Summer 1972

The role of the adrenal gland in the survival of white rats subjected to the parotid toxin of the toad, Bufo marinus

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THE ROLE OF THE ADRENAL GLAND IN THE SURVIVAL OF WHITE RATS SUBJECTED TO THE PAROTID TOXIN OF THE TOAD, BUFO MARINUS

A thesis

submitted to the Faculty of the Graduate School

of the University of Richmond

in partial fulfillment of the requirements for the

Degree of Master of Science.

by

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B. S. University of Richmond 1968

August 1972

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ABSTRACT

Six groups of white laboratory rats were utilized in this experiment. A sham-operated group and an adrenalectomized group were used as controls. Four other groups were adrenalectomized and given replacement therapy of cortisone, deoxycorticosterone acetate, epinephrine, and norepinephrine. All groups were then given injections of toad toxin from the parotid glands of Bufo marinus. Their heart and breathing rates were recorded for 60 minutes.

All of the untreated adrenalectomized rats showed altered heart and breathing rates and were killed by the toxin. Cortisone and norepinephrine aided the adrenalectomized rats to survive, but cortisone was the more effective of the two.

ACKNOWLEDGMENTS

I would like to thank the following members of the Department of Biology at the University of Richmond: Dr. Francis B. Leftwich, my major professor, for his suggestion of the problem and aid in the writing of the thesis; and Drs. Nolan E. Rice and William S. Woolcott for their constructive criticism of the thesis.

I would also like to express my gratitude to Dr. William H. Leftwich (Psychology Department, University of Richmond) for his aid with the statistical analyses of the data, and Mr. Haley F. Thomas (Modern Foreign Languages Department, University of Richmond) for his translations of foreign papers.

To my wife, Bunny, I want to express my thanks for her optimistic endurance during the research period and for her typing of this thesis.

INTRODUCTION

Toads have few defense mechanisms. The poisonous secretions of their skin glands constitute their best means of warding off enemies (Lutz, 1971). Immunity against toad toxin among potential predators is rare. Several predators on which susceptability has been tested are: Mammals including man (Musgrave and Cochran, 1930); reptiles especially snakes (Smith and White, 1955); and amphibians (Brazil and Vellard, 1926). When these animals are subjected to toad toxin, death usually occurs.

Knowledge of the toxicity of the secretions of toad skin glands goes back to ancient times (Brazil and Vellard, 1926). Toad toxin preparations have been used for medicinal purposes, e.g. diuretics against dropsy (Meyer and Linde, 1971). Meyer and Linde (1971) further stated that in modern China and Japan there is still some medicinal use of toad extracts.

Charles and Marie Phisalix were the pioneer workers in the modern study of toad toxins (Lutz, 1971). They found that the toxins were secreted by mucous and granular glands of the skin. The parotid glands, which are granular, form well defined structures posterior and lateral to the eyes. They are large in Bufo marinus, extending onto the shoulders.

The secretions from the parotid glands of Bufo contain two classes of pharmacologically active constituents (Deulofeu and Rúveda, 1971). One class is made up of steroids (bufogenins and their deriva-

tives, the bufotoxins). The bufogenins are C_{24} steroids, e.g. argentinogenin. The other class is composed of several basic compounds. The basic compounds are those derived from phenylethylamine and tryptamine, e.g. epinephrine and digitaloid compounds. A complete list of the 10 steroidal and the 10 basic compounds found in the parotid glands of Bufo marinus is given in Table 1.

Bufophagy is limited by physiological resistance to toad toxin. Smith and White (1955) found such a resistance in species of the snake genera Heterodon, Thamnophis, Natrix, Coluber, and Xenodon. Xenodon and Heterodon and some species of Thamnophis feed primarily on toads. It was reported by Surface in Smith and White (1955) that 75% of the diet of Heterodon platyrhinos consisted of toads. Uhler et al. (1939) gave a somewhat lower figure at 40%. Munro (1949) stated that H. nasicus readily feeds on toads. Smith and White (1955) found that toads may be 16-25% of the diet of some species of Thamnophis.

The reasons for the resistance of these snake species to toad toxins are unknown. However, Smith and White (1955) suggest that it is related to enlarged adrenal glands. The ratio of body weight to adrenal weight in bufophagous species of Heterodon range from 440 to 1670, whereas this same ratio in non-bufophagous snakes ranges from 3155 to 8825. Based on the statement of Hartman and Brownell (1949) that enlarged adrenals are usually hyperactive adrenals, Smith and White (1955) present two hypotheses concerning the enlarged adrenals of

Heterodon and the physiological adjustment to a toad diet. First, the enlarged adrenals produce large quantities of catecholamines which make the snake resistant to epinephrine and the digitaloid compounds of toad toxin. This resistance may be interpreted as an increased tolerance of body cells to the drugs. The adrenals would, therefore, have to secrete larger quantities of epinephrine to overcome the unusually high resistance to the drug as an adaptation to ingested epinephrine. A second hypothesis is that the enlarged adrenals secrete large quantities of adrenal steroids which enable these snakes to withstand the toxic effects of toad toxins. Thus, enlargement of the adrenal gland may be regarded as a virtually necessary adjunct of well developed bufophagy.

