A STUDY OF THE EFFECTS OF 5-HYDROXYTRYPTAMINE (SEROTONIN) ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS OF THE DOG.

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A Thesis

Submitted to

the Faculty of the Department of Biology

University of Houston

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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ACKNOWLEDGMENT

The author wishes to express his gratitude to those instrumental in the completion of this report. In particular, Dr. Donald C. Kroegor for his directional guidance, Dr. Daniel E. Sheer for his assistance in the statistical analysis, and the faculty members of the Biology Department of the University of Houston.

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EFFECTS OF 5-HYDROXYTRYPTAMINE (SEROTONIN) ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS OF THE DOG

INTRODUCTION

In 1948 Rapport, Green and Page (25) isolated and characterized a vasoactive substance present in serum and defibrinated blood. This substance they named serotonin.

Rapport (26) in the following year proposed its structure and in 1951 Hamlin and Fischer (14), Speeter, Heinzelmann and Weisblat (31) synthesized the substance. The latter group confirmed Rapport's proposed structure of 5-hydroxy-3-beta-aminoethylindole (5-hydroxytryptamine).

In 1952 Page (19) in reviewing the vascular action of natural serotonin, 5- and 7-hydroxytryptamine and tryptamine, reported similar responses to all four compounds on the dogs' arterial pressure. The intravenous injections of 5-hydroxytryptamine caused an initial transient increase in rate and amplitude of respiration, accompanied but not necessarily associated with variable arterial pressure responses, the pressor response being the prominant component.

In the same year, Douglas and Toh (7) observed a stimulation of breathing when small doses of 5-hydroxytryptamine were injected in the common carotid artery of the dog. Freyburger et al. (11) observed erratic effects on the blood pressure of anesthetized dogs and slight transient bronchoconstriction in the isolated rabbit lung.

In 1953 Page and McCubbin (20) suggested possible mechanisms involved in the variable arterial pressure responses to 5-hydroxytryptamine. Again Douglas and Teh (8), in attempting to analyse the respiratory stimulation caused by 5-hydroxytryptamine in dogs, suggested that the respiratory effects are partly due to excitation of afferents running in the carotid sinus and vagus nerves, and partly to central stimulation. They also reported variable arterial responses as did Page. Heymans and van den Heuvel-Heymans (15), from their experiments, suggested that the respiratory stimulation in dogs by 5-hydroxytryptamine is mainly of central origin.

Comroe et al. (5) observed that the administration of 5-hydroxytryptamine to chloralosed cats produced bradycardia, hypotension, apnea, bronchoconstriction and pulmonary vasoconstriction. They, as did others, attributed these responses to the reflex effects passing over the vagus nerves and concluded that the sensory receptors for both apnea and bradycardia lay in the cardiopulmonary region.

In view of the variable respiratory and cardiovascular responses reported for different species, Schneider and Yonkman (29) in 1954 characterized these variations, and, by means of pharmacological and surgical blockade of reflex pathways, endeavored to differentiate the points of attack of 5-hydroxytryptamine in the different species. In this study, they observed that the administration of 5-hydroxytryptamine to dogs elicited an initial brief respiratory stimulation which was followed in 50% of the animals by a period of apnea. The remaining dogs

demonstrated continued increases in respiratory rates lasting from five to ten minutes. The blood pressure response consisted mainly in a rise which was preceded in about 50% of the animals by a transient slight depressor response. Initial bradycardia was regularly reported and followed occasionally by tachycardia. From these observations, together with those they recorded for the cat and rabbit, they concluded that serotonin caused qualitatively and quantitatively different respiratory and cardiovascular responses in these animals. They suggested that serotonin activates the pulmonary stretch receptors, and that the species difference is largely due to reflexes originating in the heart and lungs.

Excellent reviews summarizing the conclusions of the early studies of serotonin were prepared in 1954 by Erspamer (9) and Page (21).

In the same year MacCanon and Horvath (16) studying the effects of serotonin in pentobarbitalized dogs, rapidly injected large quantities into the pulmonary artery and observed initial hyporpnea followed in about 70% of the instances by a period of apnea or reduced respiratory activity. They noted purely pressor responses in pulmonary and systemic pressures, bradycardia with occasional tachycardia, increases in pulse pressures, sequential changes in cardiac output, changes in pulmonary and systemic vascular resistance and possible bronchoconstriction.

In 1956 McCubbin et al. (17) studying the chemoreceptor stimulation of serotonin in dogs, reported that intra-arterial (carotid) and intravenous (femoral) injections of 5-hydroxytryptamine elicited hyperpneic responses together with rises in systemic arterial pressure. They

suggested that small doses produce these responses chiefly through the stimulation of chemoreceptors in the carotid sinus and that larger doses in addition acted through central mechanisms.

Rudolph and Paul (23), 1957, studying the pulmonary and systemic vascular response to the continuous infusion of 5-hydroxytryptamine in dogs, reported marked pulmonary vasoconstriction and hypertension during the entire course of the infusion. The initial pressure responses were of a variable pattern but consistently followed by a generally sustained reduction in arterial pressure. In view of the fact that tachycardia and increases in cardiac output were regularly observed, the sustained systemic depressor response was attributed to a decrease in peripheral resistance. They concluded that 5-hydroxytryptamine when continuously infused is a powerful pulmonary vasoconstrictor, due to a direct local effect of the drug, and a vasodialator of peripheral vessels.

In this same year Borst, Berglund and McGregor (2) reviewing the effects of pharmacologic agents on the pulmonary circulation in dogs, reported that the administration of small doses of 5-hydroxytryptamine into the pulmonary artery produced marked pulmonary vasoconstriction and a rise in this pressure. The systemic arterial pressure usually was unchanged until large doses were administered and the systemic pressure response then obtained was of a variable nature. They observed no evidence of bronchoconstriction.

A substance displaying pharmacological properties such as those of serotonin has been and is a challenge to the many investigators in

this field. It's physiological role is of great importance and concern, in that 5-hydroxytryptamine has been found to be an important metabolite of and present in the tissues of many animals including man. As to the mechanisms through which it manifests it's effects, many conflicting conclusions have been presented and at present, they are not clearly understood.

That respiration is reflexly dependent upon or regulated by the changes in the partial pressures of carbon dioxide and that these tensions are in turn determined to a greater extent by the concentration of carbon dioxide in the blood and tissues are well established facts (13, 24, 4). The rate of respiratory ventilation is directly proportional to the carbon dioxide tension; a decrease in carbon dioxide is followed by a decrease in respiratory rate. On the other hand, carbon dioxide tensions and concentrations are inversely proportional to the rate of respiratory ventilation; an increase in respiratory minute volumes regularly produces a decrease in arterial blood tensions and carbon dioxide concentration.

"If the partial pressure of CO_2 in the arterial blood and alveolar air is reduced 1.5 to 2.5 mm. Hg. below the normal value of 40 mm. Hg. by voluntary hyperventilation, apnea of brief duration results when the voluntary effort is suspended."[1]

It has been reported that the administration of serotonin to dogs, first induces a state of transient hyperpnea which is then quite often followed by a period of apnea (16, 29). It is the objective of this study to observe the respiratory and cardiovascular responses

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[1] Fulton's Textbook of Physiology, Sixteenth Ed. p. 842.

elicited by the administration of serotonin to dogs and to test the hypothesis that the initial respiratory stimulation lowers the blood carbon dioxide levels enough to induce the state of apnea.

EXPERIMENTAL

A total of eighteen dogs (male and female) weighing between 6 and 15 Kg. were included in this study. The experimental work was developed in two stages.

