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Phylogenetic Development of Cardiovascular Control

Thomas Kenny Akers
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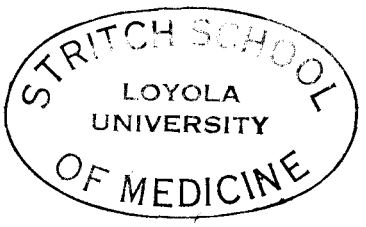
PHYLOGENETIC DEVELOPMENT

OF CARDIOVASCULAR CONTROL



BY

THOMAS KENNY AKERS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

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BIOGRAPHY

Thomas Kenny Akers was born in Brooklyn, New York, January 16, 1931.

He was graduated from Fenwick High School of Oak Park, Illinois in June, 1948. In September of the same year he entered DePaul University, Chicago, Illinois. He entered the United States Navy in March, 1951 and was released to the retired list in January, 1954. He resumed his studies at DePaul University and received the Degree of Bachelor of Science in June, 1956.

He entered the Graduate School of Loyola University of Chicago in September, 1956. He was a Standard Oil Fellow from 1958 to 1959. In June of the same year he received a Master of Science Degree. He continued his graduate studies and was awarded a Royal E. Cabell Fellowship in 1961.

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INTRODUCTION

The broad aim underlying the investigation reported in this thesis concerns the description of some aspects of the phylogenetic development of cardiovascular control. This is accomplished by comparing cardiovascular responses to specific procedures in representative species of reptiles, birds, and mammals. To better understand the basis of this investigation, it is necessary to outline the course of evolution of the reptiles.

We have sufficient knowledge to be able to trace the early stages of reptilian evolution with considerable certainty. The most amphibian-like forms are placed in the order Cotylosauria, the 'stem reptiles', known mainly from the Permian, though certainly existing already in the Pennsylvanian. Seymouria, found in the lower Permian, perhaps two hundred twenty million years ago, is the classic example of these animals. It was a lizard-like creature, about two feet long, living on insects and perhaps some larger animals. Its characteristics are so exactly intermediate between those of amphibians and reptiles that it is not possible to place it definitely with either group and many zoologists class it with amphibia. There is no doubt that these animals were very close to the earliest amphibia and therefore not far removed from the fish ancestors from which the whole group of tetrapods had arisen fifty million years earlier, at the end of the Devonian period. Yet Seymouria and its allies show distinct tendencies toward adaptation for land life, and from them there soon arose a great number of different types, which came to dominate not only the land but also the sea and air throughout the subsequent Mesozoic period.

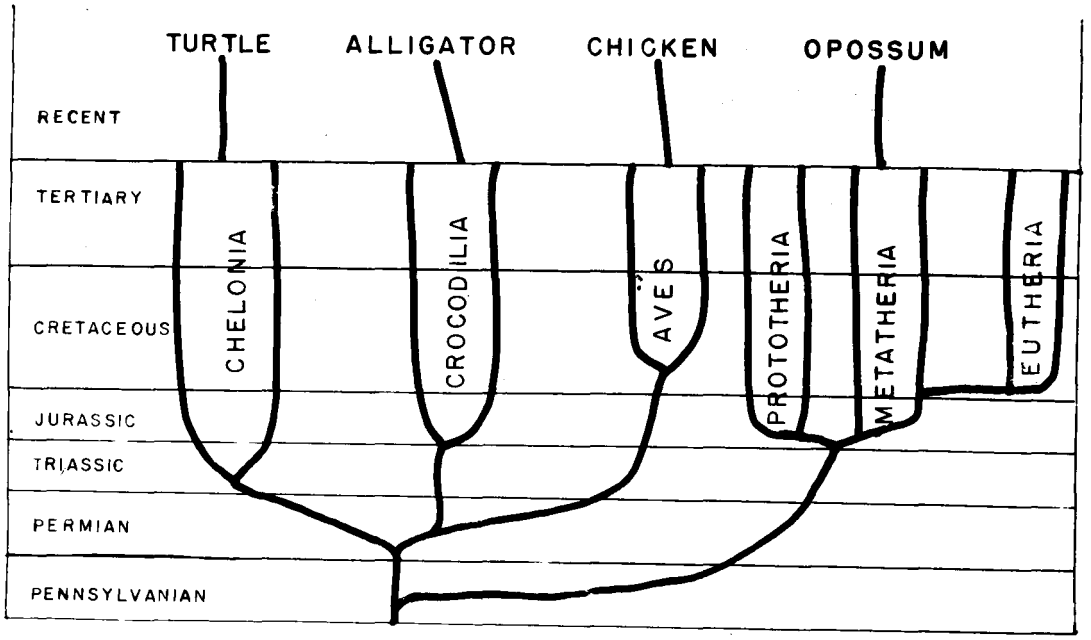


Figure 1

A brief outline of the evolution of reptiles, birds and mammals

After the Cotylosauria, the reptile tree branched into three main stems (see Fig. 1). The first, the Anapsida branch, gave rise to the Chelonia during the Triassic age and these have existed as turtles to the present. Encased in an armored shell the tortoises and turtles have retained some of the features of the earliest anapsid reptiles. Because the present turtles have so many anatomical features similar to those found in fossil remains dated two hundred million years ago, they represent a group of animals from which the function of the primitive cardiovascular system may be learned. Because of this turtles were used in this study.

A second branch, the Diapsida, split during the Permian to give rise to the Squamata, forerunner of the snakes and lizards, and the Pseudosuchia. The Pseudosuchia gave rise to a vast number of forms which became extinct by the end of the Cretaceous. Also from the Pseudosuchia arose the Crocodilia which exist today as the crocodiles and alligators. These animals date back to the end of the Triassic about one hundred sixty million years. From the same root the Ornithischia, ancester of the birds, arose. Two accidental finds have shown us an intermediate stage in the evolution of birds from reptiles. These are called the Archaeopteryx. The Solenhofen state in Germany is a fine-grained solidified clay, originally laid down in shallow fresh water in Jurassic times. About one hundred fifty million years ago, two Archaeopteryz, about the size of large crows, fell into mud and their bones and feathers were preserved in the clay. In 1861, one was discovered by workmen quarrying the slate; another was found in 1872. These toothed half-reptilian birds must be very close to the ancestors of the modern birds. Because none of the intermediate forms exist today, the alligator, a present day Crocodilia was used to present a primitive branch of the Pseudosuchia. For a more

modern branch, the chicken was used.

The third branch the Synapsida gave rise to the mammals. During the early Jurassic the Prototheria branched from the main stem. These exist today as the Montotremata of which there are only a few forms still extant. These are the egglaying mammals, the duck billed platypus and the spiny anteater. They exist only in the isolated geographical pocket, Australia. The next major branch was the Metatheria which arose during the middle of the Jurassic. This branch gave rise to the Marsupials.

The pouched mammals, Marsupials, though essentially very similar to placentals, are primitive in many characteristics and they diverged from an early stage of the main mammalian stock; with the insectivora they show us what mammals were like in the late Cretaceous period. Today they are found mainly in Australasian region, with a few representatives in North and South America, but in Eocene times they occurred in Europe and they have presumably become extinct by the competition which has been imposed by the placentals.

The opossums were the earliest group to appear and the other families have probably evolved from them. They are arboreal, mainly insectivorous animals, with a prehinsile tail, indigenous to the southern United States. Similar forms are found back to the Upper Cretaceous, about seventy million years ago, and the American opossums are certainly the closest living marsupials to the ancestors of the group. Perhaps they are the least modified of all therian mammals. Therefore because of this and their availability, opossums were used in this study.

Several different lines of evidence converge to show that all the Eutherians (placentals), have been derived from small insectivorous animals

living in the Cretaceous period, about one hundred million years ago. From these forms arose all of the modern placental mammals (1, 2).

Our knowledge of the phylogenetic development of the control of the cardiovascular system is fragmentary. A fair amount of work has been done in fish and amphibians as well as birds and mammals. The cardiovascular system in reptiles covers an important stage in the physiologic phylogeny of circulation because the reptiles are the link between fish and amphibians on the one hand, and birds and mammals on the other.

When comparing the cardiovascular responses in reptiles with those found in birds and mammals it is necessary to know what type of responses have been found in lower forms in order to see if there is any evolutionary trend present.

Fishes

No determinations of the cardiac output of fishes by direct methods, for example by application of the Fick principle, appear to exist. Hart (3) compared the volumes of blood in the ventricles isolated by ligatures in systole or in diastole and computed cardiac outputs of 2.24-5.5 gms/500 gms body weight per minute. Other workers used the same and other indirect methods and obtained results of similar magnitude. The mean blood pressure proximal to the gills in electric eels is 16-18 mm. Hg. However other workers quote higher values ranging up to 40 mm. Hg. (4,5,6,7). It was observed that the larger the fish the higher the blood pressure.

Section of the cardiac branches of the vagi in fishes causes an increase in heart rate and there is abundant evidence that stimulation of the peripheral end of the cardiac vagus has an inhibitory effect on heart rate,

which is abolished by atropine. Jullien and Ripplinger (8) reported that the perfusate collected from the eserinized hearts of fishes during vagal stimulation acted on a leech muscle in the same fashion as acetylcholine. This result is consistent with the conception of the vagus as a cholinergic nerve.

Though it is generally accepted that fishes possess a sympathetic nervous system, there is no good evidence for any effect of the sympathetic nervous system on the heart and circulation. There are however a few observations which make it unwise to completely dismiss the possibility that the sympathetic nervous system plays a role in the control of the fish cardiovascular system. The heart rate of Scyliordinous canicula increases when the ductus venosus is stimulated. Blood pressure is higher in intact fish than in pithed fish. The circulatory system responds to epinephrine and nor-epinephrine, but the production of these substances could be elsewhere than at nerve endings (9).

Small doses of epinephrine (4-10 micrograms) cause prolonged rises of blood pressure in elasmobranchs (4) and in the eel (9). The prolonged pressor effect of epinephrine suggest that the enzyme systems for the inactivation of catechol amines, if present, have a low rate of activity. Amine oxidases are present in fish as in all animals with chromaphil tissue (10).

Amphibians.