An ideal experiment to test these two hypotheses would be to adrenalectomize an appropriate bufophagous snake species and subject the species to toad toxin. This type of experiment would then be followed by tests of toxicity of toad toxins in adrenalectomized snakes that had received replacement therapy of the two types of adrenal hormones (catecholamines and steroids). However, as snakes have a low survival rate following an adrenalectomy, rats which are easily adrenalectomized and more readily available were used. In addition, rats were considered acceptable substitutes since the adrenals of rats and snakes produce similar compounds having similar physiological activity (Gorbman and Bern, 1962). Brazil and Vellard (1926) found toad skin gland secretions to be toxic to the white rat with its adrenals

intact. The present experiment was designed to show whether a particular hormone of the adrenal gland of the white rat would in some way counteract or hold down to a minimum the lethal effects of the parotid gland toxin of Bufo marinus. Bufophagous snakes are assumed to produce an excess of hormones in their adrenal glands, whereas white rats are not. If a white rat is given an excess of an adrenal hormone, this may demonstrate the action taking place in the snake.

MATERIALS AND METHODS

Twelve South American, marine toads, Bufo marinus, obtained from Tarpon Zoo, Tarpon Springs, Florida, were used in this study. The animals were maintained in screen-covered glass cages and provided with water. They fed readily on crickets which were provided approximately once every four weeks. Under this regime the toads remained healthy throughout the six month experimental period.

Toxin was obtained by squeezing the parotid glands with the thumb and index finger. The creamy white, odorless secretion was expelled into previously tared petri plates and dried overnight in a dessicator. A comparison of wet and dry weights revealed that it was about one-half water (40 - 60%). The dry toxin was then pulverized in a mortar and dissolved in 0.9% NaCl. (Preliminary work indicated that toxin in powdered form was more soluble). The final concentration of toxin was 2.5 mg/1.0 ml 0.9% NaCl. Each rat received an intraperitoneal (IP) injection of 2.5 mg toxin/100 g body weight (bw).

Thirty-eight white rats of the Sprague-Dawley strain, which were obtained from stock at the University of Richmond, were used as test animals. They were 27 male and 11 female adult rats (weight range of 152 to 464 g). Throughout the experimental period the rats were provided water and standard laboratory chow.

In the experimental groups some of the adrenalectomized animals were given injections of deoxycorticosterone acetate (DOCA), cortisone, epinephrine, and norepinephrine. The first two compounds

are adrenal steroids; the last two are adrenal catecholamines. DOCA is one of the mineralocorticoids and cortisone is a glucocorticoid. Most researchers believe now that epinephrine is the major, if not the only, catecholamine secreted by the adrenal glands (Turner, 1971).

The rats were divided into six groups. A sham-operated group and an adrenalectomized group were set up as controls. Four groups of rats were used to test the adrenal hormones.

Group 1 consisted of eight sham-operated rats. Surgery was essentially that for an adrenalectomy, but the adrenal glands were not excised. Following the operation and a recovery period of 24 hours, four animals were given subcutaneous injections of 1.0 ml of Sesame oil/100 gbw, and four were given intramuscular injections of 0.5 ml of 0.9% NaCl/100 gbw. Sesame oil and NaCl solution were used because they are solvents of the hormones used in test animals. Rats receiving Sesame oil were injected once a day for three consecutive days, whereas those receiving the NaCl solution were injected twice a day (12 hours between each injection) for five consecutive days. On the fourth and sixth day, respectively, the animals were anaesthetized with an intraperitoneal injection of 25 mg/kg bw of Nembutal. They were then attached to a polygraph provided with an oscilloscope and heart and breathing rates were monitored for 30 min. Toad toxin was then injected and heart and breathing rates were observed for an additional hour. Readings were recorded at five min. intervals after the first 10 min. post-toxin injection time. The same procedures for anaestheti-

zation, toxin injection, and recording of heart and breathing rates were used for the rats in all groups.

Group 2 consisted of six adrenalectomized rats which were subjected to toxin three days following surgery.