EXPERIMENTAL PART I. A preliminary study in which six animals were used to determine:

A. The effect, if any, of the wash-in solution, Isotonic Saline.

B. The possible influence of the anticoagulant used, Heparin/Pitkin Menstruum 'Warner'. A saline solution containing 10 mg. of heparin sodium salt per ml., no vaso-constrictors.

C. Dose of 5-Hydroxytryptamine required to elicit the response under observation.

D. Evaluation of the techniques used in obtaining and storing the blood samples.

EXPERIMENTAL PART II. In this stage, the remaining twelve animals were used in detailing the effects of 5-Hydroxy-tryptamine (13 mcgm/Kg. I.V.) on the:

A. Respiratory System

B. Cardiovascular System

C. Blood Gases

All dogs were anesthetized with pentobarbital sodium (30 mg/Kg.) in the lesser saphenous vein. The trachea was cannulated. The right and left common carotid arteries, right external jugular and left femoral veins were exposed.

Respiration was recorded by placing the pneumograph around the dog's thorax, the connecting tube from the pneumograph was then attached to a transducer of the simple, tambour-type (rubber membrane) to which was attached the writing stylus. All tracings were recorded on a smoked kymograph drum.

The blood pressure and heart rate were recorded for the purpose of observing their variations during the course of the experiment and for the assurance that the data obtained was characteristic of that recorded by previous workers. Blood pressure was recorded from the left carotid artery by means of a mercury manometer. The heart rate was registered by an electrocardiograph, or by counting the individual excursions in the blood pressure tracings.

Arterial and venous samples (carotid artery and external jugular vein) were collected simultaneously just prior to the administration of serotonin and immediately after the termination of the initial marked respiratory stimulation. The technique for obtaining and handling the blood anaerobically was a modification of that described by Scholander (27).

Eighteen gauge needles equipped with three-way Leur lock valves were inserted in the right carotid artery and right external jugular vein. To the valves were attached two 2 ml. syringes - one for washout, the other for the collection of samples. These units, excluding the collection syringes, were left in place during the course of the entire experiment. As the samples were obtained, the collection syringes were removed and stored in an ice bath at 5°C. New syringes were then placed in readiness for the drawing of the next samples.

Calculations of the volumes of carbon dioxide and oxygen in blood were made from data obtained by the use of the Van-Slyke-Neill manometric apparatus (34). The pCO_2 for arterial blood was arrived at by applying the volume percent values to the nomogram of Singer and Hastings (30).

A solution of serotonin creatinine sulfate[2] containing 130.35 mgm. of base per ml. was prepared for injection by weighing 3 mg. of the salt and dissolving it in 10 ml. of distilled water. The figures given for 5-hydroxytryptamine in this report refer to the base and not the complex.

Serotonin was administered via the left femoral vein through a cannula equipped with a 25 ml. burette containing isotonic saline. Each injection was immediately followed by a wash-in of 2 ml. of saline.

[2] Thanks are extended to Dr. R. K. Richards of Abbott Laboratories, North Chicago, Ill. for the generous supply of serotonin creatinine sulfate.

A Franz kymograph timer was used for the time tracing, upper scale in each tracing indicates one-fifth and one-second divisions of time, lower scale indicates ten-second and one-minute. A signal magnet placed at 0 mm. Hg. (blood pressure) indicated the points at which blood samples were drawn and drug injected.

Each animal was subjected to three injections. The time lapse between injections being approximately forty-five minutes.

RESULTS

EXPERIMENTAL PART I

A and B. THE EFFECTS OF ISOTONIC SALINE AND HEPARIN

The injection of 1-2 ml. of saline (wash-in solution) and the use of heparin had no apparent effect on the functions studied. Therefore, saline and heparin were used throughout the entire experiment.

C. DETERMINATION OF THE DOSE STRENGTH OF 5-HYDROXYTRYPTAMINE

Serotonin was administered intravenously to six animals at a dose of 5-25 mcgm/Kg. Immediate respiratory stimulation was observed through the entire dose range although of much less intensity at the 5 mcgm/Kg. level. The initial hyperpnea was followed by a period of apnea at least once for all dose levels, but most frequently at 10-15 mcgm/Kg. Therefore, 13 mcgm/Kg. of 5-hydroxytryptamine was chosen as the test dose.

D. EVALUATION OF THE TECHNIQUES USED IN OBTAINING AND STORING THE BLOOD SAMPLES.

On three different occasions, arterial and venous samples were drawn from an anesthetized donor dog and stored in an ice bath at 5°C. The carbon dioxide and oxygen volumes percent were determined immediately, and at one hour intervals thereafter for a period of six

hours. From this data, the regression co-efficients were calculated, CO_2 +.2667 volumes percent per hour, O_2 -.1631 volumes percent per hour. Upon calculating the t-values, it was concluded that the regression of the volumes percent of carbon dioxide on time was not significantly related to time, but that oxygen does display a significant regression - the value being between -.0510 and -.2752 volumes percent per hour.

Figure I is an illustration of the regression lines fitted to this data.