The mean arterial pressure in the frog is 30 mm. Hg. (11). Regulation of blood pressure in the amphibians contrast sharply with the regulation in the fish. Stimulation of the spinal nerves in the frog causes vaso-constriction of limb blood vessels and visceral vessels (12). It was established by experiments on the frog that acetylcholine is the substance released from the

vagus nerve (13). Gaskell (14) has shown that the frog and reptile hearts are innervated by both parasympathetic and sympathetic nervous systems.

Reptiles.

Recently attention has been focused on the circulation and hemodynamics of the reptilian heart. Considerable work has been directed toward the descriptive anatomy of the reptilian heart; however, very little work has been done to substantiate the functional interpretations that have arisen from the study of the structure. White (15) studied the blood oxygen levels in aorta, atrium and pulmonary artery and showed a relatively complete separation of aerated and non-aerated blood in the heart of Caiman sclerops. Steggerda and Essex (16) recorded simultaneously the cardiac and arterial pressures of the turtle and studied the oxygen levels on both sides of the circulatory system. Johansen (17, 18) studied the circulatory dynamics of the three chambered snake heart. An area in the medulla of the turtle has been postulated to represent an area for central control of blood pressure (19).

Birds.

Values for normal arterial blood pressure of birds range from 100 to 200 mm. Hg. However, the determinations have been made on relatively few birds and considerable variation exists among the same species. The pressure in the male is significantly higher than that in the female (20,21), and pressure tends to increase with age after maturity, particularly in the female. Rodbard and co-workers believe that there is a direct central control of blood pressure in the chicken. There is some evidence which suggests that it is in the thalamus, according to Dijk (22), who showed that stimulation of this area produces changes in blood pressure. The effects of catechol amines on the

chicken have received little attention. According to the meager data available, the effects appear to be the same as in the mammal (23, 23A).

The role of the sympathetic and parasympathetic innervation of the heart, the role of humoral agents (epinephrine, and nor-epinephrine) have not been adequately compared in the various forms.

MATERIALS AND METHODS

1. Animals and Anesthesia

The animals used in this study were chosen on two bases: their position on the phylogenetic tree, and their availability to this laboratory. The turtles used were obtained from Wisconsin and were of three varieties; Graptemys geographica, Emys blandingi, and Chrysemys pictabelli. Altogether 36 turtles were used in this study. They measured between 7 to 8 inches in shell width and weighed between 600 and 900 grams. Twenty-four alligators, Alligator mississippiensis, were obtained from Louisiana for this study. All of these animals were between 2 and 3 feet in length and their weights ranged from 500 to 700 grams.

Eleven opossums, Didelphis virginianus, were obtained from the Carolinas and were all young adult males weighing between 1.5 and 3 kg. The chickens were obtained from poultry markets and were caponized. There were twelve chickens of mixed variety; White Leg, White Rock, and Plymouth Rock, weighing on an average one and a half kilograms. The reptiles, turtles, and alligators were anesthetized with chloral hydrate. Urethane, nembutal and chloralose were tried with very poor results. In the turtles the optimal dose of chloral hydrate which kept the animal lightly anesthetized was 1 millogram per gram of body weight. It was administered intraperitoneally by inserting a long twenty-two gauge needle through the skin fold of the hind leg close to the plastron and up into the abdominal cavity. It usually took one hour from the time of injection until the animal was anesthetized. The signs of anesthesia used were the lack of corneal reflex and complete flaccidity of the neck and leg muscles. In the alligator the optimal dose of chloral hydrate

which kept the animal lightly anesthetized was one milligram per gram of body weight. It was administered intraperitoneally with a short twenty-six gauge needle inserted between the scaly plaques of the animals abdomen. The signs of anesthesia used were the lack of corneal reflex and the lack of leg withdrawal upon toe pinching. In the chickens and opossums the anesthetic used was α-chloralose disolved in carbowax. It was administered into a convenient vein. In both species the dose found effective for light anesthesia was one hundred milligrams per kilogram of body weight (29,30). The signs of anesthesia used in these animals were the lack of corneal reflex and lack of deep palm response. Chloral hydrate is a chlorinated derivative of ethyl alcohol. The chlorine acts as an oxidizing agent on tri-chloral acidaldehyde ($C\text{-}Cl_3\text{-}CHO$). Hydration of chloral yields chloral hydrate ($C\text{-}Cl_3 \cdot CHOH^2$) a crystalline substance (13). Alpha-chloralose is a condensation product of chloral and one of the glycosides. It was found that chloralose works better in smaller doses in the warm blooded animals than chloral hydrate. This is because the solvent is a very viscous substance requiring a large surface vein for proper injection. Inasmuch as in alligators and turtles the surface veins of large enough size for cannulation by a needle are difficult to find; chloral hydrate was used instead of chloralose.

2. Recording Techniques

Records of the pressure pulse, blood pressure, and heart rate from a convenient artery in all the animals were obtained by a means of a Statham P23A transducer coupled to a 5P1 Grass preamplifier which was coupled to a Grass model 5A driver amplifier. This in turn was coupled to the Grass Direct writing oscillograph. The P23A pressure transducer has a nominal

pressure range of zero to seventy five centimeters of mercury and an approximate natural frequency of thirty nine cycles per second at ninteen hundreths critical damping with a twenty gauge needle that was five centimeters in length. The 5P1 preamplifier is a chopper modulated and demodulated high gain, low noise, low frequency, DC preamplifier which has a frequency response flat to forty cycles per second. Drift is less than 3 microvolts per hour, and is random. The input is designed to drive balance controls and excite the strain gauge transducer for pressure. The model 5A driver amplifier is a push pull, two stage direct coupled amplifier with a differential input. Its primary function is to amplify signals from the polygraph preamplifier sufficiently to drive the direct writing oscillograph. It also supplies voltages to operate the associated preamplifier. The maximum sensitivity of the driver amplifier combined with the pen writer oscillograph is greater than one hundred millivolts per centimeter. The entire system has a frequency response of forty cycles per second.

The general principle of transducer operation is as follows: the pressure in arterial blood is transmitted through a closed fluid system to the transducer. Any alteration in pressure in the fluid is transmitted directly to the diaphragm of the transducer. The transducer is a strain gauge type in which a strain wire resistor (two limbs of a Wheatstone bridge) is attached to the center of the diaphragm. Any alteration in the diaphragm changes the length of the strain wire resistor and changes its resistance, thus unbalances the bridge and alters the output voltages of the transducer. The output voltage is then suitably amplified to drive the pens of the oscillograph.

An integrating cardiotachometer (24) was added to the system at this point. The input of the tachometer was taken from the driver amplifier output of the channel recording blood pressure. The active triggering signal was the pressure pulse. The output of the tachometer was connected to another channel of the polygraph.

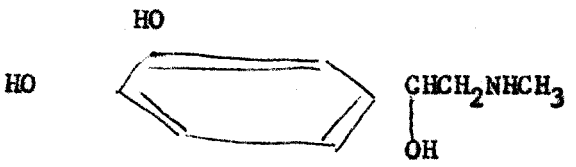
The recording techniques used in this study are capable of faithfully reproducing events occuring in the pulse pressure curve to the tenth harmonic if the frequency of the heart doesn't exceed four beats per second. This is perfectly adequate for pulse pressure study in the turtle, alligator and opossum. However this system cannot be used to study the shape of pulse pressure curves in the chicken.

3. Stimulation Techniques

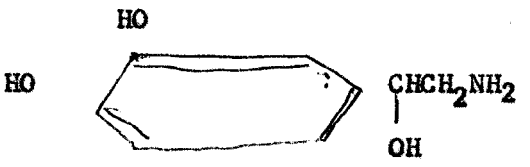
Controlled alterations of the cardiovascular system in these animals were obtained in two ways. First electrical stimulations of the vagus nerve or the sympathetic chain were obtained utilizing a Porter electrode coupled to a Grass S5 square wave stimulator with controlled, oscilloscope monitored, frequency, duration and voltage. The second method of bringing about changes in the cardiovascular system was to inject ℓ-epinephrine and ℓ norepinephrine.

Epinephrine may be viewed as composed of two major components. The aromatic portion of the molecule consists of 1,2-dihydroxybenzene (catechol); the aliphatic portion consists of ethanolamine. Beta-phenylethylamine may be considered as the parent compound. To form epinephrine, substitutions are made on the benzene ring (3,4-dihydroxy), the beta carbon (OH), and the

amino group (CH_3); only the alpha carbon remains without further substitution.



Norepinephrine, 1-2 amino 1-(3,4-dehydroxyphenyl) ethanol, differs from epinephrine merely by the absence of methyl substitution in the amino group (13).



4. Procedure

Turtles

After the turtles were anesthetized with chloral hydrate, the cephalad third of the plastron was removed by sawing through the plastron between the first and second row of bony plaques. In removing the plastron, care was taken to strip the foreleg muscle attachments from the plastron. The brachial artery was cannulated with a PE 100 polyethylene catheter for blood pressure recording. A longitudinal incision was made in the skin of the neck and the vagus nerve and sympathetic nerves were exposed. The right and left vagi were stimulated separately with varying voltages, durations and frequencies. The right and left sympathetic trunks were stimulated in the neck and on the first four ganglia in the thorax with and without the vagus nerves

intact. After the vagus nerves were cut, they were stimulated centrally and also peripherally before and after the administration of Atropine (ten gamma per kilogram of body weight). In some turtles epinephrine and norepinephrine were administered intravenously and intra-arterially. In 4 turtles the brain was stimulated electrically by passing a concentric bipolar needle electrode through the foremen magnum up into the center of the brain. The parameters of stimulation were as follows. The voltages ranged from eight to thirty volts. The duration was two milliseconds and the frequency ranged from one to one hundred and fifty per second. The brain was examined histologically to ascertain the point of stimulation. Circulation time was estimated by injecting india ink into one of the mesenteric veins and timing the appearance in the celiac artery.

Alligators

When anesthesia had been induced by chloral hydrate in the alligators, a longitudinal incision was made in the ventral aspect of the neck, and the carotid artery exposed and cannulated with a PE 100 polyethylene catheter for blood pressure recording. The vagus nerve was exposed for stimulation. However, stimulation procedures involving the vagus usually brought about death of the animal within five minutes. Therefore this procedure was abandoned and atropine administered in place of vagisection. The sympathetic trunks were exposed by a dorsal approach to the thorax. Epinephrine and norepinephrine were injected intravenously and intra-arterially.

