1956

On the neurogenesis of respiratory rhythmicity

Frederick Ware
University of Nebraska Medical Center

ON THE EUROGENESIS OF RESPIRATORY RHYTHMICITY

Frederick Ware, B.S., M.S., Ph.D.

Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

April 2, 1956

Omaha, Nebraska

TABLE OF CONTENTS

The range of topics which might be covered under the general subject of the physiology of respiration is indeed broad. It could include the physiological anatomy of the bones, joints, and muscles concerned in the respiratory act, the physics of gas diffusion and gas exchange between the external medium and the alveolar spaces, the mechanics of gas movement across the alveolar membrane, the chemistry of gas transport within the vascular system, the biochemistry of internal respiration, the reflex and chemical control over the rate and depth of respiration, and the mechanisms concerned with the origin of rhythmicity in the respiratory act. The latter topic actually has two parts, the mechanisms responsible for the repetetive firing of the respiratory neurons during a given respiratory act (e.g. inspiration), and the mechanisms responsible for the generation of alternating inspiration and expiration. This Thesis is concerned mainly with this latter question-- why does an animal breathe in and out? That the brain is essential in maintaining breathing has been known since the time of Galen, though the vital significance of the neural structures within the medulla was first demonstrated by Legallois in 1812 (1). Since that time, there has been active interest in this problem, and in general three theories have been

developed to explain how the nervous system generates the respiratory rhythm. According to the first, respiration is a reflex process, basically similar to many other processes in the body which are known to be reflex in character. The respiratory centers in the medulla (see later discussion) are supposed to be driven by afferent impulses from various receptors. By such reasoning, the isolated respiratory centers should be quiescent. According to the second theory, the basic rhythm of breathing is generated by structures or mechanisms which reside within the confines of the medulla, although, to be sure, influences from the periphery and other portions of the central nefvous system may modify this rhythm in important ways. According to this theory, the isolated medulla would continue to produce nervous activity essentially similar to that found during normal respiration. During the latter part of the nineteenth century, this concept was fairly widely accepted (1,2), although the demonstration of inspiratory cramp in the vagotomized animal after midpontine decerebration by Marckwald (3,4), plus the various researches which confirmed and extended this observation (2,5) caused it to fall into some disrepute. As will be shown later, recent developments have resulted in a re-emergence of this theory, so that at the present

time it would appear to be the one best supported by experimental evidence. The third theory holds that the isolated medullary respiratory centers would be spontaneously active, giving rise to a maintained, strong inspiratory cramp. Normally this inspiratory cramp is periodically interrupted by impulses from receptors in the lung which are stimulated by inflation (inspirato-inhibitory impulses of the Hering-Breuer reflex) or by a structure located in the brain stem near the upper reaches of the pons (the pneumotaxic center). This hypothesis has gained very wide popularity, especially in the form detailed by Pitts and his co-workers (2,5,6,7,8,9,10,11), although it seems probable that fairly recent experimental evidence, reported mainly by Hoff & Breckenridge in a series of papers beginning in 1949, will serve to decrease its acceptance in favor of the second mentioned theory (1,12,13,14,15, 16,17,18,19,20).

Although it seems clear that respiration can under some circumstances be reflex in nature, as for instance in the case where breathing can be maintained in an animal with a depressed nervous system by rhythmic single stimuli (painful or electrical) to the pad of the foot (19), it appears to be felt by the majority of workers that the basic respiratory rhythm is not reflex in nature (1).

Therefore nothing further will be said about this theory. The main emphasis of this Thesis then is to discuss evidence relating to the second and third theories mentioned above, in an attempt to decide between them, at least as far as present knowledge allows. Preceeding this discussion there will be a short presentation of neuroanatomy as it pertains to respiration and a brief historical review of the main contributions to this field.

THE NEUROANATOMY OF RESPIRATION. The pertinent nervous structures may be considered under three headings: the basic respiratory centers, central nervous system structures having an influence on respiration, and peripheral nerves concerned with respiration. The information available has been obtained using four basic techniques: focal recording from restricted areas of the brain and from small slips of nerve, focal stimulation of portions of the brain, and controlled destruction or removal of portions of the brain.