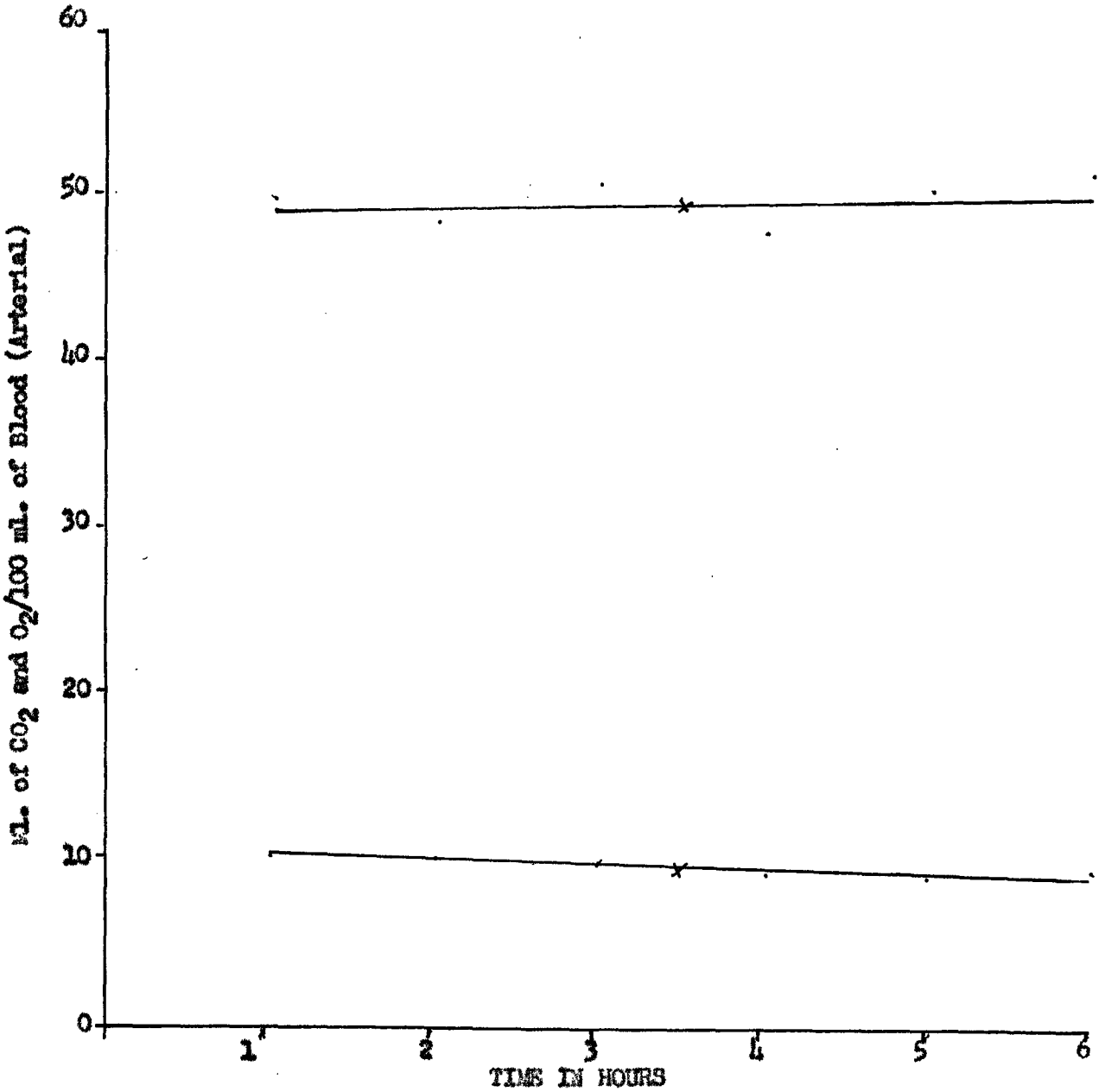

Figure 1. Regression of ml. of CO_2 and O_2/100 ml. of stored arterial blood (5°C) on time. Upper line fitted to CO_2 data - lower line to O_2 data. The cross indicates the origin for deviations.

RESULTS

EXPERIMENTAL PART II

A. RESPIRATORY EFFECTS OF SEROTONIN (13 mcgm/Kg. I.V.)

The average latent period (with standard deviation) for the respiratory response was 6.4 ± 1.8 seconds. Seventy-seven percent of the injections and fifty-eight percent of the animals fell within this range. The initial marked respiratory stimulation was a consistent observation in all animals. The average increase in respiratory rate (with standard deviation) was found to be 4.7 ± 1.6 times the pre-injection rate. Only forty-one percent of the animals, as compared to seventy-two percent of the injections, demonstrated increases that consistently were within the limits of this range.

The average duration for the initial hyperpnea (with standard deviation) was 12.1 ± 3.8 seconds. The percentage of animals repeatedly falling within these limits was less than half that observed for the total number of injections.

Generally, the immediate post-hyperpneic rate had an average increase (with standard deviation) of 1.35 ± 0.4 times the pre-injection rate (figures 2 and 6). Occasionally, this rate returned to the control level,

fell slightly below (figures 3 and 5) or was characterized by a period of apnea (figures 4 and 7), but these findings were not consistent. The initial stimulation was consistently followed by a period of respiratory arrest in the expiratory position in about sixteen percent of the animals. The duration of the over-all respiratory response ranges from three to twelve minutes.

With regard to the initial increases in respiratory rate, the data indicated that the maximum respiratory response to 13 mcgm/Kg. of serotonin was obtained in the first, second, and third injections and that the time lapse between injections in each dog was great enough to overcome any possible influence of the phenomenon of tachyphylaxis.

B <u>CARDIOVASCULAR EFFECTS OF SEROTONIN</u> (13 mcgm/Kg. I.V.)

The blood pressure response occurred one to three seconds after the onset of the respiratory effect. The average latent period (with standard deviation) was 8.4 ± 2.8 seconds. Sixty-one percent of the injections, as compared to forty-one percent of the animals, displayed latent periods that fell within these limits.

The immediate blood pressure response consisted mainly of a rise. The average increase (with standard deviation) being 41.8 ± 21.5 mm. Hg. Figures 2 and 4

are illustrative of this type of response. In some instances, the pressor effect was preceded by a transient, slight blood pressure fall (figure 3).

Occasionally, two diphasic type responses were observed. One in which the initial effect, depressor in nature, was followed by a rise in blood pressure of approximately equal degree and duration (figure 5), the other having a primary pressor response followed by a fall in blood pressure. A rise in blood pressure followed by a predominantly depressor response formed another pattern infrequently observed (figure 6). Only rarely did a sustained, solely depressor response occur (figure 7). Depressor responses recorded ranged from -6 to -68 mm. Hg.

An accurate measure of the duration of the overall circulatory response was not obtained. However, it apparently fell between ten and thirty-five minutes as it was noted that a new and generally lower control level was established during the time taken in preparing for the next injection. Initial bradycardia of various degree and duration was a consistent finding.

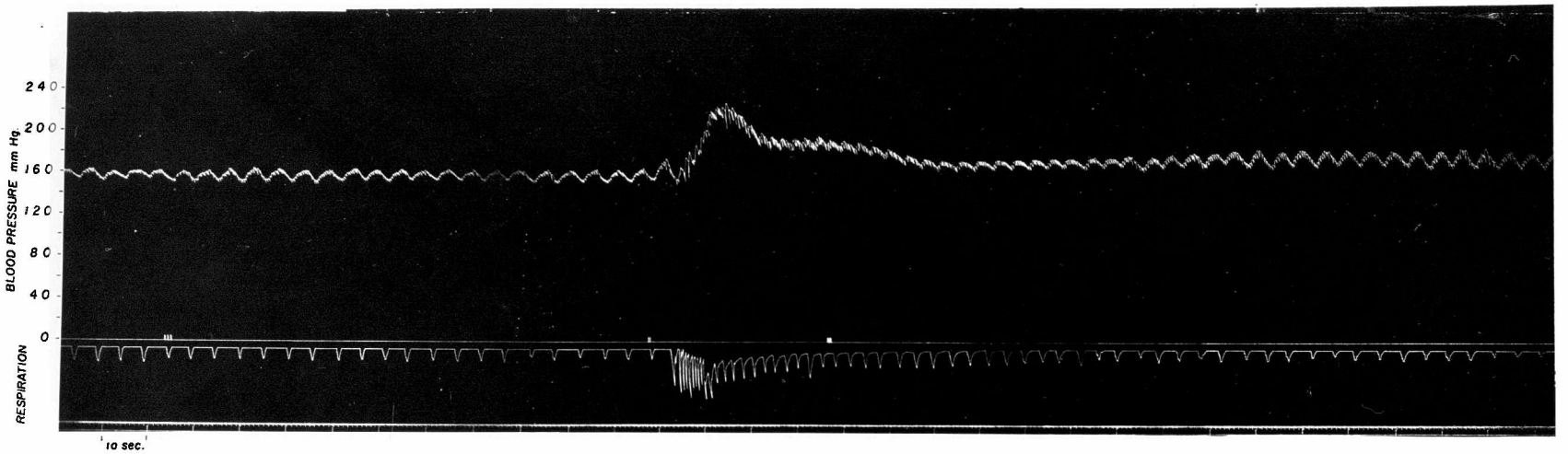

Figure 2. Dog, male, 11.3 Kg. Anesthetic; pentobarbital (30 mcgm/Kg. I.V.). Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following first injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions, administration of serotonin. Time interval - 10 seconds. Upper tracing shows a primarily pressor response. Lower tracing, the initial hyperpnea followed by a continuation of increased respiratory activity.