In 5 alligators small holes were drilled through the skull so that a concentric bipolar needle electrode could be introduced. Various areas in the brain were stimulated and the brains were kept for histological workup after the experiment was terminated. The spinal cord at the T-12 level was stimulated in one animal. Circulation time was estimated in the alligator in the same manner as in the turtle.

Opossums

Ether was used as a preanesthetic and while the opossum was unconsious a cutdown was performed on one of the hind legs and a vein was exposed for the injection of the chloralose. The carotid arteries and vagus nerves were exposed and one carotid artery was cannulated with a PE190 polyethylene catheter; for blood pressure recording. The trachea was cannulated to allow a clear airway. In some animals the vagus was stimulated, sectioned and the cut ends stimulated. In others the chest was opened and the sympathetic trunks on both sides were stimulated electrically. In some animals epinephrine and norepinephrine were administered intravenously. After the animal was sacrificed the hearts were examined for lesions.

Chickens

After the chickens were anesthetized with chloralose and in some cases nembutal, the brachial artery in the wing was cannulated with a PE60 polyethylene catheter for blood pressure recording and the brachial vein was cannulated for intravenous administration of epinephrine and norepinephrine.

5. Analysis of Data

In the normal situation the dynamic equilibrium of the arterial system is such that the stroke volume of the heart equals the outflow from the arteries into the capillaries. If however this equilibrium is altered in any way, such as changes in heart rate, changes in stroke volume, or changes in peripheral resistance, the stroke volume is disproportionate to the peripheral outflow until a new state of dynamic equilibrium exists.

Wiggers (25) studied this problem in an artificial circulation model in which each of the factors could be regulated separately. Such studies showed that an increase in heart rate caused a greater rise in diastolic pressure than in systolic pressure, thus reducing pulse pressure. Increase in peripheral resistance elevates diastolic pressure more than systolic pressure until diastolic distensibility begins to diminish drastically; then systolic pressure rises progressively faster than diastolic until the pulse pressure exceeds normal. Increase in stroke volume raises systolic pressure more than diastolic, thereby increasing the pulse pressure above normal.

Keeping in mind that in the intact animal the situation is much more complex and the majority of cardiovascular changes obtained are mixed responses, the dynamics of the artificial circulation model are applicable.

In this study, the pressure pulse curves from the polygraph were analysed in the following manner. An increase in pulse pressure obtained primarily by a rise in systolic pressure was interpreted as an increase in the force of myocardial contraction, a rise in blood pressure with a con-

comitant rise in diastolic pressure with little or no change in pulse pressure was interpreted as a vasoconstrictor response in the absence of large increases in heart rate.

Changes in heart rate were determined by direct inspection of the polygraph record of the integrating cardiotachometer.

The changes in cardiovascular function described above have been supported in general by the investigations of Cotten (26), Randall (27), and Rushmer (28).

EXPERIMENTAL RESULTS

I. Turtle

In all the turtles the control systolic pressure was between 9 and 20 millimeters of mercury (mm. Hg.). The control diastolic pressure was between 4 and 18 mm. Hg. and the control heart rate was in the range of 14 to 35 beats per minute. These values are similar to those obtained by other workers (39).

The circulation time from the mesenteric vein to the celiac artery, measured in two animals, was 20 seconds. This is about the same as the circulation time for equal anatomical distances in mammals and birds (39). But it is much shorter than that measured in the eel for a comparable distance (9).

A. Vagal Stimulation.

In the first series of 6 animals the vagi were dissected free of surrounding tissue in the neck and stimulated with Porter electrodes, using various parameters of stimulation. The voltage was varied between 0.5 and 10 volts at each step of duration and frequency. The duration was varied between 0.1 and 5 milliseconds (msec.) by the following steps: 0.1, 0.2, 0.8, 1, 2, 5 and 10 msec. The following steps in frequency were used, 1, 2, 4, 5, 10, 20, 50, 70, 100 and 200 cycles per second (cps).

The minimum frequency of stimulation necessary to elicit a significant cardiac response from either vagus nerve at any voltage and duration was 4 cps. When the frequency of stimulation was increased, the percentage

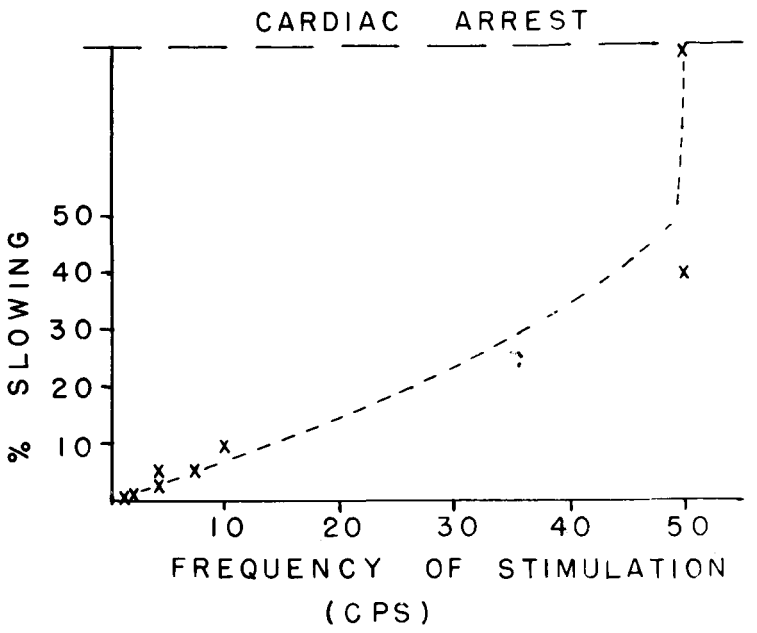


Figure 2

Effect of stimulation frequency on cardiac slowing induced by stimulation of the right vagus nerve in the turtle. Voltage=3.0 v; pulse duration=5 msec.

of slowing increased until complete cardiac arrest occurred. Figure 2 illustrates this point. When the right vagus was stimulated with 3 volts at 5 msec., there was a gradual decrease in the heart rate as the frequency of stimulation was increased. At 50 cps and above, complete cardiac arrest occurred. The least voltage required to slow the heart was found to be 2 volts if the duration exceeded 0.2 msec. in the right vagus. In the left vagus the frequency and duration characteristics were found to be the same as in the right vagus. However the intensity of stimulus required to affect the heart was found to be approximately 1.5 volts. The intensity of stimulation necessary to cause cardiac effects in these animals is of the same magnitude as that reported as rheobase for auricular effects by Fredericq and Garrey (40).

When stimulating a nerve such as the vagus and observing the effect on the contraction of a muscle, there are a number of factors to consider. In irritable tissue, the excitation process develops faster the stronger the intensity of the stimulus. Hence there is interaction between the voltage and duration. When an effector such as cardiac muscle is interposed between the stimulus and the observed effect, then the frequency of stimulus becomes a factor. Further, when stimulating a multi-fibered nerve such as the vagus the contact of the electrode on the surface alters the voltage required to obtain an effect. However, certain general relations between the parameters of stimulation and the resultant cardiac effects can be stated; 1) increases in voltage within physiological limits cause decreases in heart rate until

cardiac arrest occurs, 2) slowing of the heart rate can be obtained by increasing the duration of the stimulus at constant voltage and frequency and 3) heart rate is progressively slowed with increasing frequency of stimulation.

B. Sympathetic Stimulation

In a second series of 6 animals the right and left sympathetic trunks, including the last three cervical and first four thoracic ganglia were stimulated. The parameters of stimulation were the same as those used in the survey of the vagi. Stimulation of the right sympathetic trunk at all parameters elicited no change in blood pressure. However there was a slight increase in rate (6%) in 3 animals when the frequency of stimulation exceeded 50 cps. This response appeared to be frequency dependent because it could not be obtained with higher voltages at frequencies below 50 cps.

Only one animal with left sympathetic trunk stimulation responded with a change in blood pressure and heart rate. In this animal the blood pressure and heart rate decreased when the left cervical sympathetic trunk was stimulated. The parameters of stimulation were 3.5 volts, 4 msec. and 2 cps. This response was repeated three times before the electrode was moved. After moving the electrode it could not be obtained. It is felt that this response was due to a leakage of current into the vagal fibers. In the area in which the electrode was placed there was a close proximity of vagal and sympathetic fibers with some intermixture of the two systems. This animal was not atropinized, but the vagus nerve had been severed higher up in the neck. In other animals which were atropinized, stimulation

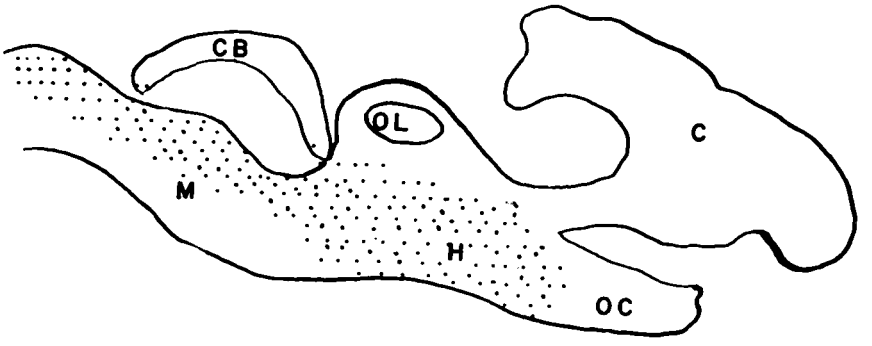


Figure 3

Sagittal section of a turtle brain.

C-cerebrum
M-medulla
OC-optic chiasma
CB-cerebellum
OL-optic lobe
H-hypothalamus

Scale above drawing is in 0.5 cm.

in this area produced no change in blood pressure or heart rate. Except for this one instance no effect could be elicited by stimulation of the left sympathetic trunk.

C. Stimulation of the Central Nervous System

In a series of 4 turtles an electrode was inserted into the brain by way of the foramen magnum. The shaded area in figure (3) indicates the areas stimulated in these animals. With the vagi intact in these animals, stimulation at 7-10 volts, 2 msec, and 60 cps. caused a great decrease in heart rate and a slight depression of blood pressure. Section of the vagi abolished this effect.