Group 3 was composed of six adrenalectomized rats treated with cortisone. Following surgery and a 24 hour recovery period, each animal was given subcutaneous injections of cortisone (1, 4-Pregnadien-17 α, 21-Diol-3, 11, 20 Trione Prednisone) in Sesame oil at a dosage of 1 mg/100 gbw. They were injected once a day for three consecutive days. On the fourth day they received the toad toxin injection.

In Group 4 were six adrenalectomized rats treated with DOCA. Operation and recovery procedures were like those for Group 3. At the end of the 24 hour recovery period the animals were given subcutaneous injections of DOCA in Sesame oil at a dosage of 25 ug/100 gbw. Hormonal and toxin injections followed the same procedure as in Group 3.

Group 5 was composed of six adrenalectomized rats treated with epinephrine. Operation and recovery procedures were the same as those for Group 3. Each animal received an intramuscular injection of epinephrine (L-Epinephrine) in 0.9% NaCl (25 ug/100 gbw) twice a day (12 hours apart) for five consecutive days. On day six, they were anaesthetized and attached to the polygraph. At the end of 30 min. each animal received a final intramuscular injection of epinephrine just

prior to toad toxin injection. Heart and breathing rates were then monitored for an additional hour.

Group 6 consisted of six adrenalectomized rats treated with norepinephrine. Operation and recovery procedures were like those of Group 3. The post-operative animals were given intramuscular injections of norepinephrine (L-Arterenol) in 0.9% NaCl at a dosage of 25 ug/100 gbw, twice a day for five consecutive days. On day six, the animals were subjected to the same treatment as those in Group 5, except each was injected with norepinephrine instead of epinephrine.

The heart rate and breathing rate data were statistically analyzed using a two factor (groups x time) analysis of variances with repeated measures on the time dimension (Winer, 1970). An unweighted means analysis was performed because of unequal sample sizes. Interaction effects were found for both heart and breathing data. Therefore, simple effects were tested at each of the 13 time intervals after the preliminary monitoring time of 30 min. Newman-Keules test of ordered means were performed for all significant simple effects. Significant differences were reported at the .05 confidence level.

RESULTS

All rats subjected to the toad toxin exhibited the same external responses. Smooth muscle relaxation was associated with a twitching of skeletal muscle. This was followed by a partial paralysis of the extremities that began in the back legs. The eyes were opaque and epiphorating. The eyelids drooped. The immediate symptoms lasted about four hours if the animals were to survive the toxin; full recovery required a longer period of time (24-36 hours).

Table 2 gives the results of the effects of the toad toxin on the rats of the six groups. Only one sham-operated animal was killed. The killing time (point of injection to death) was 150 min. All six adrenalectomized, but untreated rats, died after toxin injection (mean killing time of 230 min.). Four of the six adrenalectomized animals that received cortisone survived the toxin. The two in this group which died had a mean killing time of 330 min. Of the six adrenalectomized rats that were given deoxycorticosterone prior to toxin injection, five succumbed to the toxin (mean killing time of 349 min.). When epinephrine was used as replacement therapy for six adrenalectomized animals, five died quickly (48 min. mean killing time). The sixth rat died two days prior to toxin injection, while still receiving epinephrine replacement. Three of five adrenalectomized animals survived the injection of toxin when they were given norepinephrine prior to the toxin injection. The sixth animal of this group was dead two days prior to toxin injection.

Figure 1 compares the effects of parotid toxin injection on the heart rate. Tables 3, 5, and 7 present statistical analyses of these data. Prior to toxin injection, there were no significant differences between the groups. At the time of toxin injection the heart rate of each group dropped but did not differ significantly. Ten min. after injection the heart rates of the rats in five groups increased. Those that received DOCA increased 15 min. after toxin injection. Significant differences occurred 35 min. after toxin injection when the epinephrine treated rats showed a lower heart rate than those in all other groups. This continued for the duration of the experiment. There were no significant differences between any other groups until the last time interval (60 min.) at which time the heart rate of the cortisone replacement group was lower than that of the adrenalectomized animals.

Figure 2 compares the effects of parotid toxin injection on the breathing rate. Tables 4, 6, and 8 are statistical analyses of these data. During the pre-toxin injection period and up to 10 min. after injection, there were no significant differences in the breathing rates. Between this time and the completion of the experiment, the breathing rates of the norepinephrine and epinephrine groups were significantly lower than the sham-operated control group. Sixty min. after toxin injection the average for the norepinephrine group was also significantly lower than the adrenalectomized control group. At the 15 and 20 min. periods the average breathing rate of the epinephrine group was significantly lower than the average breathing rates of the DOCA and

untreated adrenalectomized groups. These differences persisted until completion of the experiment. At the 60 min. time interval, the average breathing rate of the epinephrine group was significantly lower than all other groups except the norepinephrine group. The average breathing rate of the cortisone group was significantly lower than the average breathing rate of the sham-operated control group at time intervals 30, 35, 40, and 45 post-toxin injection time.