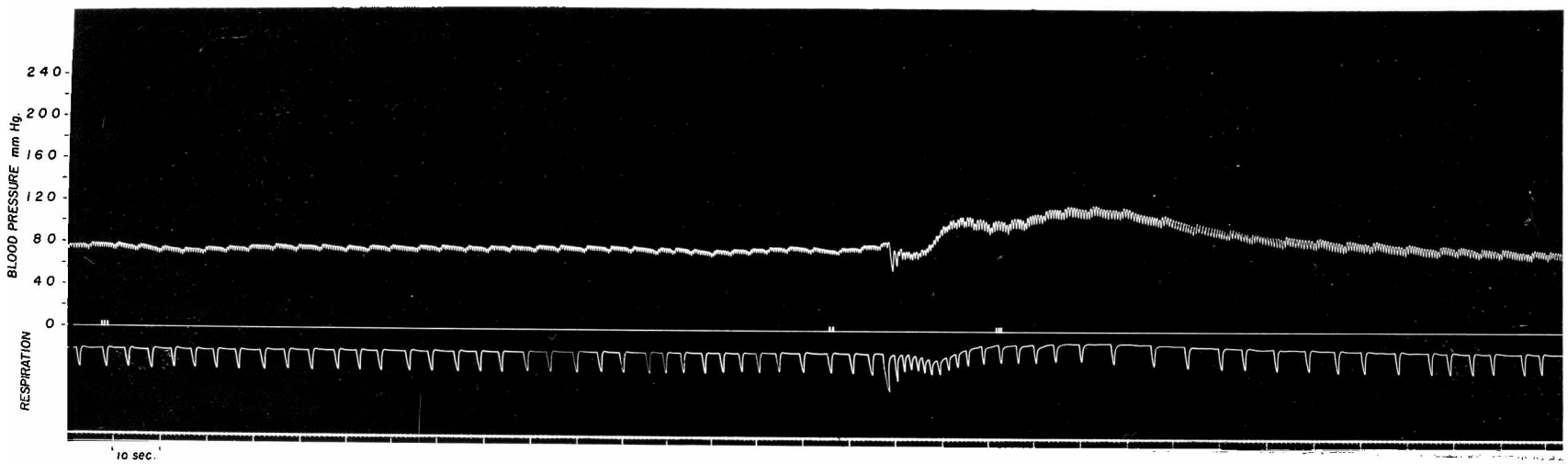

Figure 3. Dog, male, 11.8 Kg. Anesthetic; pentobarbital (30 mgm/Kg. I.V.). Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following second injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions, administration of serotonin. Time interval - 10 seconds. Upper tracing shows a transient depressor response preceding the predominately pressor response. Lower tracing, the initial hyperpnea followed by a slight decrease in respiratory activity with regards to control rate.

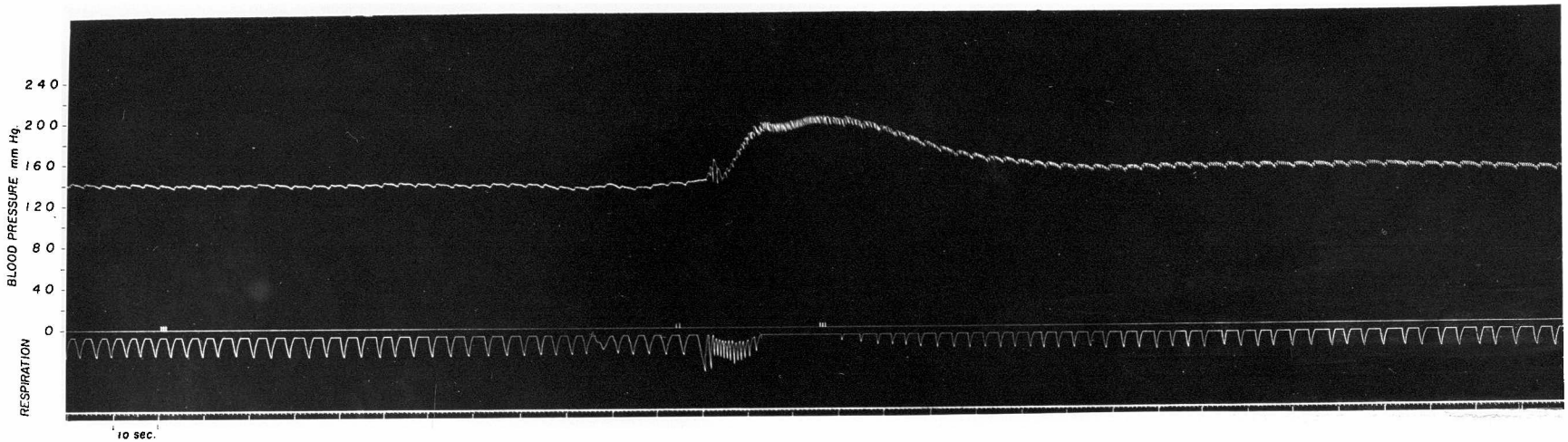

Figure 4. Dog, male, 11.3 Kg. Same animal as in Figure 2. Anesthetic; pentobarbital (30 mgm/Kg. I.V.) Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following third injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions, administration of serotonin. Time interval ~ 10 seconds. Upper tracing shows a primarily pressor response. Lower tracing, the initial hyperpnea followed by a period of respiratory arrest.

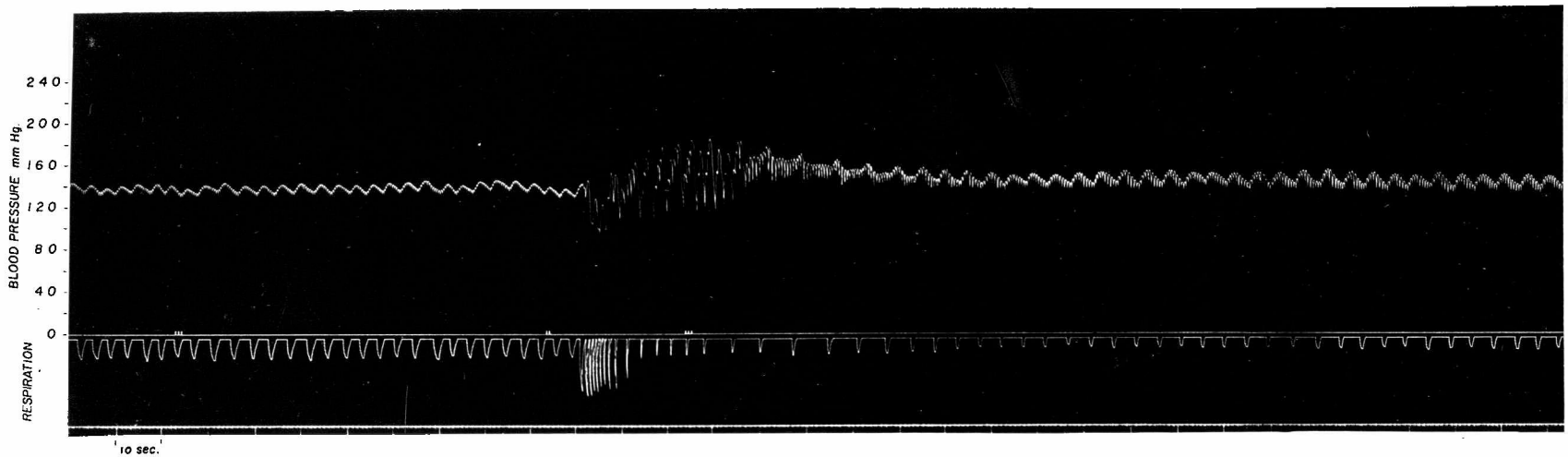

Figure 5. Dog, male, 15.3 Kg. Anesthetic; pentobarbital (30 mcgm/Kg. I.V.). Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following third injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions administration of serotonin. Time interval - 10 seconds. Upper tracing shows the diphasic type of blood pressure response. Lower tracing, the initial hyperpnea followed by a slight decrease in respiratory activity with regards to control rate.

