation of these compounds is presented below.

TRIETHYLAMINE

Triethylamine was tested at three dose levels, 44 mgm., 92 mgm., and 160 mgm./l., which were the calculated amounts that could be obtained from the breakdown of 138.2 mgm., 289 mgm., and 500 mgm. respectively of R-3588 in vivo. The doses were administered per liter of circulating blood in the heart-lung preparation and the results were similar at all three dose levels. In each case the drug reversed the state of failure as indicated by an increased cardiac output and decreased right auricular pressure. This beneficial effect did not persist for more than one minute after which time spontaneous failure occurred.

Several experiments were run on the hypodynamic cat papillary muscle preparation. A concentration of 0.43×10^{-3}M triethylamine was established in the bath and this produced an immediate marked positive inotropic response which persisted for forty-five minutes. This dose of triethylamine corresponds to the amount of drug that can be formed from 138.2 mgm. of R-3588 in the heart lung preparation. It was observed that the same dose was ineffective when given to a muscle after the previous administration of an equimolar dose of R-3588. These results agreed with similar observations in the dog heart-lung preparation

using the same concentrations of drugs.

DIETHYLAMINOETHANOL

Diethylaminoethanol was examined at three dose levels, 51 mgm., 107 mgm., and 185 mgm./l., which were the calculated amounts that could be obtained from the breakdown of 138.2 mgm., 289 mgm., and 500 mgm. respectively of R-3588 in vivo. The above doses were administered per liter of circulating blood in the heart-lung preparation and the results obtained were similar at the three dose levels. In each case the drug reversed the state of failure as indicated by an increased cardiac output and a decrease in right auricular pressure. The cardiac stimulating effect did not persist for more than 5 minutes. At this time the heart was unable to compensate for an increased load and failed rapidly. These results confirm the observations of Krayer, Farah, and Uhle (19) that diethylaminoethanol has a slight positive inotropic action. When tested on the hypodynamic cat papillary muscle at a concentration of 5×10^{-3} M, the drug produced a marked positive inotropic response which persisted for 10 minutes. At a concentration of 1×10^{-3}M diethylaminoethanol produced only a slight increase in the amplitude of muscle contraction. (See page 87).

II. Solvents Employed in the Purine Studies

In the course of this study I encountered several drugs that were practically insoluble in water. These included 7-chloroethyltheophylline, theobromine, 8-chlorocaffeine, uric acid, and 1,3-dimethyluric acid. None of the compounds were soluble in the commonly employed solvents with known pharmacological properties. I employed three solvents, lithium carbonate, nicotinamide, and sodium salicylate solutions, whose cardiac activity had not been adequately examined.

Twenty milliliters of a 0.1% solution of lithium carbonate was utilized as a solvent for uric acid (154 mgm.) and 1,3-dimethyl-uric acid (163 mgm.). The results obtained with these drugs are listed in Table 7. Control experiments with the solvent demonstrated that it had no effect upon the dog heart-lung preparation.

Nicotinamide and sodium salicylate, however, possessed definite cardiac activity when tested alone. The results obtained with these solvents are found in Table 22.

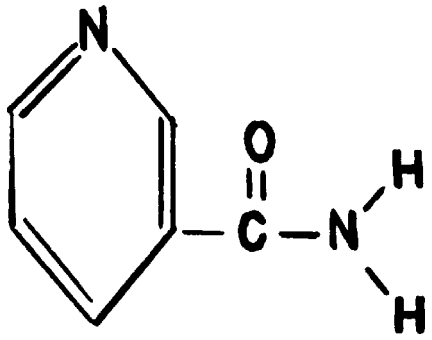
Nicotinamide (400 mgm.) exerts a definite effect upon the failed dog heart-lung preparation as demonstrated by the increase in cardiac output and change in competence index.

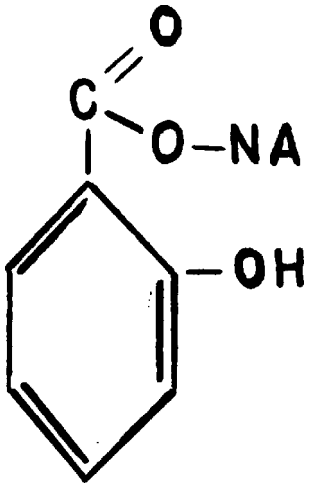
The results obtained with 7-chloroethyltheophylline plus nicotinamide (400 mgm.) suggest that the theophylline derivative may actually possess a negative inotropic action, however, the intense positive chronotropic action plus the positive inotropic action of the solvent produce a definite increase in cardiac output. The observed change in total delta C.I. and the prolonged duration of action recorded for 7-chloroethyltheophylline is probably largely due to the marked positive chronotropic activity of this molecule. The compound should be evaluated for inotropic activity in the cat papillary muscle preparation where chronotropic activity is controlled.