The effects of the toxin first appeared 10 min. after injection in the breathing rate and 35 min. after the animals were injected in heart rate.

DISCUSSION

The initial symptoms of the toad toxin on the rats (smooth muscle relaxation, twitching of skeletal muscle, partial paralysis, epiphoration, and drooping of the eyelids) persisted up to 24-36 hours. The animals were lethargic, showing little interest in their surroundings. Brazil and Vellard (1926) observed the same effects in white rats, also in mice, guinea pigs, rabbits, rattlesnakes, and even B. marinus when subjected to toad toxin.

The injection of toad toxin into a rat causes an initial increase in the rates of heart beat and breathing (Figs. 1 & 2). However, these rates decline, and if the animal dies, breathing fails first, followed by paralysis of the heart. This finding verifies the results obtained by Brazil and Vellard (1926).

The significance of the adrenal hormones is evident as seven out of eight animals with intact adrenals survived the toad toxin. Similar results with white rats were obtained by Brazil and Vellard (1926). However, these workers found that survival was related to dosage and type of injection. If the dosage exceeds the minimal lethal dose established for a particular species, death occurs so rapidly that toxic symptoms do not have time to appear. If the dosage is less than the minimal lethal dose, illness is still evident, but eventually the animals fully recover. The rats used in the present study died most quickly following intramuscular injections. Although death occurred

much slower after intraperitoneal injections, the death rate was as high as that in animals given intramuscular injections.

The fact that all adrenalectomized animals without replacement therapy were killed by the toxin, indicates that there is a relation between the presence of the adrenal gland (or its secretions) and survival. Not all adrenal compounds have the same therapeutic value. Cortisone suppressed the lethal toxic effects to a greater extent than did other adrenal hormones, although three out of five norepinephrine treated rats survived the effects of the toxin (Figs. 1 & 2; Table 2).

Two of the most important adrenal corticoids secreted from the steroidogenic tissue of the adrenal gland are cortisone and DOCA (Turner, 1971). Cortisone affects carbohydrate metabolism and is classified as a glucocorticoid. Glucocorticoids are also known to enable an organism to survive stress (Turner, 1971). DOCA is a mineralocorticoid affecting the mineral and water metabolism of an organism. Both of these compounds have been found in the adrenal secretions of rats (Phillips and Bellamy, 1963, and Gorbman and Bern, 1962). Epinephrine and norepinephrine affect the cardiovascular system, blood flow through individual organs, respiratory system, carbohydrate metabolism, and the central nervous system (Turner, 1971). Both hormones increase the heart rate, systolic blood pressure, but only norepinephrine increases the diastolic blood pressure. They cause hypertension, inhibition of the respiratory system, and an increase in carbohydrate metabolism. The over-all effect of norepi-

nephrine is to produce vasoconstriction. Even though some norepinephrine treated animals survived the toad toxin, the significance of this finding to the present study is questionable as most researchers do not consider this compound an adrenal hormone (Turner, 1971).

Turner (1971) states that adrenal steroids, particularly glucocorticoids, restore the resistance of adrenalectomized animals to various kinds of stressors such as toxin. This involves Seyle's General Adaptation Syndrome (Gorbman and Bern, 1962). The concept of the General Adaptation Syndrome (GAS) is an attempt to explain the ability of an organism to adapt to stressors through the mobilization of its adrenocortical hormones. In the GAS there is a release of adrenocorticotrophin (ACTH) from the pituitary gland which in turn stimulates the steroidogenic tissue of the adrenal cortex to secrete more adrenal corticoids, e.g. cortisone. This is the first stage of the GAS which occurs immediately upon exposure to stress. Following this is the resistant stage which occurs if the organism continues to be exposed to the stress. This may last for several months. The stage of exhaustion is the third stage and occurs when the adrenals atrophy and the animal loses its adaptation to the stress. This usually results in death. The results of the present study suggest that the General Adaptation Syndrome is involved in the survival of white rats subjected to toad toxin. Whether the GAS is also involved in the survival of bufophagous snakes remains to be determined.

From the results of the present study, two hypotheses are presented. The first is that if in the snake as is indicated in the rat, a glucocorticoid does counteract the effects of the toad toxin, adrenal enlargement may be the result of hypertrophy independent of ACTH action on the adrenal glands. Secondly, in snakes the enlarged adrenal gland may result from the GAS in which the resistant stage is much longer than known, or the stage of exhaustion may never be reached.

A possible practical result from the study is, pending further investigation, that cortisone is an effective agent to prevent sickness or death when animals are subjected to the parotid gland toxin of Bufo marinus.