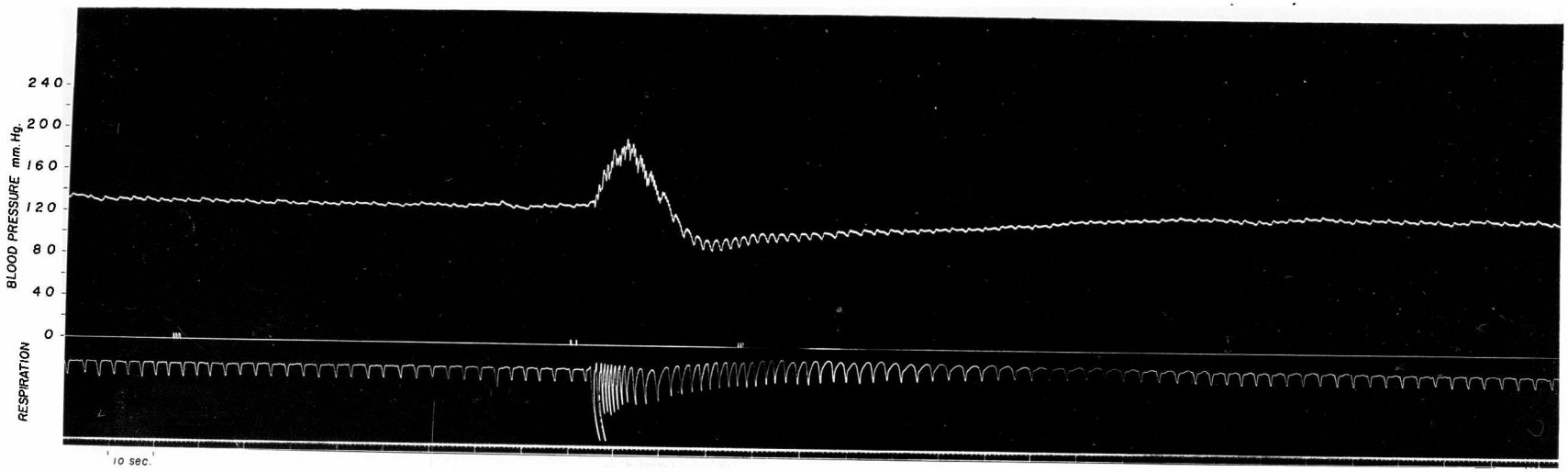

Figure 6. Dog, female, 9.0 Kg. Anesthetic; pentobarbital (30 mcgm/Kg. I.V.). Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following second injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions, administration of serotonin. Time interval - 10 seconds. Upper tracing shows a rise in blood pressure followed by a prolonged depressor response. Lower tracing, the initial hyperpnea followed by a continuation of increased respiratory activity.

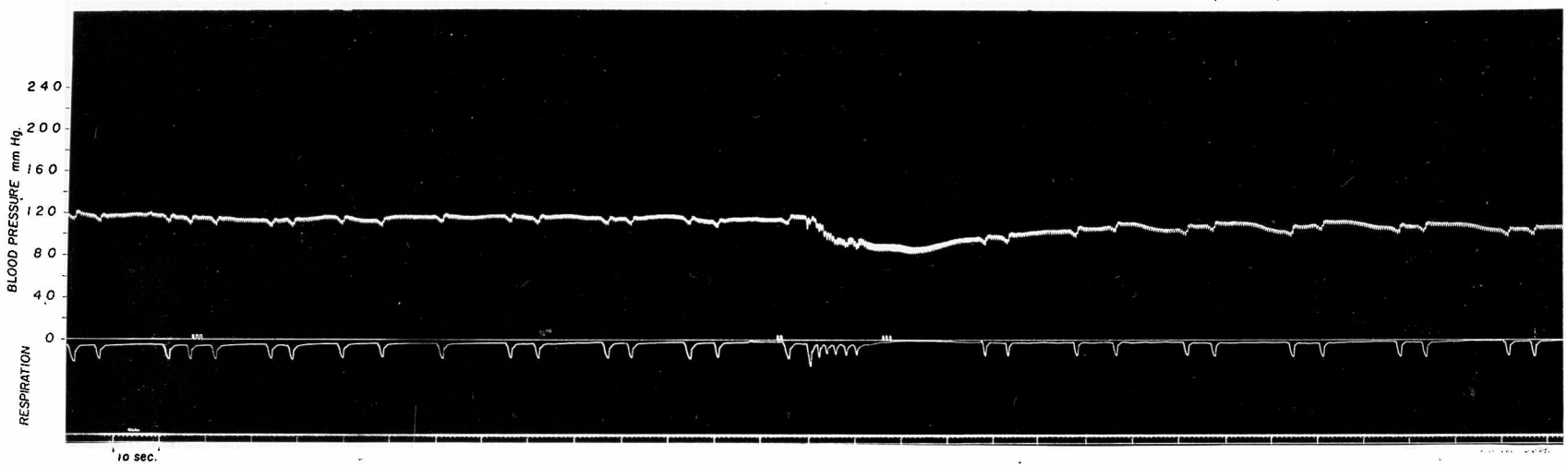

Figure 7. Dog, male, 10.5 Kg. Anesthetic; pentobarbital (30 mgm/Kg. I.V.). Effects of serotonin base (13 mcgm/Kg. I.V.) on blood pressure and heart rate, and respiration following second injection. Blood pressure in mm. Hg., Heart rate by individual excursions of blood pressure tracing. Respiration: pneumographic record; inspiration down. Signal magnet at 0 mm. Hg. Three interruptions in signal magnet tracing indicate time at which blood samples were drawn (arterial and venous); two interruptions administration of serotonin. Time interval - 10 seconds. Upper tracing shows a soley sustained depressor response. Lower tracing, the initial hyperpnea followed by a period of respiratory arrest.

C BLOOD GASES

Arterial and venous samples were obtained and stored, and the analysis for the oxygen and carbon dioxide contents were made by the methods previously cited.

The control samples were withdrawn one to two minutes prior to the administration of serotonin. The oxygen and carbon dioxide contents for both arterial and venous blood closely followed those of the normal dog as found by Stewart (32). The occasional slight variations were attributed to the influence of the anesthetic used.

The oxygen and carbon dioxide contents for the arterial and venous samples taken at the termination of the initial marked respiratory stimulation, or not longer than thirty seconds thereafter, also displayed values similar to those in the normal state.

All analyses for the volume percents of the blood gases were completed within eight hours from withdrawal time and any necessary corrections as detailed in the results in Experimental Part I - Section D were made.

When comparing the mean oxygen and carbon dioxide contents of the samples taken at the pre-in-

jection time with those drawn at the post-injection time, a slight increase in the oxygen content and decrease in the carbon dioxide content were found for both arterial and venous blood samples.

The statistical study of these data was carried out by the analysis of variance. The only source of variance where the oxygen and carbon dioxide contents displayed significant changes was between individuals, for both arterial and venous samples, and between repetition, for the oxygen contents of venous blood. Tables I, II, III, and IV (pages 26 through 29) are a summary of the statistical evaluations.

Upon further examination of the changes in blood gases of the animals demonstrating a respiratory response with a distinct apneic component following the initial marked respiratory stimulation, it was noted that: most frequently, the volume percent of carbon dioxide in arterial and venous blood showed a fall; the percent of change ranging from .06 to 1.1 and .11 to 1.3 respectively. Occasionally, increases were recorded.

The oxygen contents followed a similar pattern, only in the direction of a rise. Table V is a summary

of the blood gas analyses of these animals. The arterial pCO_2 values followed the directional changes of the volumes percent and will be discussed later.