Nicotinamide was also employed as a solvent for 8-chlorocaffeine. No control experiments were performed at the dose level of nicotinamide utilized (600 mgm.) in view of the results obtained with smaller doses (400 mgm.). It was assumed that 600 mgm. would produce a response qualitatively similar to that observed with 400 mgm. of nicotinamide. If this assumption is correct, then 8-chlorocaffeine is definitely a

Table 22

Solvents Employed





DRUG	DOSE MGM./L.	% INCREASE IN C.O.	TOTAL △C.I.	% INCREASE IN H.R.	(min.) DURATION OF △C.I.	NO. OF EXP.
7-CHLOROETHYLTHEOPHYLLINE (201 mgm.) DISSOLVED IN 20 ML. OF 2% NICOTINAMIDE	201 400	15	0.9	29	>75	3
NICOTINAMIDE	400	18	0.3	0	3	3
8-CHLOROCAFFEINE (190 mgm.) DISSOLVED IN 30 ML. OF 2% NICOTINAMIDE	190 600	2	-0.1	0	0	2
THEOBROMINE (150 mgm.) PLUS SODIUM SALICYLATE (133 mgm.)	150 133	34	0.9	0	51	6
SODIUM SALICYLATE	133	-16	-0.1	0	0	2

myocardial depressant since the positive inotropic activity and change in competence index obtained with this drug in combination with nicotinamide (600 mgm.) is less than that observed with nicotinamide (400 mgm.) alone. The possibility exists that the larger doses of nicotinamide (600 mgm.) may have a depressant effect upon the myocardium, however, this appears doubtful.

In the case of theobromine combined with sodium salicylate, the salicylate has a definite negative inotropic action as demonstrated by the decrease in C.O. and the negative change in competence index (Table 22).

The results of the above studies indicate that the values obtained for 7-chloroethyltheophylline and reported in the preceding tables do not give a true picture of the activity of this molecule. The per cent increase in C.O. and total delta C.I. are influenced by the solvent employed. For this reason the compound should be thought of as being less active than the tabular figures indicate. The same situation applies to the results obtained with 8-chlorocaffeine. This compound is probably a cardiac depressant with no real positive inotropic activity. A pharmacologically inactive solvent must be found for these two drugs before a true evaluation of their activity can be determined.

The situation with theobromine and sodium salicylate presents a somewhat different picture. In this case the sodium salicylate present acts as a cardiac depressant due to a negative inotropic action. The positive results obtained with this combination, therefore, are less than those that might be expected if an inactive solvent had been employed. This suggests that much of the work present in the literature

with respect to the quantitative evaluation of theophylline, caffeine, and theobromine on the cardiovascular system may have to be revised. The necessity for such a revision would be based upon the demonstration that sodium salicylate antagonizes the positive inotropic action of theobromine since this preparation has frequently been used to represent theobromine when compared with other methyl xanthines. In a similar manner theophylline ethylenediamine has often been used to represent theophylline in comparable experiments. The work presented in the section on experimental results (Part II, A and B) demonstrates that ethylenediamine has cardiovascular properties of its own and under certain conditions a statistically significant difference can be demonstrated between the cardiac activity of theophylline and theophylline ethylenediamine.

Addenum

An Investigation of the Cardiac Activity of 7-Diethylaminoethyltheophylline (R-3588) and its Potential Products of Hydrolysis

If R-3588 is hydrolyzed at position 7 in vivo, then any beneficial effect observed with this compound in conditions of congestive heart failure would be almost entirely due to the amount of theophylline liberated on hydrolysis. This statement is based upon the observations that neither triethylamine nor diethylaminoethanol have sufficient cardiac activity to be of any therapeutic importance in the doses employed. At the present time, however, there is no adequate proof that hydrolysis occurs at the 7 position in vivo.

A recent report by Livesay and Moyer (24) indicates that R-3588 does not have the same diuretic potency as aminophylline. They further observed that R-3588 depressed glomerular filtration, renal

plasma flow, and urine volume in normal humans and patients with cardiac failure. An occasional patient showed a natriuretic effect of tubular origin which suggested to the authors that R-3588 might be used as an adjunctive therapeutic measure in patients with intractable heart failure. I cannot agree with this proposition on the basis of my experimental data (Table 4) and feel that R-3588 should be regarded as a dangerous drug until more data is available on its cardiovascular effects in the intact animal. The metabolic breakdown of this compound as well as the rate of the possible conversion to theophylline should be determined before further clinical studies are warranted.

III. The Original Data Utilized in Preparing Tables for Section I of Experimental Results

A. Data Utilized in Calculating " % Increase in Contractile Force" for Table 1

All drugs were tested at a dose equivalent to 8.3×10^{-4}M theophylline on the hypodynamic cat papillary muscle preparation.