If the stimulus intensity is increased to 20 volts in the vagotomized animal, pressor responses are elicited. The responses are summarized in the following table:

Table 1

Stimulation Parameters				Pressure Response			
						After 1mg. C10	
Animal	Volts	msec.	cps.	Control	Experimental	Control	Experimental
12	20	2	60	28/18	45/33	27/15	29/16
13	26	2	60	32/13	35/22	26/15	27/15
14	30	2	60	35/18	40/22	37/21	37/21
15	40	2	60	31/16	40/27	31/13	30/13

Pressures in the above table are expressed as systolic/diastolic in mm. Hg. The heart rate decreased slightly in animals 13 and 14 and remained the same in the others. In all cases the systolic and diastolic pressures were increased by the central stimulation. But generally the pulse pressure did

not change. It should be noted that the responses produced by central stimulation were characterized by a rapid onset (less than 3 sec.) after the stimulus was applied and a sudden decay as soon as the stimulus was discontinued. Concomitant with the blood pressure effects there were also gross sustained contractions of body muscles. These responses could be abolished by the administration of 1 mg./kg. of Decamethonium (C10) intravenously. It thus appears that cardiovascular responses to stimulation of the central nervous system in the turtle are indirect, and result from hemodynamic changes in the turtle associated with skeletal muscle contraction.

D. Cardiovascular Changes Induced by the Administration of Epinephrine and Norepinephrine.

Typical cardiovascular responses to epinephrine and norepinephrine in one turtle are shown in figure 4. In this experiment there was a greater change in diastolic pressure induced by epinephrine than by norepinephrine. However, there is considerable variability in this response. The collective data are shown in Tables 2 and 3. Both agents increase diastolic pressure significantly. Epinephrine causes a slightly greater increase in pulse pressure sure than epinephrine. However both agents significantly increase pulse pressure. The heart rate changes induced by the two agents are relatively small. The rate of change induced by norepinephrine is statistically not significant. If we roughly equate increased diastolic pressure with increased peripheral vasoconstriction; then epinephrine is the more effective vasoconstrictor substance in the turtle. If we equate increased pulse pressure with increased myocardial contraction; then both drugs seem to be acting equally. It should be noted that the total duration of blood pressure

response to both drugs was of the magnitude of 500 seconds for all of the animals regardless of the dose level. Furthermore no correlation could be obtained between the size of the dose and the response elicited. However there does appear to be a tendency for the smallest doses of epinephrine to be more effective than equal doses of norepinephrine. The lack of any response from sympathetic stimulation seem to indicate that these catechol amines may not play any great role in normal cardiovascular control as direct neuroeffector transmitters. The long response time appears to indicate either absence of or very low activity of amine oxidases. This animal may be quite like the fish (3) in this respect.

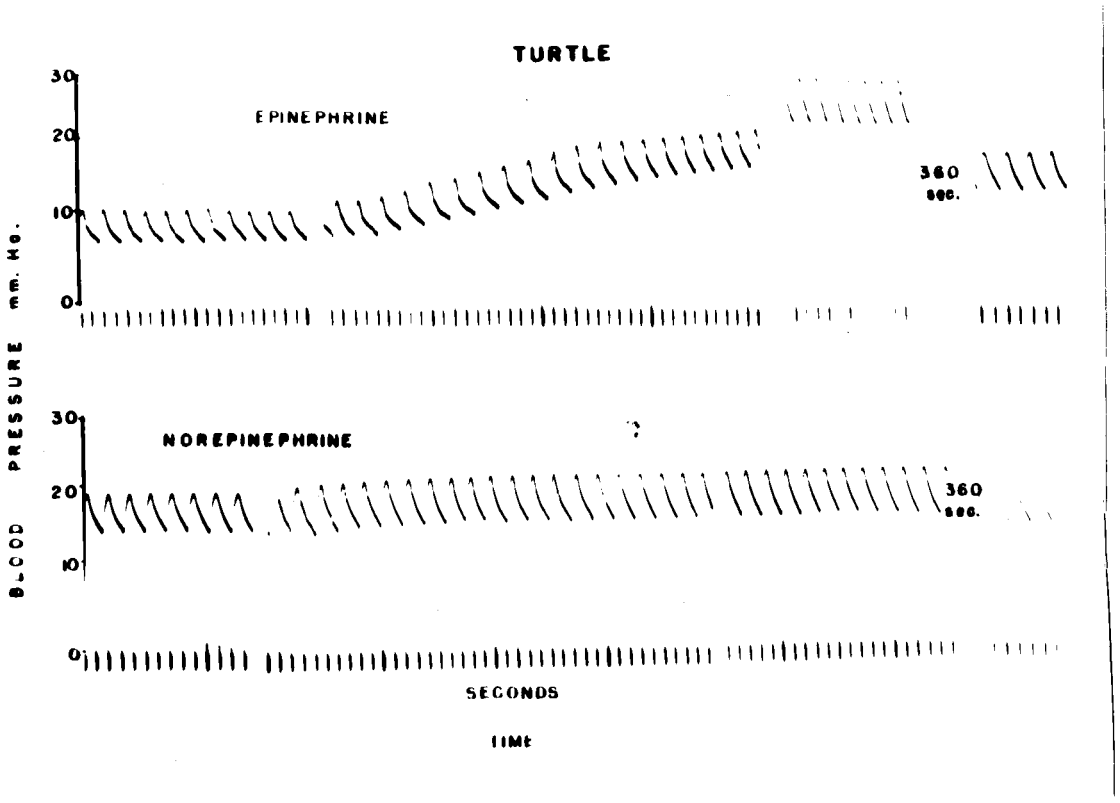


Figure 4

Comparison of the epinephrine and norepinephrine response in the turtle. The amount of epinephrine administered was 0.5 gamma per kilogram. The amount of norepinephrine administered was 3 gamma per kilogram.

Table 2

Cardiovascular Changes in the Turtle Induced by Epinephrine

Animal T	Dose /kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
2	0.5	10	7	3	35	25	18	7	40
3	0.2	18	13	5	35	22	14	8	35
7	2	20	13	7	30	20	13	7	30
8	3	16	12	4	18	18	14	4	23
9	0.1	18	12	6	18	25	16	9	22
11	0.25	19	16	3	24	30	18	12	32
14	1	20	12	8	16	19	12	7	15
15	1	19	13	6	14	26	15	11	14
16	4	9	4	5	15	18	9	9	15

Note that averages are expressed as % change from control.

Percent Change Above Control

	% S.E.	P
Diastolic Pressure	40 ± 18	.05
Pulse Pressure	88 ± 29	.05
Heart Rate	10 ± 10	.05

Table 3

Cardiovascular Changes in the Turtle
Induced by Norepinephrine

Animal T	Dose /kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
2	3	18	13	5	30	22	14	8	40
3	0.25	19	13	6	35	20	14	6	35
7	0.5	18	12	6	30	20	13	7	30
8	2	17	12	5	18	18	14	4	25
9	1	11	7	4	21	15	9	6	15
11	2	16	12	4	24	25	13	12	15
14	10	17	13	4	24	33	18	15	14
15	4	11	6	5	18	24	15	9	22
16	0.1	10	6	4	16	14	6	8	16
4	0.25	10	5	5	15	13	6	7	15

	Percent Change Above Control % S.E.	P
Diastolic Pressure	27 ± 14	.05
Pulse Pressure	80 ± 27	.05
Heart Rate	2.7 ± 8.5	---

E. Pressure Pulse

The pressure pulse of the turtle was analysed under three different conditions: control, after epinephrine administration and after norepinephrine administration. The pulses were analysed for the following: 1) duration of one cycle, 2) the percent represented by systole as marked by the dicrotic notch, 3) the percent represented by diastole and 4) the rate of change of arterial pressure (43) given in mm. Hg. per msec. The results in the turtle are averaged in the following table:

Table 4

	Control	Epinephrine	Norepinephrine
Cycle (sec.)	2.0	1.8	2.0
Systole (%)	35.0	43.0	41.0
Diastole (%)	65.0	58.0	59.0
Rate of change (mm. Hg./msec.)	0.013	0.029	0.023

The decrease in cycle time is indicative of an increase in heart rate with administration of epinephrine. Under epinephrine there has been a decrease in the proportion of the cycle taken up by diastole indicating that with a modest heart rate increase in the turtle the diastolic filling time is reduced. However the same thing occurs with norepinephrine without an increase in rate. Perhaps this means that the prolongation of systole is due to a more complete contraction due to the action of norepinephrine on the ventricle. Without analysis of ventricular pulses to determine the role

of the isometric period in this effect, no conclusion can be made. By examining the rate of change of ejection data, it may be concluded that epinephrine and norepinephrine both increase the initial velocity of myocardial contraction. However epinephrine increases it to a greater extent. This data supports the conclusions derived from pulse pressure values in Tables 2 and 3.

F. Other Observations and Discussion.

In the turtle which has a low pressure cardiovascular system, the systolic-diastolic pressure relationship is proportional to the same relationship found in birds and mammals. The average control blood pressure as measured in this study and as reported by others (39, 16) is 16 mm. Hg. for systole and 10 mm. Hg. for diastole. Both of these values are approximately one-seventh to one-eighth of the values seen in man. The circulation time is approximately the same as that of man for the same relative distances.

The turtle heart contracts relatively slowly. However there is a relatively rapid phase of ejection. This is to be expected as the pulmonary and systemic aortas are invested with stiff connective tissue (visually observed). With careful observation, the volume of blood ejected with each beat can be seen to fill the arterial system. Between each beat the major arteries with the exception of the aortas appear to decrease in size and flatten. When blood is ejected its course can be followed quite easily. Therefore in the turtle the cardiovascular system is characterized by; 1) a slowly contracting heart, 2) a stiff aorta and 3) very low resistance arteries. These factors account for the relatively rapid ejection of blood

out of the heart.

It should be noted that bilateral vagotomy in the neck does not alter the blood pressure and heart rate in these turtles. This indicates that the vagus is not exerting a tonic inhibition under the conditions used in these experiments. The central stimulation experiments indicate that there is a cardio-inhibitory area in the brain. Because vagisection abolishes the effect of central stimulation it may be concluded that the pathway for cardio-inhibitory impulses is the vagus.