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Table 1

A List of the Known Chemical Constituents

Present in the Parotid Gland Toxin of Bufo marinus.*

Steroids

Bufogenins

- Argentinogenin
- Bufalin
- Bufotalidin = hellebrigenin
- Gamabufotalin
- Hellebrigenol
- Marinobufagin
- Resibufogenin
- Telocinobufagin
- Jamaicobufagin

Bufotoxin

- Marinobufotoxin

Basic Compounds

Phenylethylamine Bases

- Dopamine
- N-methyldopamine
- Norepinephrine
- Epinephrine

Tryptamine Bases

- 5-hydroxytryptamine, serotonin, enteramine, thrombocytin, thrombotonin
- N-methyl-5-hydroxytryptamine
- Dehydrobufotenine
- Bufotenine
- Bufotenidine
- Bufothionine

*Compiled from: Deulofeu and Rúveda, 1971

Daly and Witkop, 1971

Meyer and Linde, 1971

Table 2

The Effects of Adrenal Corticoids and Catecholamines on the Survival of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

Experimental Group	Animal #	Sex	BW (g)	# of Animals Killed by Toxin	Killing time (min.) after Toxin Injection	Mean Killing Time (min.)
Sham-Operated Control Animals	1	M	464.0	1	150	150
	2	M	346.0		---	
	3	F	277.0		---	
	4	M	259.0		---	
	5	F	228.0		---	
	6	F	222.0		---	
	7	M	249.0		---	
	8	M	272.0		---	

Table 2 (continued)

Experimental Group	Animal #	Sex	BW (g)	# of Animals Killed by Toxin	Killing time (min.) after Toxin Injection	Mean Killing Time (min.)
Adrenalectomized Control Animals	1	M	298.0	6	300	230
	2	M	193.0		180	
	3	M	199.0		240	
	4	F	209.0		360	
	5	F	152.0		300	
	6	M	195.0		0	
Adrenalectomized Animals with Cortisone Replacement	1	F	200.0	2	---	330
	2	M	214.0		---	
	3	M	224.0		---	
	4	F	185.0		---	
	5	M	157.0		420	
	6	M	176.0		240	

Table 2 (continued)

Experimental Group	Animal #	Sex	BW (g)	# of Animals Killed by Toxin	Killing time (min.) after Toxin Injection	Mean Killing Time (min.)
Adrenalectomized Animals with Deoxycorticosterone Replacement	1	M	267.0	5	480	349
	2	M	250.0		600	
	3	M	202.0		---	
	4	F	236.0		0	
	5	M	210.0		420	
	6	M	255.0		240	
Adrenalectomized Animals with Epinephrine Replacement	1	M	153.0	5	120	48
	2	M	*			
	3	F	298.0		45	
	4	M	209.0		0	
	5	M	161.0		0	
	6	F	172.0		75	

*Death prior to toxin injection.

Table 2 (continued)

Experimental Group	Animal #	Sex	BW (g)	# of Animals Killed by Toxin	Killing time (min.) after Toxin Injection	Mean Killing Time (min.)
Adrenalectomized Animals with Norepinephrine Replacement	1	M	*	2		120
	2	M	220.0		120	
	3	M	228.0		120	
	4	F	162.0		---	
	5	M	165.0		---	
	6	M	154.0		---	

*Death prior to toxin injection.

Table 3

Analysis of Variance of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Heart Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

Source	d.f.	M. S.	F (observed)	F (critical value)
Between:	31			
Groups (G)	5	159570.22	5.10	2.59
Error Between	26	31311.64		
Within:	384			
Time (T)	12	7365.26	3.40	1.79
G x T	60	7697.87	3.50	1.57
Error Within	312	2168.43		

Table 4

Analysis of Variance of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Breathing Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

Source	d.f.	M.S.	F (observed)	F (critical value)
Between:	31			
Groups (G)	5	101708.56	6.45	2.59
Error Between	26	15770.37		
Within:	384			
Time (T)	12	3494.10	9.36	1.79
G x T	60	1329.76	3.56	1.57
Error Within	312	373.20		

Table 5

Analysis of Variance (Simple Effects) of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Heart Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

Source	d.f.	M.S.	F (observed)
Groups			
30 min. pre injection	5	4748.20	1.08 n.s.
0 min. (toxin injection)	5	3708.48	.84 n.s.
10 min. post injection	5	1518.01	.34 n.s.
15 min. post injection	5	1943.63	.44 n.s.
20 min. post injection	5	3496.54	.80 n.s.
25 min. post injection	5	4658.22	1.06 n.s.
30 min. post injection	5	4199.91	.95 n.s.
35 min. post injection	5	27340.11	6.20*
40 min. post injection	5	29902.38	6.78*
45 min. post injection	5	40623.72	9.21*
50 min. post injection	5	43227.79	9.80*
55 min. post injection	5	42872.24	9.72*
60 min. post injection	5	43705.39	9.91*