EXP. NO.	(%) CONTRACTION AT FAILURE	(%) CONTRACTION AFTER DRUG	DIFFERENCE
		Theophylline	
1	48	72	24
2	53	69	16
3	47	126	79
4	61	89	28
			$\bar{x}$ = 37%
		Desoxytheophylline	
1	44	42	-2
2	42	48	6
3	71	85	14
4	66	78	12
5	34	60	26
6	54	76	22
7	50	90	40
			$\bar{x}$ = 17%
		Caffeine	
1	52	82	30
2	57	57	0
3	67	77	10
4	48	92	44
5	39	66	27
			$\bar{x}$ = 22%
		8-Chlorotheophylline	
1	50	50	0
2	48	48	0
3	53	57	4
4	48	48	0
			$\bar{x}$ = 1%
		Xanthine	
1	40	40	0
2	35	35	0
3	60	63	3
4	27	37	10
			$\bar{x}$ = 3%

EXP. NO.	(%) CONTRACTION AT FAILURE	(%) CONTRACTION AFTER DRUG	DIFFERENCE
	8-Bromotheophylline		
1	40	42	2
2	57	68	11
3	50	59	9
4	55	52	-3
			$\bar{x}$ = 5%
	Imidazole		
1	45	51	6
2	41	37	-4
3	59	59	0
			$\bar{x}$ = 1%

B. Purine Derivatives Evaluated in the Dog Heart-Lung Preparation

EXP. NO.	DOSE (mgm./l.)	CONTROL C.O.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.	(min.) DURATION C.I.
					Adenine Sulfate						
1	336	556	136	1.0	470	136	0.1	556	144	1.0	56
2	336	556	132	0.9	365	132	0.1	470	132	0.6	30
3	336	536	136	1.0	350	136	0.0	470	136	0.9	41
										Avg.	42
					Adenosine						
4	222	556	140	0.9	395	138	0.1	250	122	0.1	0
5	222	517	136	0.9	395	136	0.1	395	121	0.4	29
6	222	500	124	0.9	375	124	0.0	316	120	0.0	0
										Avg.	10
					Benzimidazole						
7	98	525	144	1.0	385	144	0.1	375	152	0.0	10
8	98	652	142	0.9	250	144	0.0	250	134	0.3	11
										Avg.	10
					8-Bromotheophylline						
9	231	517	140	1.0	316	160	0.2	273	160	0.0	0
10	231	330	140	0.9	250	140	0.0	305	140	0.2	12
11	231	566	134	0.5	500	132	0.5	517	132	0.5	0
										Avg.	4
					Caffeine						
12	161	600	148	1.0	435	148	0.1	577	148	0.9	51
13	161	612	140	0.8	250	150	0.0	652	144	0.7	51*
14	161	430	152	1.0	250	124	0.0	440	148	0.3	30
15	161	430	140	1.0	250	132	0.0	316	132	1.0	30
										Avg.	40

* preceded by another drug

EXP. NO.	DOSE (mgm./l.)	CONTROL C.O.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.	DURATION (min.) C.I.
					8-Chlorocaffeine						
16	190	536	152	0.9	440	136	0.1	365	132	0.0	0
17	190	500	100	1.0	375	104	0.1	455	104	0.0	0
										Avg.	0
					7-Chloroethyltheophylline						
18	201	470	132	1.0	357	132	0.0	485	152	0.9	105
19	201	589	144	0.9	455	144	0.1	536	240	1.0	60
20	201	470	136	0.9	340	134	0.0	405	144	1.0	60
										Avg.	75
					8-Chlorotheophylline						
21	193	517	144	0.9	250	144	0.0	285	172	0.0	0
22	193	455	114	0.9	273	116	0.1	350	120	0.2	7
23	193	430	122	0.9	323	124	0.1	357	140	0.0	0
										Avg.	2
					Desoxytheophylline						
24	152	500	144	0.9	290	144	0.1	430	160	0.9	42
25	138	546	160	0.9	470	156	0.1	556	194	0.9	33
26	138	517	136	0.9	500	140	0.0	517	164	1.0	49
										Avg.	41
					2,6-Diaminopurine Sulfate						
27	205	625	140	0.9	500	136	0.1	625	136	0.9	32
28	205	577	160	1.0	333	152	0.0	600	132	0.9	10*
	* Cardiac arrhythmias developed after drug									Avg.	21
					7-Diethylaminoethyltheophylline						
29	289	508	136	0.7	400	148	0.6	250	152	0.0	0
30	289	470	146	1.0	344	132	0.0	250	132	0.0	0
31	289	577	120	0.9	465	120	0.0	250	120	0.0	0
										Avg.	0