This study did not demonstrate any effect of sympathetic stimulation on the heart and blood pressure. We cannot completely rule out sympathetic control in the turtle because of the effects of the catechol amines. It is known that these particular catechol amines are produced in the adrenals of these animals (37). If the catechol amines from the adrenals are active then 1) there must be a slow rate of destruction because of the long duration of response, and 2) they must be relatively unspecific in their action on myocardial contractility because both increase pulse pressure about equally.

No gross lesions were seen on the endocardial surfaces of any of the turtle hearts.

II. Alligator

The average control value of blood pressure of all the alligators used was as follows; systolic pressure was 25 mm. Hg., diastolic pressure was 15 mm. Hg. The average heart rate was 18 beats per minute. These values are slightly less than those reported in the literature for crocodiles (39).

Circulation time was obtained in these forms in the manner previously described. The average time for the circulation of india ink from the mesenteric vein to the mesenteric artery was 20 seconds. This was repeated in 2 animals. This value is similar to that found in the turtles.

A. Vagal Stimulation

When the vagus nerve in the alligator was dissected out for stimulation procedures, many of the animals died. In every animal in which the vagi were sectioned death occurred within 5 minutes. Stimulation procedures were carried out on the vagi in 3 animals in which the vagi were left intact. In one animal stimulation of the right vagus brought about cardiac arrest. The only effect observed from vagal stimulation (right or left) in the rest of the animals, was a slight decrease in both systolic and diastolic blood pressure. Because of the rapid deterioration of the animals after vagisection, the vagus was left intact for the other procedures applied (drug injections).

B Sympathetic Stimulation

In a series of 4 alligators the right and left sympathetic trunks were stimulated. There was no change in blood pressure and heart rate observed in this procedure. This finding is in agreement with the results

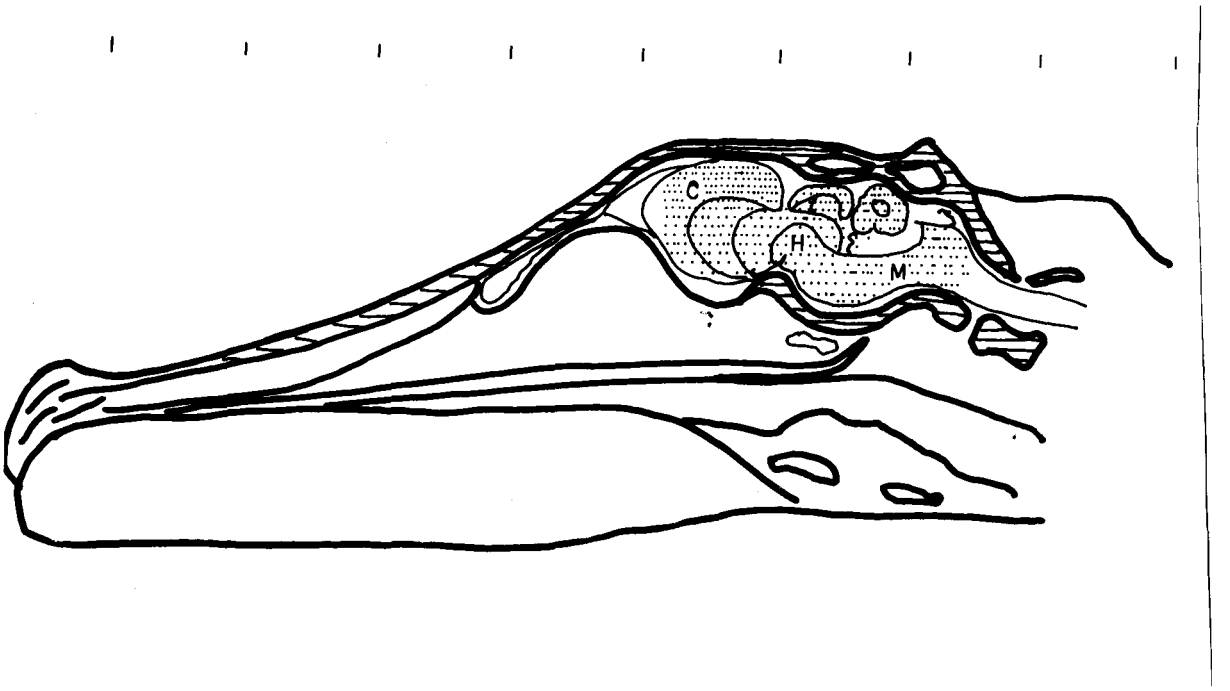


Figure 5

Sagittal section of an alligator head. C-cerebrum, H-hypothalamus, M-medula. The cross-hatched area represents the bone of the skull. The shaded area represents the areas that were stimulated. Each mark is 1 cm.

reported in the literature (14).

C. Central Stimulation

Holes were drilled in the skulls of 5 alligators and electrodes placed in verious parts of their brains. Figure 5 shows the areas of the brains that were stimulated. When the electrode was in the area of the medulla, stimulation of 3 volts, 2 msec. and 30-100 cps. decreased the heart rate and caused it to become very irregular. Stimulation in the posterior hypothalamic area usually produced about 25% increase in heart rate. In this area a slightly higher voltage was necessary. None of the other areas had any effect on heart rate. Altogether 70 stimulations of intensity in excess of 5 volts were applied to various areas in the brains of the alligators. There was no significant change in blood pressure from these stimulations. In some areas the stimulus produced eye movements and nictitating membrane contractions. In other areas the stimulus produced contraction of the neck muscles. The data are shown in Table 5.

In one animal a laminectomy was performed at the level of the twelvth thoracic vertebra. The spinal cord was then stimulated at 20 volts, 2 msec. and 50 cps. This stimulus produced gross motor movements of the tail but did not produce any change in blood pressure or heart rate.

D. Cardiovascular Changes Induced by the Administration of Epinephrine and Norepinephrine.

The results of all the experiments in this series are summarized in Tables 6 and 7. In all the animals receiving epinephrine, the diastolic pressure was increased very significantly above the control values. This

Table 5

Site	Stimulation V	Stimulation D	Stimulation D	Control S	Control D	Control HR	Experimental S	Experimental D	Experimental HR
Medulla	3	5	30	25	9	19	25	9	17
Medulla	3	2	100	28	11	17	25	8	15
Medulla	8	5	70	25	8	17	17	5	8
Medulla	5	2	50	24	7	16	23	7	12
Post Hypo.	10	2	70	21	6	15	20	6	18
Post Hypo.	5	2	30	20	5	16	20	5	20
Post Hypo.	8	2	50	20	5	16	20	5	22
Post Hypo.	5	5	50	45	36	20	45	36	24
Post Hypo.	15	2	50	32	22	24	33	22	27

Table 6

Cardiovascular Changes in the Alligator Induced by Epinephrine

Animal A	Dose γ/kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
1	1	22	12	10	12	35	22	13	16
2	5	14	2	12	6	28	12	16	8
3	2	28	16	12	17	47	35	12	18
4	1	17	10	7	15	27	18	11	16
5	10	25	15	10	16	45	35	10	21
7	1	24	13	11	15	32	21	11	16
8	3	27	18	9	25	38	25	13	25
9	1	32	22	10	24	36	26	10	24
11	.1	26	17	9	18	32	20	12	19
12	10	28	19	9	35	48	36	12	40

	Percent Change Above Control % ± S.E.	P
Diastolic Pressure	79 ± 14	.001
Pulse Pressure	23 ± 5	.05
Heart Rate	12 ± 3	.001

Table 7

Cardiovascular Changes in the Alligator Induced by Norepinephrine

Animal	Dose	Control				Experimental			
	γ/kg	S	D	P	HR	S	D	P	HR
1	3	22	9	13	12	26	12	14	15
2	1	22	9	13	12	16	8	8	18
3	10	26	17	9	18	32	20	12	19
4	3	27	21	6	18	31	19	14	18
5	5	35	25	10	16	37	25	12	16
8	2	27	12	15	13	25	6	19	13
9	1	21	2	19	10	22	2	20	16
10	1	31	20	11	22	35	22	13	23
12	0.1	31	20	11	22	32	22	10	22

	Percent Change Above Control	
	% S.E.	p
Diastolic Pressure	0±15	---
Pulse Pressure	21±15	---
Heart Rate	12±7	.05

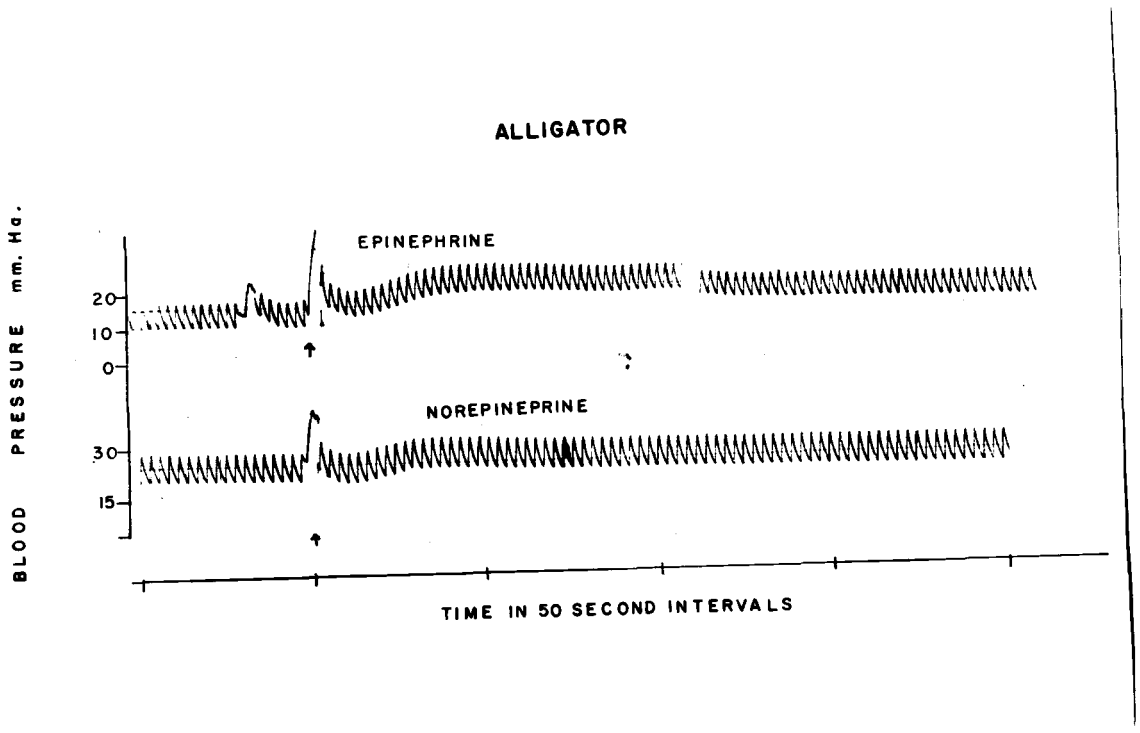


Figure 6

Comparison of the epinephrine and norepinephrine response in the alligator. The amount of epinephrine administered was 1 gamma per kilogram. The amount of norepinephrine administered was 3 gamma per kilogram.

is illustrated in Figure 6. The pulse pressure was increased with epinephrine but only slightly more than with norepinephrine. The average change in heart rate was the same for both agents. However there was considerably less variability for this response with epinephrine than with norepinephrine. The changes in diastolic pressure with norepinephrine were so variable that no significance could be attached to them. In fact, when averaged there was no change.