TABLE I

Results of the Analysis of Variance Applied to the

Volumes Percent of Oxygen in Arterial Blood

SOURCE OF VARIANCE	VARIANCE	DEGREES OF FREEDOM	F-RATIO	SIGNIFICANTS
Between Conditions*	2.64	1	0.868	NS
Between Repetition**	5.13	2	1.688	NS
Between Individuals***	24.77	11	8.148	****
Condition x Repetition	.395	2	.130	NS
Error	3.04	55		
Total	453.76	71		

* The inj. of 13 mcgm/Kg. of Serotonin
** 1st inj., 2nd inj., 3rd inj.
*** Dogs - 1 through 12 inclusive
**** .001 - level of confidence
NS Not Significant

TABLE II

Results of the Analysis of Variance Applied to the

Volumes Percent of Carbon Dioxide in Arterial Blood

SOURCE OF VARIANCE	VARIANCE	DEGREES OF FREEDOM	F-RATIO	SIGNIFICANTS
Between Conditions*	7.42	1	0.844	NS
Between Repetition**	18.96	2	2.157	NS
Between Individuals***	150.11	11	17.077	****
Condition x Repetition	1.94	2	.221	NS
Error	8.79	55		
Total	2184.01	71		

* The inj. of 13 mcgm/Kg. of Serotonin
** 1st inj., 2nd inj., 3rd inj.
*** Dogs - 1 through 12 inclusive
**** .001 - level of confidence
NS Not Significant

TABLE III

Results of the Analysis of Variance Applied to the

Volumes Percent of Oxygen in Venous Blood

SOURCE OF VARIANCE	VARIANCE	DEGREES OF FREEDOM	F-RATIO	SIGNIFICANTS
Between Conditions*	6.26	1	1.459	NS
Between Repetition**	42.0	2	9.790	****
Between Individuals***	67.56	11	15.74	****
Condition x Repetition	3.77	2	.879	NS
Error	4.29	55		
Total	1073.25	71		

* The inj. of 13 mcgm/Kg. of Serotonin
** 1st inj., 2nd inj., 3rd inj.
*** Dogs - 1 through 12 inclusive
**** .001 - level of confidence
NS Not Significant

TABLE IV.

Results of the Analysis of Variance Applied to the

Volumes Percent of Carbon Dioxide in Venous Blood

SOURCE OF VARIANCE	VARIANCE	DEGREES OF FREEDOM	F-RATIO	SIGNIFICANTS
Between Conditions*	2.7	1	0.254	NS
Between Repetition**	8.85	2	.834	NS
Between Individuals***	69.80	11	6.579	****
Conditions x Repetition	5.18	2	.488	NS
Error	10.61	55		
Total	1302.5	71		

* The inj. of 13 mcgm/Kg. of Serotonin
** 1st inj., 2nd inj., 3rd inj.
*** Dogs - 1 through 12 inclusive
**** .001 - level of confidence
NS Not Significant

TABLE V

Oxygen and Carbon Dioxide Contents of Arterial and Venous Blood in Dogs before and after the Administration of Serotonin

Dog No.	Wgt.	Inj. No.	ARTERIAL BLOOD Oxygen Before Inj.	Oxygen After Inj.	% Change	Carbon Dioxide Before Inj.	Carbon Dioxide After Inj.	% Change	VENOUS BLOOD Oxygen Before Inj.	Oxygen After Inj.	% Change	Carbon Dioxide Before Inj.	Carbon Dioxide After Inj.	% Change
	Kg.		Vol. %	Vol. %		Vol. %	Vol. %		Vol. %	Vol. %		Vol. %	Vol. %	
2	10.65	1*	16.22	18.06	1.13	45.09	42.83	-0.50	14.73	14.97	0.13	47.55	45.91	-0.34
		2*	16.17	17.10	.58	42.27	39.66	- .62	15.19	11.68	-2.31	47.79	45.52	- .48
		3*	16.15	13.54	1.48	44.47	39.47	-1.12	11.84	12.83	.84	44.18	45.59	.32
3	11.25	1	17.79	15.42	-1.33	48.97	39.07	-2.02	15.28	21.68	4.19	52.76	50.50	- .43
		2	18.44	19.31	.47	45.55	43.67	- .41	16.80	17.82	.61	51.02	50.50	- .10
		3*	17.33	20.66	1.92	38.51	45.23	1.76	15.42	4.73	-6.93	49.51	55.17	1.14
10	7.48	1*	13.04	13.75	.54	42.43	40.93	- .34	6.46	7.23	1.27	47.90	47.38	- .11
		2*	14.04	14.47	.31	42.00	38.88	- .74	5.73	6.75	1.78	46.75	46.23	- .11
		3*	13.31	14.47	.87	42.00	41.74	- .06	6.22	6.75	.85	45.92	39.92	-1.31
11	10.48	1	13.95	14.36	.65	39.30	33.35	- .24	9.62	9.08	- .56	44.07	43.97	- .02
		2*	15.89	15.46	- .27	34.60	36.04	.42	6.00	5.72	- .47	41.88	43.50	.39
		3*	14.18	13.47	-0.50	32.10	34.20	0.65	4.71	5.77	2.25	41.01	43.48	0.60

NOTE: The animals included in this table are only those that demonstrated a distinct apneic response following the initial hyperventilation elicited by the administration of Serotonin base (13 mcgm/Kg. I.V.). All % changes are positive unless noted.

* Injections in which apnea was observed.

DISCUSSION

That the initial respiratory response in dogs, under pentobarbital anesthesia, to the intravenous injections of 5-hydroxytryptamine is increased respiratory activity was a consistent finding and in accordance with the observations of others (19, 7, 8, 27, 17). Douglas and Toh (7, 8), Heymans and van den Heuvel-Heymans (15), Schneider and Yonkman (29) and McCubbin et al (17) have suggested mechanisms possibly involved in this response, but at present there is much disagreement. However, in view of the published data, the effects of serotonin upon respiration seem to be manifested through both peripheral and central mechanisms.

During the preliminary phase of this study, it was noted that the intensity of the initial respiratory response was to a certain degree governed by the depth of anesthesia. This has also been previously observed (19, 8) and can more than likely be accounted for in part by the depressant effects of the barbiturates upon the respiratory centers (12). As previously noted by Douglas and Toh (8), the magnitude of the initial response to serotonin was found to be also influenced by the dose. Small doses elicited only minimal responses, sometimes in the nature of a single gasp, while larger doses produced a marked increase in the rate and amplitude of respiration. However, it was observed in these experiments that the maximum effect for the initial response in the majority of the animals was obtained at 13 mcgm/Kg.

Upon the rapid administration of 13 mcgm/Kg. of 5-hydroxytryptamine, the initial respiratory response displayed a mean latent period of 6.4 seconds and an average duration of 12 seconds, the former figure being a few seconds less than that obtained for the latency of the initial circulatory response. The initial respiratory rate increased approximately five times the pre-injection rate and was accompanied by an increase in respiratory amplitude as illustrated by the respiratory tracings.

It would seem quite evident from these experiments that the initial respiratory effect is independent of the circulatory responses in view of the differences in their latent periods and the variable circulatory responses observed with the consistent finding of respiratory stimulation. This lack of interdependence between the two responses has also been previously noted and more definitely illustrated in experiments where neural pathways were blocked by mechanical and pharmacological measures (19, 20, 8, 29, 17).

Immediately after the initial hyperventilation, four response patterns were observed: one - continuation of the increased respiratory activity, only of far less intensity than that of the initial response; two - a decrease in the respiratory rate with regards to the control value; three - a period of respiratory arrest; four - a period in which the respiratory rate returned to that observed at control time (listed in order of decreasing frequency of occurance). Generally each animal displayed the same pattern for all of the three injections.