*$F_{.95}$ (critical value) = 2.59

Table 6

Analysis of Variance (Simple Effects) of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Breathing Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

Source	d.f.	M. S.	F (observed)
Groups			
30 min. pre injection	5	510.88	.33 n.s.
0 min. (toxin injection)	5	765.88	.49 n.s.
10 min. post injection	5	4587.05	2.95*
15 min. post injection	5	6358.07	4.08*
20 min. post injection	5	7433.73	4.77*
25 min. post injection	5	9064.81	5.82*
30 min. post injection	5	10300.97	6.61*
35 min. post injection	5	12619.65	8.10*
40 min. post injection	5	13231.89	8.50*
45 min. post injection	5	13428.11	8.62*
50 min. post injection	5	13381.79	8.59*
55 min. post injection	5	13078.37	8.40*
60 min. post injection	5	12904.43	8.29*

*$F_{.95}$ (critical value) = 2.59

Table 7

Newman-Keuls Analysis of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Heart Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

(Groups as shown on left are significantly different than group number at specific time interval)

Group	Group No.	Time Interval - Minutes Post Injection					
		35	40	45	50	55	60
Sham-operated Control	1	5	5	5	5	5	5
ADX* Control	2	5	5	5	5	5	3, 5
ADX + Cortisone	3	5	5	5	5	5	2, 5
ADX + DOCA**	4	5	5	5	5	5	5
ADX + Epinephrine	5	1, 2, 3, 4, 6	1, 2, 3, 4, 6	1, 2, 3, 4, 6	1, 2, 3, 4, 6	1, 2, 3, 4, 6	1, 2, 3, 4, 6
ADX + Norepinephrine	6	5	5	5	5	5	5

*ADX = Adrenalectomized.

**DOCA = Deoxycorticosterone.

Table 8

Newman-Keuls Analysis of the Data of the Effects of Adrenal Corticoids and Catecholamines on the Breathing Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

(Groups as shown on left are significantly different than group number at specific time interval)

Group	Group No.	Time Interval - Minutes Post Injection										
		10	15	20	25	30	35	40	45	50	55	60
Sham-operated Control	1	5,6	5,6	5,6	5,6	3,5,6	3,5,6	3,5,6	3,5,6	5,6	5,6	5,6
ADX* Control	2			5	5	5	5	5	5	5,6	5,6	5,6
ADX + Cortisone	3					1,5	1,5	1,5	1,5	5	5	5
ADX + DOCA**	4		5	5	5	5	5	5	5	5	5	5
ADX + Epinephrine	5	1	1,4	1,2,4	1,2,4	1,2,3,4	1,2,3,4	1,2,3,4,6	1,2,3,4,6	1,2,3,4,6	1,2,3,4	1,2,3,4
ADX + Norepinephrine	6	1	1	1	1	1	1	1,5	1,5	1,2,5	1,2	1,2

*ADX = Adrenalectomized.

**DOCA = Deoxycorticosterone.

Figure 1. The Effects of Adrenal Corticoids and Catecholamines on the Heart Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

▪ Mean of Eight Sham-Operated Control Animals

⊙ Mean of Five Adrenalectomized Control Animals

△ Mean of Six Adrenalectomized Animals with Cortisone Replacement

□ Mean of Five Adrenalectomized Animals with Deoxycorticosterone Replacement

+ Mean of Three Adrenalectomized Animals with Epinephrine Replacement

▲ Mean of Five Adrenalectomized Animals with Norepinephrine Replacement

The toad toxin injection was made immediately after the 30 min. pre-toxin injection period. The next reading (0 post-toxin injection) followed immediately after toxin injection.