The total duration of response to both agents was the same, 200 seconds. This is a long time when compared to the duration in higher animals but is much shorter than the duration in the turtle. It may indicate that the catechol amine breakdown system is present, but because of the lower body temperature of this form, the rate of metabolism is slower. The duration and amplitude of the response could not be correlated with the size of the dose of the catechol amine. As in the turtle, however, it appears that the smaller dose of epinephrine is more effective in producing cardiovascular responses then the smaller dose of norepinephrine.

E. Pressure Pulse Analysis.

The pressure pulse of the alligator was analysed in the same way as that of the turtle. The results are summarized in the following table:

Table 8

	Control	Epinephrine	Norepinephrine
Cycle (sec.)	3.2	2.9	3.0
Systole (%)	28.0	14.0	25.0
Diastole (%)	72.0	86.0	75.0
Rate of Change (mm. Hg/msec.)	0.045	0.060	0.042

The decrease in cycle time indicates an increase in heart rate. This data indicates a greater increase in heart rate under epinephrine than under norepinephrine and supports the data derived from heart rate counts. Under epinephrine there was a great reduction in the systolic portion of the cycle. But it must be noted that these animals had a very high diastolic pressure as compared to control levels due to the vasoconstrictor effect of epinephrine. Examination of rate change of arterial pressure indicates that epinephrine has a greater effect on the velocity of myocardial contraction than norepinephrine. However it must be remembered that this measurement is derived from a brachial pulse and not a true picture of the pressure rise in the ventricle. Undoubtedly this is much greater because of the greater pressure that the ventricle must overcome to eject blood into the arterial system.

F. Other Observations and Discussion

In the alligator the systolic-diastolic pressure relationship is proportional to the same relationship found in mammals and birds. The average control blood pressure as measured in this study is 25 mm. Hg. systolic and 15 mm. Hg. diastolic. This is about one-fifth of the values

seen in man. The circulation time is approximately the same as that of man for a comparable anatomical distance.

Because of the deleterious effects associated with vagisection, it appears that an intact parasympathetic innervation of the heart is essential in this form. This may be due to the fact that the alligator is a diving animal and the vagus is involved in diving reflexes or it may be involved in the respiratory mechanism. Autopsy of a vagotomized alligator revealed that the right heart, especially the atrium, is congested with blood and the lungs are light in color.

This study did not demonstrate any effect of sympathetic stimulation on the heart and blood pressure. However sympathetic activation can not be ruled out completely because of the central effects and the catechol amine effects. Stimulation in the area of the posterior hypothalamus caused an increase in rate. This increase in rate could be due to a number of possible mechanisms; 1) direct effect on the heart by way of the sympathetics, 2) indirect effect by an inhibition of vagal tone and 3) an indirect effect by the release of catechol amines from the adrenals.

Because no effects could be obtained from stimulation of the sympathetic trunk the first possibility may be ruled out. Because norepinephrine and epinephrine both affect the heart it is possible that this central effect is mediated by release of norepinephrine and/or epinephrine from the adrenals. However the magnitude of effect on heart rate is not as great as that found with central stimulation. Therefore the third possibility may play a part in this response. Due to the fact that the vagi were not sectioned

in these animals, the third possibility cannot be assessed. However vagisection is deletorious to the heart and there is a cardio-inhibitory center in the medulla. At least in one animal vagal stimulation caused cardiac arrest.

Therefore the posterior hypothalamic stimulation effects are probably mediated by both, inhibition of the cardio-inhibitory center (41) and indirect action through catechol amine release from the adrenals.

No gross lesions were seen in any of the alligator hearts.

III. Opossum

The normal control systolic pressures in the opossum ranged from 55 to 185 mm. Hg. and diastolic pressures ranged from 30 to 130 mm. Hg. The control heart rates ranged from 140 to 240 beats per minute.

A. Vagal Stimulation.

In a series of 6 animals the vagus nerves were stimulated with various parameters. Stimulation of the peripheral end of a sectioned left vagus produced decreases of blood pressure and heart rate. The parameters of stimulation necessary to produce the first change from control were 6 volts, 5 msec, 5 cps. No further changes could be obtained after the voltage was raised to 8 volts. If after this the frequency was increased to 20 cps. the pressure dropped to 50% of control levels and the heart rate dropped to 25% of control levels. At higher frequencies the heart stopped.

When the peripheral end of a sectioned right vagus was stimulated with 8 volts, 5 msec. and 30 cps, cardiac arrest occurred and was followed by vagal escape.

Stimulation of the central end of cut vagi produced increases in systolic, diastolic and pulse pressures as well as heart rate, similar to those reported following central vagal stimulation in the cat (42). The minimum stimulation generally required is 4 volts, 5 msec., 20 cps. This response occurred 3 seconds after the stimulation was applied and did not return to control levels until 4 minutes after the stimulation had ceased.

The following table summarizes these results.

Table 9

	Parameters			Control			Experimental		
Lt. Vagus Cent.	V	D	C	S	D	HR	S	D	HR
A	4	5	20	60	40	140	175	130	170
B	4	5	30	155	120	170	165	120	170
C	8	5	30	100	75	190	122	85	210
Rt. Vagus Cent.									
A	4	5	20	80	60	150	220	170	180
B	4	5	20	85	75	140	200	170	180
C	4	5	30	100	70	190	120	80	210

These animals all developed endocardial lesions. None of these animals were used in the norepinephrine and epinephrine survey but were used in the work on the vagus and sympathetic trunks.

B. Sympathetic Stimulation

The thoracic sympathetic trunks including the stellate ganglia were stimulated in 4 animals. The parameters of stimulation were 4-10 volts, 5 msec. 10-30 eps. Stimulation of the right stellate ganglion caused an average increase of 20% in heart rate, 5% in pulse pressure and 8% in diastolic pressure. Left stellate ganglion stimulation caused no change in heart rate and an average change of 5% in pulse pressure and 10% in diastolic pressure.

In one animal the left stellate ganglion was stimulated while both the inferior vena cava and descending aorta were occluded. In this case the diastolic and pulse pressure both increased approximately 20% above control levels.

C. Cardiovascular Responses Induced by the Administration of Epinephrine and Norepinephrine.

Tables 10 and 11 summarize the effects of epinephrine and norepinephrine in the opossum. Both agents caused a significant increase of diastolic pressure above control levels. However epinephrine appears to cause a greater increase in diastolic pressure and pulse pressure. Again the response to epinephrine was greater than that to norepinephrine. However only epinephrine caused a significant increase in heart rate. (Fig. 7)

It should be noted that the total duration of response differed for the two durgs. On the average epinephrine responses lasted 50 seconds while norepinephrine responses lasted 200 seconds. There appeared to be no correlation between the dose given and the amplitude of the response.

The first three animals were in very poor physical condition, due to their transportation during very hot weather. They were dehydrated and appeared to have some respiratory ailment. Therefore they were not used in determining the average changes.

It should be noted that in the animals numbered 1, 3 and 7, there were gross endocardial lesions. In animals 1 and 7 they were very small but in animal 3 there were two very large lesions, one on the papillary muscle and the other just opposite on the septum. All the lesions were found in the left ventricle. Animal 3 had been used for sympathetic stimulation.

D. Pressure Pulse Analysis

Pressure pulses were analysed in the manner previously described.