EXP. NO.	DOSE (mgm./l.)	CONTROL C.O.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.		DURATION (min.) C.I.
					1,3-Dimethyl 5,6-diaminouracil							
32	142	525	112	0.9	410	112	0.0	415	112	0.0		0
33	142	525	132	0.8	357	132	0.0	405	132	0.0		0
											Avg.	0
					1,3-Dimethyluric Acid							
34	163	500	168	0.9	430	164	0.1	430	164	0.3		35
35	163	500	160	0.8	285	148	0.0	250	144	0.5		58*
36	163	460	132	0.9	333	132	0.0	250	132	0.0		0
											Avg.	17
	*Cardiac massage necessary after drug, therefore, omitted from calculation											
					7-Ethyltheophylline							
37	173	546	142	0.9	357	140	0.0	485	144	0.9		75
38	173	500	120	0.8	410	120	0.0	500	120	1.0		50
39	173	546	112	0.8	415	116	0.0	508	116	0.7		30
											Avg.	51
					Guanidine HCl							
40	159	566	120	0.9	405	128	0.3	536	184	1.0		76
41	159	470	144	1.0	273	136	0.0	455	152	1.0		80
											Avg.	78
					Hypoxanthine							
42	113	525	144	1.0	385	144	0.1	462	144	0.0		7
43	113	517	144	0.9	375	144	0.1	455	144	0.6		10
											Avg.	9
					Imidazole							
44	57	600	140	1.0	333	136	0.0	280	136	0.0		0
45	57	600	144	0.9	485	140	0.0	430	144	0.0		0
46	113	652	152	0.9	357	152	0.0	0	0	0.0		0
											Avg.	0

EXP. NO.	DOSE (mgm./l.)	CONTROL C.O.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.	(min.) DURATION C.I.
					Isocaffeine						
47	161	577	140	0.9	430	140	0.0	546	140	0.0	0
48	161	500	160	1.0	250	160	0.0	430	144	0.0	0
										Avg.	0
					Nicotinamide						
49	400	589	144	0.9	455	144	0.1	577	156	0.9	10
50	400	460	132	1.0	333	132	0.0	350	128	0.0	0
51	400	556	144	0.9	300	144	0.0	365	140	0.0	0
										Avg.	3
					Orotic Acid						
52	130	566	140	0.9	316	136	0.0	485	136	0.0	0
53	130	440	136	0.9	316	136	0.0	285	140	0.0	0
										Avg.	0
					Sodium Salicylate						
54	133	556	142	0.9	430	144	0.2	333	144	0.0	0
55	133	556	144	0.9	415	144	0.1	300	144	0.0	0
										Avg.	0
					Theobromine						
56	150	525	112	0.9	410	112	0.0	536	104	0.9	30
57	150	577	134	0.9	430	120	0.0	577	132	1.0	83
58	150	500	136	0.9	405	136	0.0	485	132	0.9	72
59	150	500	116	0.9	415	132	0.0	500	132	0.9	32
60	150	556	142	0.9	333	144	0.0	546	140	0.9	39
61	150	460	132	0.9	333	132	0.0	440	132	0.9	50
										Avg.	51

EXP. NO.	DOSE (mgm./l.)	CONTROL C.C.	CONTROL H.R.	CONTROL C.I.	FAILURE C.O.	FAILURE H.R.	FAILURE C.I.	AFTER DRUG C.O.	AFTER DRUG H.R.	AFTER DRUG C.I.	(min.) DURATION Δ C.I.
					Theophylline Monohydrate						
62	165	470	144	1.0	250	144	0.0	492	144	1.0	56
63	165	485	160	1.0	316	160	0.2	357	184	1.0	63
64	165	440	164	0.9	308	152	0.1	588	200	1.0	40
65	165	500	168	1.0	308	164	0.2	517	212	1.0	77
66	165	500	140	1.0	250	140	0.1	500	168	1.0	90
67	165	770	136	0.9	477	136	0.0	750	160	1.0	45
68	165	566	120	0.7	308	120	0.0	455	152	0.9	35
										Avg.	58
					1,3,7-Triethylxanthine						
69	196	536	140	0.9	405	136	0.0	440	160	0.9	41
70	196	430	136	0.9	250	132	0.3	415	164	0.9	31
										Avg.	36
					Uracil						
71	93	600	144	0.9	500	140	0.0	430	144	0.0	0
					Uric Acid						
72	154	455	130	1.0	250	132	0.0	250	132	0.1	8
73	154	500	120	0.9	385	120	0.1	375	120	0.1	0
										Avg.	4
					Xanthine						
74	139	600	160	0.9	500	164	0.1	577	172	0.7	22
75	139	600	136	0.7	508	136	0.1	500	136	0.5	20
76	139	652	136	0.9	455	140	0.1	556	136	0.8	26
										Avg.	23

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