Legallois (1) first demonstrated that the medulla contained the essential respiratory centers. He showed this by demonstrating that rabbits continued to breathe after all serial brain sections except those which removed the medulla. An attempt to make more precise localization of the centers within the confines of the

medulla was made by Pitts, Magoun, & Ranson (6). They stimulated minute areas (1-2 mm^3) within the brain stem of cats and noted the respiratory responses which resulted. It was found that in some areas stimulation caused a maximal inspiration which, if the stimulation were continued, would be maintained until death of the animal from asphyxia. Other electrode locations gave apneic or expiratory responses, although never prolonged to death. Alternate stimulation of appropriate areas might cause alternate inspiration and expiration resembling the normal respiratory rhythm. On the basis of their experiments, the authors concluded that the essential respiratory centers are located in the medulla in the reticular formation overlying the inferior olive, deep to the nuclear masses on the floor of the fourth ventricle. In addition, they interpreted their results to indicate that a ventral and posterior inspiratory center was imperfectly separated from a more dorsal and anterior expiratory center. It should not be thought, however, that these respiratory "centers" were considered to be discrete nuclear masses analogous to, for instance, the nucleus solitarius or the red nucleus (21). On the contrary, it was felt that neurons serving respiratory functions are mixed in the reticular formation of the medulla in the regions mentioned

with only approximate segregation. On the basis of evidence gained using focal recording, Gesell, Bricker & Magee (22) have concluded that the respiratory centers are indeed located in the medulla (there appears to be little if any evidence refuting this fact) but that the respiratory neurons serving inspiratory and expiratory functions are quite randomly assembled. From an examination of the evidence, Hoff & Breckenridge (1) conclude that the localization suggested by Pitts et al may in fact be true in general, but there is a large degree of mixing of the neurons. Axons from the respiratory neurons descend in the anterior and anterolateral columns of the spinal cord (9) to make synaptic connection with the anterior horn cells which innervate the various respiratory muscles.

In the broadest sense, almost any portion of the central nervous system has potential connection with the respiratory centers in the medulla. Thus the greatest breathing may be attained by voluntary hyperventilation (influence of cerebrum); in hysterical states increased breathing may be severe enough to produce marked respiratory alkalosis (thalamus-hypothalamus); and the respiratory pattern is characteristically modified by panting (2) (hypothalamus). While these influences are of considerable importance in regulating the rate and depth of respiration, they do not appear to be of significance

in generation of the basic respiratory rhythm, for classical mid-collicular decerebration of an animal (which obviously removes the centers mentioned) does not produce breathing fundamentally different from normal eupnea (14,19). Within the confines of the lower brain stem, however, there appear to be structures of more basic importance to respiration. Thus, if the vagus nerves be cut in an animal, and then the brain stem be sectioned in the region of the upper pons, it will immediately develop a prolonged inspiratory cramp (3,4), while if the brain section be made just above the pons (e.g. immediately below the inferior colliculi), no such inspiratory cramp will develop (23). Obviously, some structure of importance residing in the upper reaches of the pons is removed by the former section. This structure, which is widely referred to as the "pneumotaxic center" (23), has recently been accurately localized by Tang (24) in the extreme dorsolateral anterior pontine tegmentum. Although Lumsden (23) observed that animals continued to show breathing after a brain section below the pons, he regarded it as a "gasping" respiration without physiological significance, and regarded the medullary centers as "gasping centers". Actually this appellation is synonomous with "respiratory centers" as used by others. Lums-

den coined the term "apneusis" (from the Greek for breath-holding) for the inspiratory cramp seen in the vagotomized animal after pontine section (the term will be used henceforth), and considered that it represented unrestrained activity of the true respiratory center in the pontine region. This he termed the "apneustic center". These various names very possibly imply a more specific segregation of function of neural structures within the pons than is warranted (1,19). Possibly these "centers" only represent partially specialized portions of the reticular facilitatory and inhibitory systems (1,19,25). Nevertheless, the terms have become firmly entrenched in the literature, and it seems unlikely that they will be abandoned in the near future.