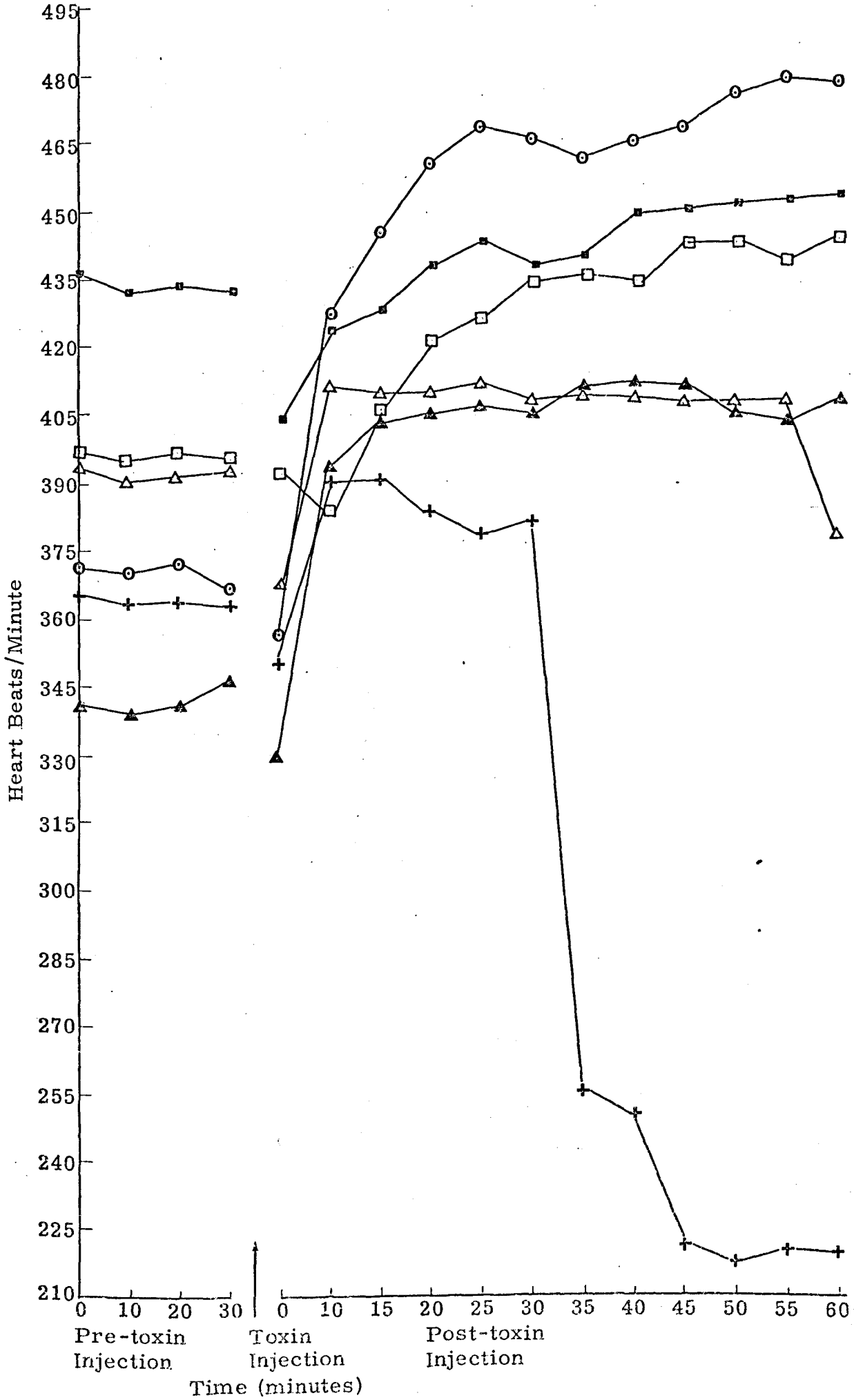

Heart Beats/Minute
495
480
465
450
435
420
405
390
375
360
345
330
315
300
285
270
255
240
225
210
0 10 20 30
0 10 15 20 25 30 35 40 45 50 55 60
Pre-toxin Injection
Toxin Injection
Post-toxin Injection
Time (minutes)

Figure 2. The Effects of Adrenal Corticoids and Catecholamines on the Breathing Rate of Adrenalectomized White Rats Subjected to Parotid Toxin of Bufo marinus.

