Rama Rao, P. B., *Fed. Proc.,* 1960, v19, 1038.

3. Mellette, S. J., Leone, L. A., *ibid.,* 1960, v19, 1045.

4. Mellette, S. J., *Am. J. Clin. Nutr.,* 1961, v9, 109.

5. Malhotra, O. P., Reber, E. F., *Fed. Proc.,* 1960, v19, 421.

6. Paolucci, Anna Maria, Rama Rao, P. B., Johnson, B. C., *ibid.,* 1961, v20, 452.

7. Metta, V. C., Nash, L., Johnson, B. C., *J. Nutrition,* 1961, v14, 473.

8. Quick, A. J., *J. Am. Med. Assn.,* 1938, v110, 1658.

9. Schjeide, O. A., Binz S., Ragan, N., *Growth,* 1960, v24, 401.

Received November 13, 1962. P.S.E.B.M., 1963, v112.

## Comparative Study of Effect of Epinephrine and Norepinephrine on Cardiovascular System of Turtle, Alligator, Chicken and Opossum.* (28056)

THOMAS K. AKERS† AND CLARENCE N. PEISS (Introduced by W. C. Randall)
*Department of Physiology Stritch School of Medicine and the Graduate School, Loyola University, Chicago, Ill.*

Our knowledge of the phylogenetic development of the control of the cardiovascular system is fragmentary. Relatively little is known about the role of humoral agents in the control of blood pressure in the lower vertebrates. Lutz(1) and Mott(2) have shown that small doses of epinephrine cause prolonged rises of blood pressure in elasmobranchs and eels. Recently, attention has been focused on the circulation and hemodynamics of the reptilian heart. There have been many studies of the reptilian heart, but relatively few correlating structure and function. White(3) studied the blood oxygen levels in the aorta, atrium and pulmonary artery and showed relatively complete separation of aerated and non-aerated blood in the heart of *Caiman sclerops.* Steggerda and Essex(4) recorded simultaneously the cardiac and arterial pressures of the turtle. The circulatory dynamics of the 3-chambered snake heart have been studied(5,6). To our knowledge there have been no studies on the effects of catecholamines in the reptilian heart, and few in the chicken(7). According to the data available, the effects appear to be the same as in the cat and dog, for which there is much experimental evidence. This report, therefore, concerns the comparative reactivity of turtles, alligators, opossums and chickens to epinephrine and norepinephrine.

*Materials and methods.* All animals were anesthetized with chloral derivatives. In the reptiles, chloral hydrate (1 mg per g of body weight) was administered intraperitoneally. In the chicken and opossum the animals were anesthetized with chloralose (100 mg/kg) dissolved in polyethylene glycol 200 (Carbowax), injected intravenously. After induction of anesthesia in the turtles (*Graptymys geographica*), the cephalad third of the plastron was removed and the brachial artery was cannulated with a PE 100 polyethylene catheter for blood pressure recording. Alligators (*Alligator mississipiensis*) were prepared by a longitudinal incision made in the ventral aspect of the neck and cannulation of the carotid artery with a PE 100 polyethylene catheter for blood pressure recording. In the opossum (*Didelphis virginianis*), a hind leg vein was exposed under ether for injection of chloralose. The carotid artery was exposed in the neck and cannulated with a PE 190 polyethylene catheter for blood pressure recording. Epinephrine and norepinephrine were administered intravenously in all species. Records of the pressure pulse, blood pressure and heart rate were obtained by means of a Statham P23A transducer coupled to a Grass Polygraph. The body tempera-

* Supported by a grant from Chicago Heart Assn.

† Royall E. Cabell Fellow, Loyola University. Present address: Dept. of Pharmacology, Stritch School of Medicine.

TABLE I. Average Normal Values.

| Species | No. of animals | Pressure: Systolic mm Hg | Pressure: Diastolic mm Hg | Heart rate, beats/min |
|---|---|---|---|---|
| Turtle | 9 | 15 | 11 | 25 |
| Alligator | 10 | 25 | 15 | 18 |
| Opossum | 9 | 120 | 80 | 190 |
| Chicken | 9 | 112 | 94 | 300 |

ture in the alligators was monitored by a thermistor probe in the cloaca and in the turtle by a thermistor probe in the body cavity underneath the plastron. The body temperature of all reptiles was 23-25°C.

*Results.* The normal values obtained for all species in this study are shown in Table I. These values in general compare favorably with those previously reported(8).

A. Turtle. Typical cardiovascular responses to 3 gamma doses of epinephrine and norepinephrine in one turtle are shown in Fig. 1. The collective data are shown in Table II. Both agents increase diastolic pressure significantly in the turtle, epinephrine becoming more potent than norepinephrine. Both agents significantly increase pulse pressure. The two drugs appear to cause insignificant changes in heart rate. In the absence of significant changes in heart rate, increased diastolic pressure can be attributed to peripheral vasoconstriction(9). Therefore, epinephrine appears to be the more effective vasoconstrictor substance in the turtle. In the absence of significant changes in distensibility, the increases in pulse pressure must result from augmentation of myocardial contraction. Both drugs appear to affect myocardial contractibility equally. It should be noted that total duration of response to both drugs was of the magnitude of 500 seconds for all of the turtles regardless of dose level. The long response time suggests either absence of or very low activity of amine oxidases.