Table 10

Cardiovascular Changes in the Opossum Induced by Epinephrine

Animal O	Dose /kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
1	5	25	10	15	200	215	160	55	270
2	0.5	55	30	25	240	110	85	25	270
3	1.25	40	20	20	210	100	70	30	260
4	2	57	42	15	150	180	140	40	220
6	1	185	130	55	150	270	200	70	210
6	1	155	110	45	170	190	140	50	180
7	5	145	110	35	160	180	135	45	175
7	5	160	110	50	150	200	130	70	170
8	3	150	100	50	140	180	115	65	150
8	2	55	30	25	160	85	60	25	270
9	1	80	50	30	240	145	105	40	270

Percent Change Above Control

	% S.E.	-p
Diastolic Pressure	72±25	.01
Pulse Pressure	41±16	.01
Heart Rate	23±11	.05

Table 11

Cardiovascular Changes in the Opossum
Induced by Norepinephrine

Animal O	Dose /kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
1	2.5	45	25	20	210	187	135	50	250
1	2.5	35	15	20	210	160	120	40	240
2	1	35	15	20	200	80	45	35	240
3	2.5	30	10	20	200	135	105	30	250
4	3	160	110	50	140	165	115	50	140
6	1	130	90	40	180	150	105	45	150
6	5	115	80	35	160	140	100	40	140
7	5	155	105	50	150	180	120	60	150
8	2	75	40	35	170	180	150	30	360
9	2	85	55	30	120	105	70	35	130
9	2	80	50	30	110	125	75	50	110

Percent Change Above Control

	% S.E.	P
Diastolic Pressure	57±26	.01
Pulse Pressure	16±10	.01
Heart Rate	12±8	---

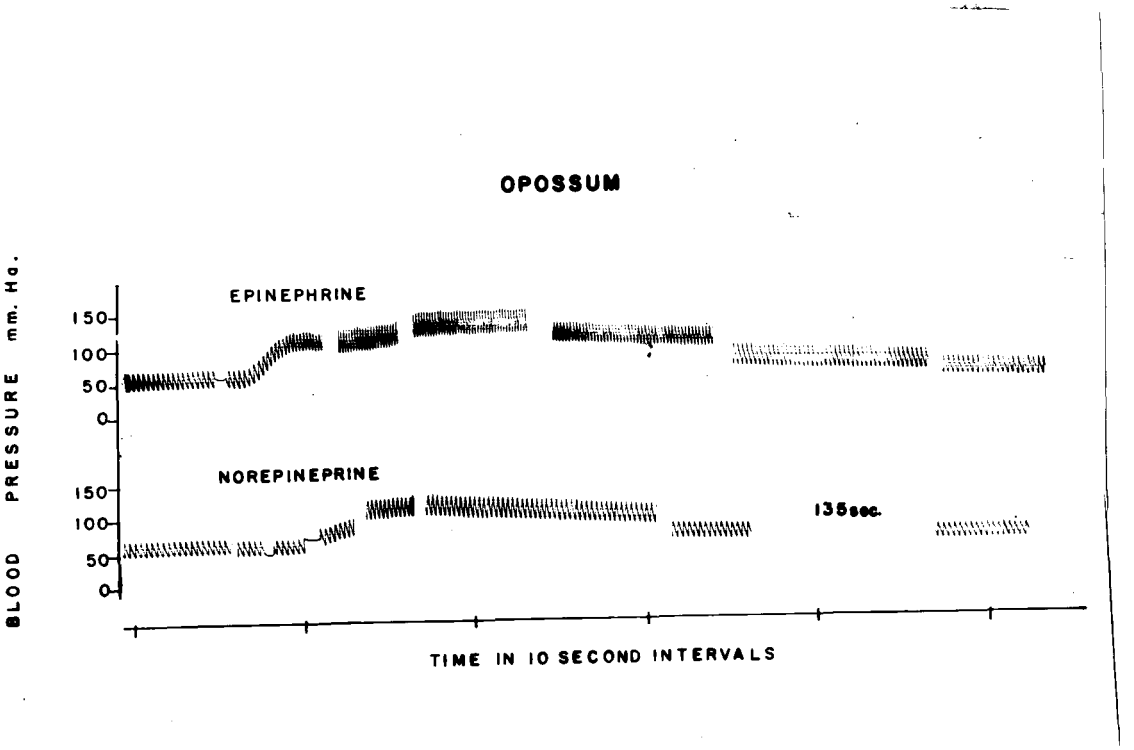


Figure 7

Comparison of the epinephrine and norepinephrine response in the opossum. Dose was 2 gamma/kg.

The results on the opossum are summarized in the following table.

Table 12

	Control	Epinephrine	Norepinephrine
Cycle (sec.)	.40	.30	.35
Systole (%)	30.0	44.0	40.0
Diastole (%)	70.0	56.0	60.0
Rate of Change (mm. Hg./msec.)	0.225	0.375	0.325

The decrease in the duration of the cycle indicates an increase in heart rate with both epinephrine and norepinephrine. However the duration of the cycle decreases to a greater extent with epinephrine. This supports the data derived from the cardiotachometer. The results indicate that the diastolic portion of the pressure pulse was decreased. This means that the diastolic filling time in the opossum is reduced with increases in heart rate. In this respect the opossum is similar to the turtle but is different from the alligator. Examination of the rate of change of the anacrotic limb of the pulse shows that with epinephrine and norepinephrine the heart increases pressure at a more rapid rate than normal. Epinephrine is slightly more effective than norepinephrine in this respect.

E. Discussion

The opossum has a cardiovascular system which reacts in the same manner as other mammals. Bilateral carotid occlusion, done in two animals, produced increases in diastolic pressure, pulse pressure and heart rate. Vagal stimulation produced cardiac arrest followed by vagal escape. The

vagal escape was much easier to obtain with the right vagus. Central vagal stimulation produced increases in systolic, diastolic and pulse pressure as well as heart rate. Right stellate stimulation produced heart rate changes primarily while the left stellate stimulation resulted in diastolic and pulse pressure changes without heart rate changes. All of these effects are similar to those found in eutherian mammals.

Endocardial lesions were found in the left ventricles in 6 of 11 of these animals. These lesions were found in animals which showed a greater than average pulse pressure change. In fact the largest lesions were found where the pulse pressure change exceeded 40% above control.

IV. Chicken

The average control values of blood pressure for the anesthetized chickens used in this study were as follows: 1) systolic pressure was 112 mm. Hg., 2) diastolic pressure was 94 mm. Hg. The average heart rate was 300 beats per minute.

A. Cardiovascular Responses Induced by the Administration of Epinephrine and Norepinephrine.

In the chicken both catechol amines significantly increased diastolic pressure. However epinephrine was almost twice as effective as a vasoconstrictor substance. Epinephrine had a much greater effect on the change in pulse pressure than norepinephrine. Heart rate decreased with epinephrine and did not change with norepinephrine. In this series the vagi were intact. If the vagi are cut or atropine administered the heart rate increases with epinephrine. Under these conditions norepinephrine causes a slight increase in heart rate. The experimental results of the animals with intact vagi are summarized in tables 13 and 14.

Figure 8 illustrates the typical cardiovascular responses to the catechol amines in one chicken. Careful inspection of the records shows that the duration of the response to epinephrine is twice as long as the duration of response to norepinephrine. In general the duration of the epinephrine response was 60 to 80 seconds while the duration of the norepinephrine responses was 25 to 40 seconds. As in the other forms, it appears that the smaller doses of epinephrine are more effective in producing cardiovascular responses than the smaller doses of norepinephrine.

Table 13

Cardiovascular Changes in the Chicken Induced by Epinephrine

Animal C	Dose /kg	Control S	Control D	Control P	Control HR	Experimental S	Experimental D	Experimental P	Experimental HR
1	1	105	85	20	340	125	100	25	320
1	2	105	85	20	340	130	100	30	320
2	5	125	110	15	350	165	135	30	280
2	5	120	105	15	350	165	135	30	330
3	1	115	100	15	340	145	125	20	320
3	5	125	105	20	330	180	135	45	252
4	0.5	115	100	15	330	150	125	25	260
5	3	130	110	20	315	165	130	35	260
6	1	150	125	25	240	180	135	45	180
7	2	100	35	65	240	120	60	60	250
8	5	65	45	20	370	120	100	20	370
9	5	75	60	15	370	155	125	30	375

	Percent Change Above Control % S.E.	P
Diastolic Pressure	39±4	.001
Pulse Pressure	58±13	.01
Heart Rate	-12±3	----

Table 14

Cardiovascular Changes in the Chicken
Induced by Norepinephrine

Animal C	Dose /kg	Control S	D	P	HR	Experimental S	D	P	HR
1	1	110	90	20	340	115	95	20	360
1	2	100	80	20	340	120	100	20	360
2	5	115	100	15	350	150	125	25	340
2	5	115	100	15	350	155	130	25	375
3	1	120	105	15	310	135	120	15	300
3	5	110	95	15	300	140	115	25	300
4	0.5	105	80	25	330	130	110	20	300
4	3	110	95	15	330	130	110	20	365
5	0.1	105	80	25	350	130	110	20	360
6	5	165	135	30	330	185	145	40	330
7	2	100	60	40	300	155	55	100	300
8	5	70	50	20	360	80	65	15	370
9	5	65	50	15	370	75	65	10	380

Percent Change Above Control

	% S.E.	P
Diastolic Pressure	20 ± 3	.001
Pulse Pressure	26 ± 13	----
Heart Rate	1 ± 1.6	----

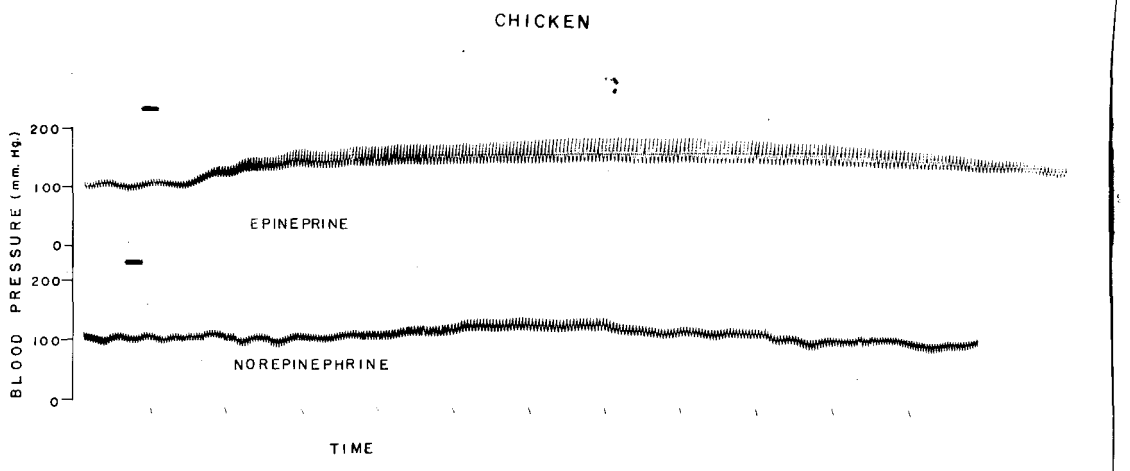


Figure 8

Comparison of the cardiovascular effects of 5 gamma per kilogram of epinephrine and 5 gamma per kilogram of norepinephrine. Each time mark is 5 seconds.

B. Pressure Pulse Analysis

The pressure pulse of the chicken was analysed in the same manner as the other species in this study. The results are summarized in Table 15.