■ Mean of Eight Sham-Operated Control Animals

⊙ Mean of Five Adrenalectomized Control Animals

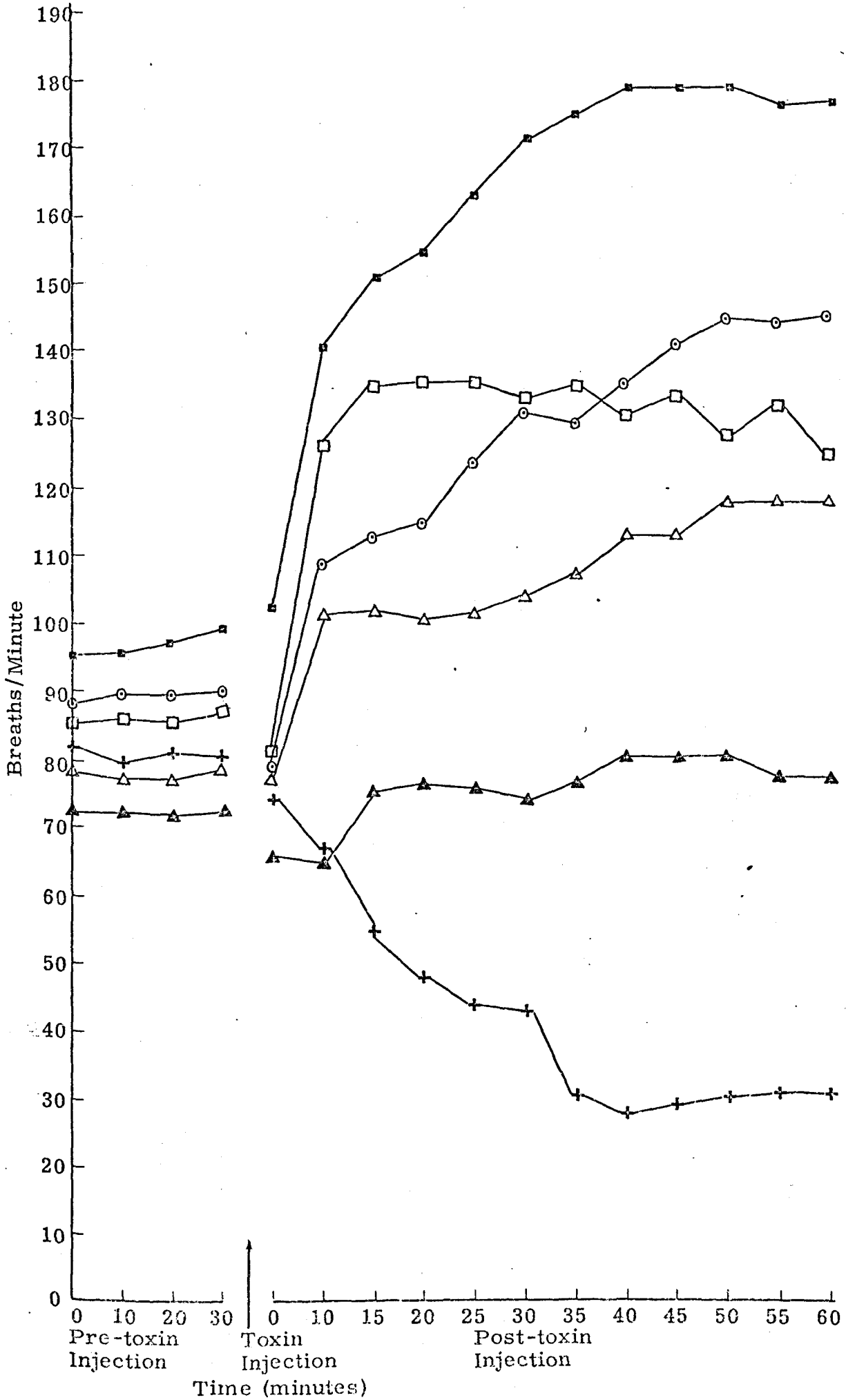
△ Mean of Six Adrenalectomized Animals with Cortisone Replacement

□ Mean of Five Adrenalectomized Animals with Deoxycorticosterone Replacement

+ Mean of Three Adrenalectomized Animals with Epinephrine Replacement

▲ Mean of Five Adrenalectomized Animals with Norepinephrine Replacement

The toad toxin injection was made immediately after the 30 min. pre-toxin injection period. The next reading (0 post-toxin injection) followed immediately after toxin injection.


Breaths/Minute
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30
Pre-toxin Injection
Toxin Injection
0 10 15 20 25 30 35 40 45 50 55 60
Post-toxin Injection
Time (minutes)

VITA

Mark A. Green was born October 31, 1943, in Buena Vista, Virginia. He attended Parry McCluer High School in Buena Vista, Virginia, and completed his secondary education in June, 1962. He entered Ferrum Junior College in Ferrum, Virginia, in September, 1963. After the completion of his first year he transferred to Richmond College of the University of Richmond entering in February, 1965. While at Richmond College he was initiated into the Beta Beta Beta Honorary Biological Society. During his Senior year he was married to the former Miss Delores Ann Humphries of Buena Vista, Virginia. He was graduated in June, 1968, with the Bachelor of Science degree in Chemistry and Biology, and began graduate study at the University of Richmond in September, 1968. After having completed his first semester work, he was drafted into the United States Army. He completed his active duty in January, 1971, and returned to his graduate study in February, 1971. He completed the requirements for the Master of Science Degree in August, 1972. He will enter the Medical College of Virginia, Health Sciences Division of the Virginia Commonwealth University, in September, 1972, to work toward the degree of Doctor of Philosophy.