The type, intensity and duration of the post-hyperpneic response was not related to the degree of the initial respiratory stimulation rate. Animals showing only a slight decrease in respiratory activity in the post-hyperpneic period may have displayed an initial hyperventilation of three to six times the control rate. In one instance, the injection of a small dose elicited an initial response of a single gasp followed by a period of apnea. These variations also apply to the duration of the initial response, and are of a conflicting nature if one considers the rather soundly established linear relationships between the duration and magnitude of over-ventilation and the existance and duration of the following apneic period, shown as early as 1905 by the observations of Weil (35).

Schneider and Yonkman (29) reported that the intravenous injections of 50 mcgm/Kg. of serotonin consistently produced an initial respiratory stimulation followed in fifty percent of the animals by a period of apnea, thirty percent demonstrating increases in respiratory activity five to ten minutes after the drug injection. MacCanon and Horvath (16) observed that the injection of 500 mcgm. of 5-hydroxytryptamine into the pulmonary artery constantly elicited the initial hyperventilation followed by a period of reduced respiratory activity with apnea in about seventy percent of the instances.

In this study, the initial marked hyperventilation was most frequently followed by a continuation of increased respiratory activity, the rate of breaths per minute being increased to about 1.3 times

the control value. The apneic response was observed in thirty-three percent of the animals with only sixteen percent consistently demonstrating this pattern for all 3 injections. Both of the previous investigators recorded responses of considerably larger doses of serotonin, McCamon and Horvath using a different route of administration. When comparing the data furnished by these workers with that obtained in this report, in regards to the extent of increase for the initial respiratory response, the similarities that existed seem to further strengthen the opinion that the maximum initial effect for most of the animals in this study was obtained at the 13 mcgm/Kg. dose, and suggest that the initial response was governed by the dose strength only to a limited degree.

On the other hand when considering the variations in the occurrence of apnea, the dose response effect could have had a direct bearing on the post-hyperpneic response if one considers Paintal's (23) explanation of the possible mechanisms involved in the initial apneic response as seen in the cat. He considers the period of respiratory arrest to be a resultant of the stimulation of pulmonary deflation receptors found in the lungs and normally these receptors are inactive, but upon moderate stimulation, they cause a reflex respiratory acceleration, and when greatly stimulated they initiate respiratory inhibition. A stimulation such as that evoked pharmacologically by the influence of a drug. He notes that these receptors are stimulated by serotonin and could be sensitized by smaller quantities. He suggests the possibility

that impulses from these receptors may be produced during hyperpnea when the lungs are inflated and deflated more rapidly.

If this action is a responsible factor, it could in part explain why McCanon and Horvath report a higher incidence of apnea than Schneider and Yonkman and why in this study a lower incidence than either of the above was observed. It could also in part account for the recorded apnea following the respiratory response of a single gasp observed in this study, and the appearance of apnea in only the second and/or third injections. However, it would be necessary that in the dog, these receptors act secondarily to those more directly responsible for the consistent initial hyperventilation.

Although the same anesthesia was employed in all three studies, the exact depth is unknown. Suskind and Rahn (33) report that dogs under pentobarbital anesthesia display an initial respiratory depression which reduces the minute volume and respiratory rate approximately forty-five percent from the recovery value. After the initial ventilatory depression (30 min.) the respiratory center slowly recovers and is eventually able to maintain fairly normal alveolar - arterial gas concentrations. The changes observed being more pronounced during the first three hours of the six-hour observation period. This constantly changing state is certainly an important factor in a study of this nature.

In these experiments, the animals that consistently displayed apneic responses also demonstrated gradual increases in the pre-injection

respiratory rate, from injection to injection. This was also observed in the animals that displayed apnea only in the second and/or third injections. This observation together with the fact that once the period of respiratory arrest was obtained, it appeared again in each of the following injections (Illustrated in Table V) seems to establish a relationship between the diminishing effects of the anesthesia on the respiratory center and the occurrence of the apneic response. It must be mentioned however, that some animals displayed higher control respiratory rates than those demonstrating apnea, and their post-hyperpneic responses were of continued respiratory stimulation. It is believed that the absence of the apneic phase in these animals is due in part to animal variation or possibly an individual response pattern governed by an oppositely acting mechanism such as that described by Paintal as previously mentioned.

It is with understanding that statistically significant changes were found between individuals for the volumes percent of both oxygen and carbon dioxide in the arterial and venous blood. These differences in the majority of the instances can more than likely be attributed to animal variation and/or to the difference in respiratory activity due to the varying responsiveness of the individual animals to the anesthetic.

The significant change in the volumes percent of oxygen in venous blood that was observed between repetition was in the direction of a fall, and considered in this report to be due to general failure of the animal, a condition also inferred by the observation of Daly et

al (6). The studies of Suskind and Rahn (33) and Farhi and Rahn (10) indicate that the oxygen reservoirs in venous blood as opposed to the carbon dioxide reservoirs are chiefly governed by the cardiac out-put rate.

As the source of variance in this part of the statistical analysis is the resultant of time, the data was reviewed with this consideration. It was noted that the majority of the animals displayed a decrease in heart rate as the experiment progressed. It was further noted that the parallelism between the general decreases or occasional increases in heart rate and the fall or rise in the venous oxygen content were in the majority of the instances so close that one could predict the change in one by the other. The occasional differences being due to the variations in the pre-injection respiratory rate.

It must be mentioned here that the techniques used for obtaining and analysing the blood samples are in themselves very reliable, but they were by no means adequate for this type of study, in particular the method used in drawing the test sample. For example, the drawing of the test sample was carried out manually; therefore, once the drug had been administered, it was necessary to observe the respiratory tracing and visually determine at what point the initial hyperventilation ceased; this being the signal for withdrawal of the test sample. This situation, together with the unpredictable duration time of the initial hyperventilation, allowed considerable error in evaluating the changes in the volumes percent and partial pressures of the

blood gases. The third injection of animal number three (Fig. 4 and Table V) is an illustration of how, due to the inadequacy of the technique, the test sample was taken while the animal was well in the apneic period rather than immediately at the end of the hyperventilation. It is this explanation that is offered for the discrepancies in Table V, listing animals which displayed a distinct apneic period yet a rise in the volumes percent of carbon dioxide. It is realized that had the blood gas and pH determinations been obtained by continuous flow techniques, this mechanical error would have been eliminated.

Milroy (13), Bransfield and Behrmann (3) in observing the change in the pH of arterial blood in dogs subjected to over-ventilation reported that the blood always showed an alkaline trend. The varying changes depending upon the magnitude of the ventilation. The latter noted that the blood pH began to increase in about 5 to 20 seconds after the hyperventilation had begun. In view of the fact that in these experiments the duration of hyperpnea was about twelve seconds, it was believed that a mean pH value could be employed in obtaining the pCO_2, in the method previously cited, and that any significant change in the partial pressure of carbon dioxide could be detected.

The partial pressures of the carbon dioxide in the arterial blood followed the directional changes of the volumes percent. In the animals consistently displaying apnea the fall ranged from -.5 to -4.3 mm. Hg., the majority of these changes being larger than that consider-

ed adequate to induce a state of apnea (24). However, it should be noted that a change of -.5 is considerably small and that there were many instances in which the animals displayed considerably large drops in the pCO_2 and no apneic response. As the incidence of apnea was far less than that anticipated a statistical evaluation of this data could not be made.

Bancroft et al (1) in attacking a similar problem infused noradrenaline into five conscious male subjects and at the same time administered varying amounts of carbon dioxide. They observed that the transient hyperpneic response due to the infusion of noradrenaline could be converted to one of a sustained nature in the majority of the instances when the subjects inhaled five percent carbon dioxide air mixtures.