Table 15

	Control	Epinephrine	Norepinephrine
Cycle (sec.)	0.2	0.24	0.2
Systole (%)	50	33	50
Diastole (%)	50	67	50
Rate of Change (mm. Hg./msec.)	0.575	6.25	2.0

Increase in the cycle time with epinephrine supports the cardiotachometer evidence of heart rate decrease. In the chicken the systolic and diastolic portions of the pressure pulse are equal during the control periods and with norepinephrine. However the rate of change of arterial pressure increases greatly with norepinephrine. These pulses have a slightly lower dicrotic notch than the control pulses. With epinephrine the systolic portion of the pulse quantitatively decreases and the dicrotic notch is very high on the pulse. The decrease in the systolic portion is probably due to the great increase in the rate of change in arterial pressure with epinephrine. The high diastolic pressure and steep slope of the pulse with a high dicrotic notch all indicate that the distensibility of the aorta has been diminished.

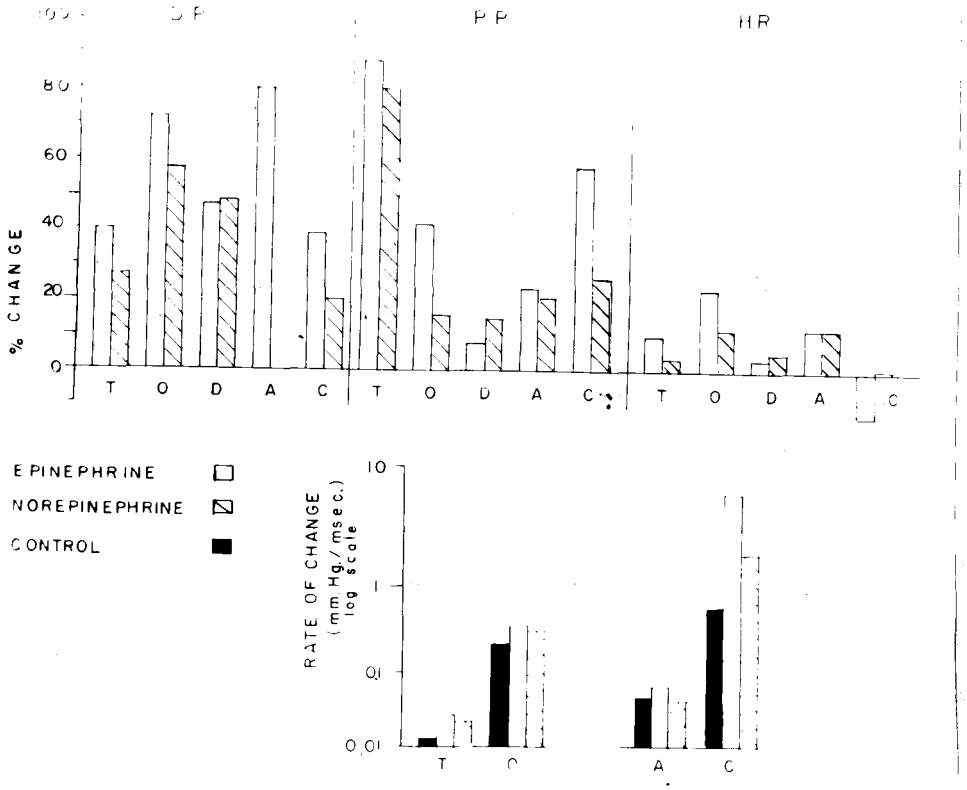


Figure 9

This figure summarizes the results of epinephrine and norepinephrine in all the animals.

T=Turtle **A=Alligator**

O=Opossum **C=Chicken**

D=Dog

However this data may very well not be a true representation of pulse pressure in the chicken. Because of the high heart rate, the time transients exceed the fidelity of the recording system.

V. Comparison of the Epinephrine and Norepinephrine Responses in all the Species.

For a more complete comparison of reptiles with birds and mammals, it was necessary to obtain data on the action of epinephrine and norepinephrine in a dog or cat. Recently a series of dogs were given small doses of epinephrine and norepinephrine and their cardiovascular responses were studied in our laboratory (44).

Figure 9 summarizes all the results of catechol amine administration in this study.

If increased diastolic pressure is equated with increased vasoconstriction due to the catechol amine action, epinephrine acts as a good vasoconstrictor substance in all of the animals, particularly in the alligator. Norepinephrine acts as a vasoconstrictor substance in all animals except the alligator. With the exception of the dog epinephrine is more effective.

Epinephrine increases the heart rate to a greater extent in the turtle and opossum than norepinephrine. In the alligator both catechol amines increase the heart rate to the same extent but epinephrine was effective at a lower dose. In the chicken heart rate actually decreases with epinephrine.

Increased pulse pressure and increased rate of change of arterial pressure may be equated with increased myocardial contraction until diastolic distensibility diminishes with high diastolic pressure. Epinephrine acts on the myocardium of all the animals to a greater extent than norepinephrine though both act on the myocardium.

General Discussion

Very little is known about the function of the autonomic nervous system in the protochordates and agnatha but the vagus seems to exert some control over the heart and gut of cyclostomes. Clear-cut domains of autonomic function become recognizable in the elasmobranchs, in which vagus inhibitory control of the heart (46) and sympathetic motor effects on the abdominal viscera (31) have been established. In teleost still further effectors come under sympathetic control, particularly chromatophores and swim bladder but more important the blood vessels. In the Amphibia there is good evidence of extension of sympathetic control to the heart (12, 14).

Analysis of evidence leads Colin Nicol to the conclusion that the autonomic nervous system of vertebrates shows two main independent lines of evolution from some simple level such as that found in extant elasmobranchs. These two lines occur in actimopterygians, leading to modern teleosts, and in choanichthyes, leading to Dipnoi and tetrapods (31).

This concept fits the data from protochordates through Amphibia. However there appears to be another division of development in the Reptiles between the Chelonia, Diapsid and Synapsid branches.

In the Chelonia, a very primitive branch, no evidence of sympathetic control of the heart could be demonstrated in this study or by Gaskell (14). The vagus in this form inhibits the heart. However section of the vagus

does not cause increase in heart rate. This indicates that the turtle heart is not under tonic inhibitory action of the vagus. Evidence is presented for a centrally located cardio-inhibitory area. However this study could not show any active areas in the brain for acceleration or augmentation of the heart or for vasoconstriction.

We cannot completely rule out sympathetic control in the turtles because of the effects of catechol amines that were demonstrated. The circulatory system of turtles appears to be slightly more sensitive to epinephrine than norepinephrine.

Analysis of the evidence indicates that the turtle is primitive in cardiovascular control as well as in anatomy. Being one of the earliest branches off of the stem reptiles it is reasonable to expect that the physiology of this form would be closer to the Dipnoi fish physiology than any of the other present reptiles. And it would probably be more primitive than the present day amphibians. This appears to be the case.

The Diapsid group of reptiles, those which were the root of the birds and crodilia, show a different picture of cardiovascular control in present day forms.

In the alligator the vagus nerve seems to be very important in normal function of the heart, because vagisection has very deletorious effects on the animal. Stimulation of the vagus causes cardiac arrest while stimulation of the medulla causes cardiac slowing. This indicates a central cardio-inhibitory area which probably acts through the vagus. In this latter aspect of vagal control both the turtle and the alligator are alike.

This study did not demonstrate any effect of sympathetic stimulation on the heart and blood pressure. This agrees with the findings of Gaskell (14) in the crocodile. But we cannot rule out sympathetic effects entirely. Stimulation in the area of the posterior hypothalamus caused an increase in rate. This rate increase could be due to either inhibition of vagal tone or increase in sympathetic discharge or release of catechol amines from the adrenals.

When administering catechol amines it was noted that epinephrine acted as a vasoconstrictor substance and both are cardio-accelerator substances.

It would appear that cardiovascular control in the alligator is primarily a vagal function. The facts that vagisection is deleterious, that there appears to be a medullary center which is cardio-inhibitory and vagal stimulation slows and stops the heart, establish that the vagus controls the heart. The lack of cardiac response to sympathetic stimulation and catechol amine administration rule out sympathetic control of the heart, at least of such nature as to over-ride the intact vagal control. The effects of hypothalamic stimulation then are probably due to both inhibition of the cardio-inhibitory area in the medulla by the hypothalamus and epinephrine release from the adrenal gland. In the chicken the catechol amine results are quite similar to those seen in the alligator except that the vasoconstriction caused by epinephrine is not as great. No work has been done on the sympathetic system of the chicken so that no conclusions as to the nature of the cardiovascular control can be reached.

In the Synapsid branch of reptiles there appears to be a balanced system of cardiovascular control with both the sympathetic and vagus taking part. This has been well established in the Eutherian mammals. It would appear that in the Metatherians, represented by the opossum, the same basic controlling systems obtain. This is borne out by the fact that vagal and sympathetic stimulations as well as catechol amine administration have the same qualitative results in the opossum as are found in dogs and cats.

This study has established some basis for the supposition that along with different morphological development in reptiles there has been different types of cardiovascular control developed. Furthermore this study adds to the general knowledge of cardiovascular physiology of the reptiles.

SUMMARY

1. Cardiovascular control has been studied in the turtle, alligator, opossum and chicken.

2. In the turtle, no evidence of sympathetic control of the heart could be demonstrated. The vagus decreases heart rate and has central representation in the medulla. The catechol amines effect the cardiovascular system of the turtle.

3. In the alligator, section of the vagi cause death. No evidence of sympathetic control of the heart could be demonstrated. Two areas in the brain were shown to effect the heart rate. Epinephrine acts as a peripheral vasoconstrictor while both catechol amines increase pulse pressure and heart rate.

4. The opossum has a cardiovascular system which reacts in the same manner as other mammals. Carotid occlusion, afferent vagal stimulation, sympathetic stimulation and catechol amine administration increase blood pressure and heart rate. Efferent vagal stimulation causes cardiac arrest.

5. In general epinephrine was more effective than norepinephrine in changing the diastolic and pulse pressures in all the species studied.

6. This study indicates that cardiovascular control has assumed several different forms from the amphibians through the reptiles to the birds and mammals.

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APPROVAL SHEET

The dissertation submitted by Thomas K. Akers has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

June 5, 1961
Date

Clarence M. Lewis
Signature of Adviser