Like the centers within the central nervous system, all peripheral afferent nerves potentially have connection with the respiratory centers in the medulla. However, in this situation also, only a few have special significance for the present discussion. Of foremost importance are the vagus nerves, which contain fibers serving the Hering Breuer reflexes. The end organs are within the lungs and are stimulated by inflation (inspirato-inhibitory fibers) or deflation (inspirato-excitatory fibers) of the lungs (5). The exact significance of the vagus nerves in respiration appears still to be in some

doubt, and it will be discussed later how the function of the vagi has been differently interpreted by different workers. The carotid and aortic bodies (the chemoreceptors) are another peripheral mechanism of respiratory significance. These structures respond to low oxygen tendion (and to acid and carbon dioxide, to a lesser degree) and have the net effect of stimulating respiration and the cardiovascular system. Experimentally, the chemoreceptors may be stimulated by ventilation with low oxygen mixtures, by occlusion of the carotid arteries, by injection of small amounts of cyanide, or by injection of lobeline. Other sensory receptors, such as pain end organs, receptors along the respiratory passages, and cold sensitive endings, have importance in regulation of rate and depth of breathing but do not appear to have basic significance in generation of the breathing rhythm. The direct action of carbon dioxide and acid on the medullary centers may be mentioned here, for it has been suggested (26) that the potent stimulating action of these agents on respiration is mediated through chemosensitive receptors in the medulla. At any rate, the direct action of CO_2 does appear to be pertinent to this discussion.

The efferent influence of the medullary centers is

widespread. From the inspiratory center fibers pass to neurons controlling the diaphragm, inspiratory chest muscles, and accessory muscles of inspiration (e.g. facial and neck muscles). Thus there is a central nervous system synapse interposed between neurons of the inspiratory center in the medulla and the muscles of respiration. Fibers from the expiratory center pass to the expiratory muscles of the abdomen and chest. Also, it appears (7) that important inhibitory connections exist between neurons of the inspiratory and expiratory centers, so that in eupnea the main expression of activity of the expiratory center is in the inhibition of the inspiratory center.

THE DEVELOPMENT OF CONCEPTS CONCERNING RESPIRATORY RHYTHMICITY. By the middle of the nineteenth century, both the theory of inherent medullary rhythmicity and the concept of basic inspiratory drive had been enunciated (1,2,3,4,5). At the end of the century Marckwald (3,4) made the important discovery that an animal subjected to a classical (mid-collicular) decerebration continues to breathe in an essentially normal manner when the vagi are cut, while if the decerebration is performed below the inferior colliculi, subsequent vagotomy leads to a prolonged inspiratory cramp. Marckwald concluded that the tonic inspiratory drive of the medullary centers could

be periodically interrupted by afferent impulses from the lungs or from a structure which he concluded was located in the inferior colliculi. He also attributed special respiratory function to the trigeminal nerves, though subsequent work has not confirmed this.

Lumsden (23,27,28,29) named the inspiratory cramp apneusis, and he also felt that vagotomy was not necessary to produce apneusis after pontine decerebration. It may be mentioned that this finding has not been confirmed by other workers, and Breckenridge & Hoff (13) have suggested that unrecognized damage to the vagal mechanisms (pressure, hemorrhage) may have influenced Lumsden's results. Lumsden observed that if section of the brain were made at the upper end of the medulla in an animal with apneusis, the inspiratory cramp disappeared at once, leaving occasional jerky respirations. He felt that these breaths were not of physiological significance, but rather represented activity of a primitive "gasping center" in the medulla. The true respiratory centers were envisoned as being within the pons, and were termed the apneustic center. He considered that the rhythm of breathing was generated entirely by fractionation of activity of the apneustic center by influence of the pneumotaxic center, which he determined to be in the upper reaches of the pons (rather than in the inferior collic-