Young (36) in studying the possible mechanisms involved in adrenaline hyperpnea observed a similarly sustained period of overventilation in the adrenaline infused, anesthetized animal without the influence of added carbon dioxide. In comparing this response and other similar responses, which in man were altered by the addition of carbon dioxide, she suggested that the explanation of the continued hyperventilation might be in the accumulation of carbon dioxide due to the previously reduced respiratory activity. She also considers the depressant action of the anesthetic themselves. She further recorded different respiratory response patterns dependent on the dose, larger doses of adrenaline introducing a period of inhibition into the respiratory response. This observation is similar to the possibility consider-

ed in this report with regards to the direct action of serotonin on both the primary and secondary respiratory patterns.

In view of the observed fall in the partial pressures of carbon dioxide following the initial hyperpnea, the apneic response in these experiments could have been due in part to the lack of respiratory stimulation furnished by carbon dioxide. It is also believed that the absence of the apneic response in the majority of the animals is due chiefly to the depressant effects of the anesthetic on the respiratory center. The possible influence of a more direct action of the drug on the apneic phase has been discussed.

The vascular responses are of a variable and complex nature. Many factors such as dose of the drug, methods and routes of administration, the effect on special vascular areas, or any pre-existing general or specific condition of the animal definitely to some extent influence the patterns of response. Many investigators have attempted to analyse these responses, but there is still much confusion. A recent review summarizing the investigations carried on in the past four years has been presented by Page (22).

The findings in this study are no less variable than those of others. The intravenous injections were followed by variable arterial pressure responses. Most frequently, the initial response was pressor in nature. Occasionally the rise in arterial pressure was preceded or followed by a transient depressor response. Biphasic patterns were also obtained and only rarely a solely sustained depressor response was

observed.

The direct primary circulatory response, pressor in nature, observed in these experiments is attributed chiefly to the vascular response to the vasoconstrictor action of the drug, as bradycardia was also an initial and consistent observation. It seems quite apparent that the slowing of the heart is due to a direct action of the drug on the heart, for the observed bradycardia was an initial and consistent observation regardless of the variable blood pressure patterns. It is also unlikely that this effect was influenced by the initial hyperventilation for the differences in the latency periods was but one to two seconds. The occasional secondary pressure responses, depressor in nature, are considered to be the resultant of the observed bradycardia.

When comparing the pre-injection arterial pressure levels for the first, second and third injections of all animals, it was noted that the initial variable effects were followed by a prolonged depressor response. The fall ranging from six to twenty-six mm. Hg. Seventy-five percent of the animals consistently demonstrated this effect between each injection while the remaining animals re-established approximately the same pre-injection arterial pressures for the second injection but then they also demonstrated a fall between the second and third.

With this in view, the initial pressure responses for the successive injections were reviewed and it was noted that a slight change from a predominately pressor response to one preceded by a transient fall

was evident. This latent prolonged depressor response has been previously observed (19, 20, 29) and considered by Rudolph and Paul (23) to be the resultant of peripheral vasodilation. As to what degree this effect in these experiments is due to the action of serotonin or general failure of the animal is not known. But if one considers the very convincing evidence that this latent fall is influenced in part by preexisting vascular tone (20), directional pressure changes being inversely proportional to the tone, it seems likely that the initial pressure responses to the same dose of 5-hydroxytryptamine in successive injections would become more pressor, as previously noted (19). But the findings of this report are to the contrary. It was observed in the preliminary study that the pressor response was increased with the dose, also previously observed (7, 8) but in the following experiments the successive injections, when separated by at least a forty-five minute interval of the same dose strength had no consistently appreciable effect on the pressor response neither in the direction of magnitude or duration. It is recognized however that this latent depressor effect differs from the initial transient fall (20) the former being attributed to the bradycardia. As a slowing of the heart was a regular finding in this study regardless of the arterial pressure changes, it is believed that the initial transient fall, as previously mentioned, is due to a direct action on the heart, which possibly becomes more sensitive upon repeated injections. The predominately pressor response attributed in part to the direct vasoconstrictor action of the drug which in this study was generally forceful enough to mask the effect of the initial bradycardia.

CONCLUSIONS

1. The intravenous injection of 13 mcgm/Kg. of 5-hydroxytryptamine to the pentobarbitalized dog elicits initially a marked increase in respiratory activity and variable circulatory responses.
2. The initial respiratory effect is generally followed by a continuation of increased activity only of a much less intensity, and occasionally by a period of respiratory arrest.
3. The initial respiratory effect appears to be due to the direct action of the drug and is independent of the circulatory responses.
4. The apneic phase is also independent of the circulatory effects.
5. The apneic response is accompanied by a decrease in the partial pressure of carbon dioxide and is in part more than likely a resultant of the decrease of this important respiratory stimulant.
6. The circulatory responses are of variable pattern, the more prominent initial effects being pressor in nature.
7. This pressor effect is due chiefly to the vasoconstrictor action of the drug.
8. Bradycardia was a consistent observation and considered to be the chief contributing factor to the recorded initial depressor responses and appears due to the direct action of the drug on the heart.
9. The initial variable circulatory response was generally followed by a prolonged depressor effect suggesting a latent vasodepressor activity of 5-hydroxytryptamine.

SUMMARY

1. The effects of the intravenous injections of 13 mgm/Kg. of 5-hydroxytryptamine (serotonin) on the respiratory and cardiovascular systems of the dog were studied. The respiratory response pattern consistently displayed an initial marked increase in respiratory activity, followed by variable secondary responses, one of which being characterized by a period of apnea.
2. The circulatory response displayed highly variable patterns, the initial component being chiefly pressor in nature, generally followed by a latent prolonged depressor response. Bradycardia was a consistent finding. The similarities between the observations in this study and those of previous investigators has been discussed.
3. Arterial blood samples were drawn at control times and again at the termination of the initial hyperventilation and the pCO_2 values were determined. Generally the initial hyperventilation was accompanied by a fall in the arterial blood pCO_2. The possible influence of the changes in arterial blood pCO_2, the effects of the anesthetic and the dose of serotonin used upon the secondary respiratory response patterns has been discussed.

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ABSTRACT

In this report, the effects of the intravenous injections of 5-hydroxytryptamine (serotonin) on the respiratory and cardiovascular systems of the dog have been investigated. This study also includes a test of the hypothesis that the initial hyperpnea elicited by the injection of 5-hydroxytryptamine produced a state of acapnia which in turn is responsible for the subsequent period of apnea.

Eighteen dogs anesthetized with pentobarbital sodium were studied - recording respiration, blood pressure and heart rate. Arterial and venous blood samples were drawn at control times and again immediately following the initial respiratory effect produced by the drug. Arterial pCO_2 values were determined.

From the data obtained, the following conclusions were made: the intravenous femoral injections of 5-hydroxytryptamine (13 mcgm/K$_g$.) elicites initially a marked increase in respiratory activity and variable circulatory responses; the initial hyperpnea is followed by variable respiratory patterns; the circulatory response generally consisted of an initial pressor effect followed by a latent prolonged depressor response; the respiratory response appears to be independent of the circulatory responses and due to the direct action of the drug; the apneic response is accompanied by a decrease in the arterial pCO_2 and may in part be a resultant of the decrease of this important respiratory stimulant.

The possibility of a more direct role of the drug on the secondary respiratory response and the correlations of the findings of this report with those of previous investigators is discussed.

A STUDY OF THE EFFECTS OF 5-HYDROXYTRYPTAMINE (SEROTONIN) ON THE RESPIRATORY AND CARDIOVASCULAR SYSTEMS OF THE DOG.

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A Thesis

Submitted to

the Faculty of the Department of Biology

University of Houston

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

** *******

by

Louis Joseph Lucco

August 1958