B. Alligator. In the alligator epinephrine was a far more effective vasoconstrictor than norepinephrine, as shown by the changes in diastolic pressure in Fig. 1 and Table II. Both catecholamines showed moderate cardiac action as indicated by pulse pressure and heart rate data. However, there was considerably less variability for these responses with epinephrine than with norepinephrine.

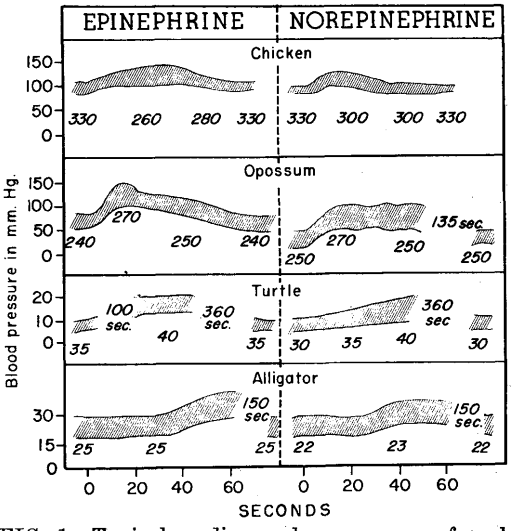


FIG. 1. Typical cardiovascular responses of each species seen with 2 catecholamines. Italic numbers are heart rates counted at that point in the response. Dose used for turtle and alligator was 3 gamma/kg of epinephrine and norepinephrine; for opossum and chicken it was 1 gamma/kg.

Total duration of response to both agents was the same (200 sec), much longer than in higher animals but much shorter than the duration in the turtle.

C. Opossum. Both epinephrine and nor-

TABLE II. Cardiovascular Changes Due to Catecholamines.

| | Turtle | Alligator | Opossum | Chicken |
|---|---|---|---|---|
| | % change ± S.E. | | | |
| *Epinephrine* | | | | |
| Diastolic pressure | 40 ± 18 | 79 ± 14 | 72 ± 25 | 39 ± 4 |
| Pulse " | 88 ± 29 | 23 ± 5 | 41 ± 16 | 58 ± 13 |
| Heart rate | 10 ± 10 | 12 ± 3 | 23 ± 11 | −12 ± 3 |
| *Norepinephrine* | | | | |
| Diastolic pressure | 27 ± 14 | 0 ± 15 | 57 ± 26 | 20 ± 3 |
| Pulse " | 80 ± 27 | 21 ± 15 | 16 ± 10 | 26 ± 13 |
| Heart rate | 2.7 ± 8.5 | 12 ± 7 | 12 ± 8 | 1 ± 1.6 |

epinephrine caused a significant increase of diastolic pressure in the opossum (Table II). However, only epinephrine causes significant changes in pulse pressure and heart rate (Fig. 1).

Total duration of response differed for the 2 drugs. Epinephrine responses averaged 50 seconds in length while norepinephrine responses lasted 200 seconds.

D. Chicken. In the chicken both catecholamines significantly increased diastolic pressure, but epinephrine was almost twice as effective as a vasoconstrictor substance. Epinephrine also had a much greater effect on pulse pressure. Heart rate decreased with epinephrine and did not change with norepinephrine when the vagi were intact. When the vagi were cut (9 exp.) or atropine administered (4 exp.) heart rate increased slightly with both catecholamines. The experimental results in the animals with intact vagi are summarized in Table II.

Fig. 1 illustrates the cardiovascular responses to the catecholamines in one chicken. In general, duration of the epinephrine response was 60 to 80 seconds while that of the norepinephrine response was 25 to 40 seconds.

E. Pressure pulse slope analysis. The pressure pulses of all species were analyzed under 3 different conditions: control, immediately after epinephrine administration, and after norepinephrine administration. The initial rate of rise of the arterial pressure pulse was measured and expressed in mm Hg per msec. The results are averaged in Table III.

By examining the amplitude and initial ejection slope of the pressure pulse in the turtle, it may be concluded that epinephrine and norepinephrine both increase the initial velocity of myocardial contraction. However, epinephrine increases it to a greater extent.

A similar analysis of pressure pulse data in the alligator indicates that only epinephrine has an effect on the velocity of myocardial contraction. In the opossum, however, both drugs are effective but epinephrine produced the greater change.