uli). Lumsden's records show that apneusis does not completely obliterate all signs of rhythmic respiration, but rather a "fringe" of rhythmic activity is superimposed on the apneusis. Also, the apneusis is not maintained tonically until death from asphyxia, but instead persists for 2-3 minutes, then a forceful expiration occurs, a few quick phasic breaths are taken, and then another breath holding supervenes. Lumsden was of the opinion that this behaviour was entirely the result of the pronounced fall in blood pressure during prolonged apneusis, with resultant asphyxia of the "apneustic center" and emergence of the "gasping center" for a few breaths, during which the improved ventilation restored the original conditions. Lumsden also observed that progressive anoxia or anesthetics can produce progressive functional "brain stem sections". Thus, with deepening anesthesia, there might first be slow, deep breathing (seen after vagotomy in the intact or midcollicular animal), then apneusis (equivalent to midpontine section), and lastly gasping respiration (as only the medulla is left functioning). He further observed that continuous stimulation of the central end of a cut vagus during/apneusis can eliminate the inspiratory cramp and replace it with phasic breathing. Lumsden's work has been mentioned in moderate detail to show that many of the pieces of evidence on which present day concepts are based were known in 1923, but Lumsden and some of the subsequent

workers either disregarded or (apparently) misinterpreted them; and those data which were emphasized seemed to corroborate the theory of tonic inspiratory drive.

In 1929, Henderson & Sweet (30) confirmed the occurrance of apneusis in vagotomized animals after pontine section, but (contrary to Lumsden) emphasized that the vagotomy was an essential step. Also, in contradistinction to Lumsden, they concluded that the true inspiratory centers are located in the medulla (and almost all subsequent wokers have concurred in this opinion). Henderson & Sweet felt that apneusis is merely a manifestation of decerebrate rigidity, and it will be seen later that this general concept is included in the postulates of Hoff & Breckenridge. It goes almost without saying that section of the brain stem anywhere in the region from the corpora quadrigemina to the striae acousticae produces decerebrate rigidity of the voluntary muscles, due to removal of important portions of the suppressor systems with concomitant retention of significant reinforcing elements in the brain stem (25). Since the respiratory muscles also have postural function, it is not surprising that apneusis might be correlated with decerebrate rigidity. Inasmuch as the spasticity of the body musculature occurs with intact vagi, while apneusis appears only af-

ter section of these nerves, Henderson & Sweet assigned a special "anti-decerebrate rigidity of the respiratory muscles" function to the vagi. Teregulow (31) and Hess (32) also suggested that apneusis was closely associated with decerebrate rigidity.

Stella (33,34,35,36) also studied the activities of the brain respiratory centers. He disagreed strongly with the concept that apneusis is a manifestation of decerebrate rigidity, for he found several functional dissimilarities between the two phenomena. For instance, decerebrate rigidity appears after midcollicular section, while apneusis does not, regardless of whether the vagi are sectioned. Also, after pontine section, vagotomy is necessary to produce apneusis, while this act is without apparent effect on decerebrate rigidity (see later discussion, however). Furthermore, unlike decerebrate rigidity, apneusis is not affected by removal of the vestibular afferent or by deafferentation of the respiratory muscles. Lastly, apneusis is markedly influenced by carbon dioxide acting directly on the brain stem and by impulses from the chemoreceptors, while decerebrate rigidity is not so affected. Stella ruled out the inferior colliculi, the red nucleus, and the trigeminal nerves as of significance in relation to apneusis. Although he ob-

served "gasping" respiration in the medullary animal (after section at the rostral end of the medulla), he agreed with Lumsden that this had no physiological significance. He suggested that some apparent discrepancies of results and/or interpretations in the literature might be reconciled by assuming that the pneumotaxic center extends farther down in the pons and upper medulla than usually assumed (see later discussion for argument refuting this).