In the chicken, the rate of rise of arterial pressure with norepinephrine increases more than 3-fold. These pulses have a slightly lower dicrotic notch than the control pulses. With epinephrine the rate of rise increases more than 12-fold, with a decrease in the systolic portion of the pulse and a very high dicrotic notch. The decrease in the systolic portion is probably due to the large increase in the rate of rise in arterial pressure. The high diastolic pressure and steep slope of the pulse with a high dicrotic notch all indicate that distensibility of the aorta has been diminished.

TABLE III. Pulse Pressure Analysis.

| | Control | Rate of rise (mm Hg/msec) | Dose ($\gamma$/kg) |
|---|---|---|---|
| Turtle | Control | .013 | – |
| | Epinephrine | .029 | 2 |
| | Norepinephrine | .023 | 2 |
| Alligator | Control | .045 | – |
| | Epinephrine | .060 | 2 |
| | Norepinephrine | .042 | 2 |
| Opossum | Control | .225 | – |
| | Epinephrine | .375 | 1 |
| | Norepinephrine | .325 | 1 |
| Chicken | Control | .575 | – |
| | Epinephrine | 6.25 | 1 |
| | Norepinephrine | 2.0 | 1 |

*Discussion.* From the data on changes in diastolic pressure, it appears that epinephrine acts as a good vasoconstrictor substance in all 4 species, particularly in the alligator. Norepinephrine acts as a vasoconstrictor substance in all species except the alligator. Epinephrine increases the heart rate to a greater extent in the turtle and opossum than does norepinephrine. In the alligator both catecholamines increase the heart rate to the same extent at optimal doses, but the threshold for epinephrine is lower. In the chicken, heart rate actually decreases with epinephrine, presumably due to reflex activation of the vagal outflow to the heart. Increased pulse pressure and increased rate of change of arterial pressure may be ascribed primarily to increased myocardial contractility until aortic distensibility diminishes with high diastolic pressure(9). Epinephrine acts on the myocardium of all the species to a greater extent than norepinephrine.

It is not known if there are naturally oc-

curring enzyme systems present in all of the forms studied, which are capable of catecholamine degradation. If there is a breakdown system present which is a specific for either epinephrine or norepinephrine, it would suggest that either of these substances could be the adrenergic transmitter responsible for cardiac effects.

In the turtle, total duration of response was long (500 seconds) and equal for both amines. This would indicate that there is an absence or at best very low activity of a relatively nonspecific breakdown system. The long duration of response may simply be the result of non-enzymatic oxidation of the catecholamines. Total duration of response in the alligator was much shorter than in the turtle, although both animals were at the same body temperature. Again, the duration was equal for both amines. This may indicate that a nonspecific catecholamine degradation system is present, but because of the lower body temperature of this form (as compared to mammals), the rate of metabolism is lower, hence a longer duration of response than is found in mammals.

In the opossum, however, average norepinephrine response was 4 times as long as that for equal doses of epinephrine. This may indicate that in this species there is an enzyme system which is much more specific for epinephrine than norepinephrine. From the data on magnitude and duration of response, a tentative hypothesis may be formed that epinephrine is the actual neurotransmitter. However, much more evidence is required in this species concerning specific enzyme systems, catecholamine concentration etc., to document this hypothesis.

The chicken is just the opposite in this respect. In this species the duration of the norepinephrine response is one-half that of the epinephrine response. Thus, it would appear that in the chicken the catecholamine breakdown system is more specific for norepinephrine than epinephrine.

There is evidence that in the fowl adrenal medulla the total proportion of pressor active norepinephrine is from 45 to 65% higher than in mammals(10). This supports the conclusion that norepinephrine may be the adrenergic agent most active in the cardiovascular system of chickens while epinephrine is the adrenergic agent most active in the opossum.

*Summary.* Turtles, alligators, opossums and chickens were given epinephrine and norepinephrine intravenously and their cardiovascular responses compared. The cardiovascular system of all species seems to be more reactive to epinephrine than to norepinephrine, but to various degrees. A tentative hypothesis, that epinephrine is the neurotransmitter in the opossum and norepinephrine is the neurotransmitter in the chicken, is considered.

1. Lutz, B. R., *Am. J. Physiol.,* 1930, v94, 135.
2. Mott, J. C., *J. Physiol.,* 1951, v114, 387.
3. White, F. M., *Anat. Rev.,* 1956, v125, 417.
4. Steggerda, F. R., Essex, H. Z., *Am. J. Physiol.,* 1957, v190, 320.
5. Johansen, K., *Circ. Res.,* 1959, v7, 828.
6. ———, *Acta Physiol. Scand.,* 1960, v43, 346.
7. Rodbard, S., Tolpin, M., *Am. J. Physiol.,* 1947, v151, 509.
8. Altman, P. L., (editor) *Handbook of Circulation,* W. B. Saunders Co., Philadelphia, Pa., 1959.
9. Wiggers, C. J., *Circulatory Dynamics,* Grune and Stratton, N. Y., 1952.
10. West, G. B., *Quart. Rev. Biol.,* 1955, v30, 116.

Received November 16, 1962. P.S.E.B.M., 1963, v112.