Pitts and associates (2,5,6,7,8,9,10,11,37,37a) confirmed many previous findings using more precise techniques. The inspiratory and expiratory centers were localized in the medulla, and the pneumotaxic center was placed in the anterior pontine tegmentum. Electrical stimulation of the inspiratory center was found to be very similar to apneusis, and it was found possible to terminate apneusis either by strong stimulation of the central end of the vagus or by stimulation of the expiratory center. On the basis of their findings, it was concluded that the theory outlined by Marckwald was essentially correct--that the inspiratory center is tonically active and must be periodically interrupted by impulses from the vagi or the pneumotaxic center. The formulations of Pitts, in regard to the localization of the various structures within the central nervous system as well as to

the mechanism of their interaction, have been widely accepted.

A short abstract in 1942 by Nicholson & Hong (38) revived interest in the problem. They reported that, contrary to the opinion expressed by Pitts, apneusis is not a permanent thing, leading to death from asphyxia, but on the contrary is periodically interrupted to produce apneustic breathing, which is adequate to sustain life for hours. Also, the apneusis in dogs (note that almost all previous workers used cats, and there appear to be quantitative species differences, as will be mentioned later) is not complete, but instead a "respiratory fringe" is superimposed on the inspiratory spasm. Furthermore, section at the rostral end of the medulla immediately abolishes apneusis, leaving an irregular but non-difficult breathing-- therefore a type of eupnea. It should be noted that Lumsden had reported these findings many years before, but their importance had never been emphasized.

Hoff & Breckenridge (12) essentially repeated the work of Lumsden and Nicholson & Hong, showing that apneusis is not complete or permanent, that a dying preparation shows a loss of apneusis with emergence of eupnea, and that medullary section abolishes apneusis and estab-

lishes eupnea. They concluded that the medullary respiratory centers are basically rhythmic and that the theory of tonic inspiratory drive is incorrect. They have followed up this original paper with a series of studies, all of them consistent with the concept of inherent medullary periodicity. The points of evidence will be discussed shortly.

To be sure other workers have made contributions which have influenced the development of this field (39, 40,41,42,43,44,45,46,47,48,49,50), but the researches mentioned appear to be the ones most often quoted in the literature on this subject.

THEORY OF TONIC INSPIRATORY DRIVE. The essence of this theory was outlined by Marckwald in his original papers. Lumsden regarded his researches as being in harmony with it except for a few particulars, and Stella confirmed Marckwald's findings, agreed with his conclusions, and felt that he successfully refuted the opinions of Henderson & Sweet which were contrary to the theory of tonic inspiratory drive. It was, however, Pitts, Magoun & Ranson (6,7,8) who provided the most precise experimental results, and it is their statement of the theory which is usually regarded as the definitive one. Thus it will be outlined here, and the main points of evidence on which it is based will be mentioned.

The inspiratory and expiratory centers are regarded as physiologically distinct and anatomically partially separated, as evidenced by the respiratory responses to focal stimulation in the medulla. The fact that the stimulating current was limited to less than 3 mm^3 of tissue while the centers occupied more than 10 times this volume, plus the fact that strong stimulation of this small fraction of the inspiratory center could lead to a maximal inspiration led to the conclusion that rich excitatory connections exist among the cells of a given center. The neurons of the inspiratory and expiratory centers are linked by inhibitory fibers, as evidenced by the fact that stimulation of (for instance) the expiratory center during inspiration occasioned by stimulation of the inspiratory center will decrease the magnitude of the inspiration. Since, in most instances, stimulation of the expiratory center only decreases or abolishes inspiration, it was concluded that the main influence of the expiratory center during eupnea is simply inhibition of the inspiratory center. More intense stimulation (analogous to hyperventilation) is required to cause active contraction of the expiratory muscles. The inspiratory center was demonstrated to be the dominant one by simultaneous maximal stimulation of both centers and showing that the net

response was strong inspiration. According to the theory, then, the medullary organization may be summarized as follows: in the beginning, a few inspiratory neurons are discharged (perhaps as a result of excitation by some one of the numerous afferents having respiratory function). Due to the excitatory interconnections, this incipient activity is rapidly magnified, leading to maintained inspiration. Coincidentally, all expiratory activity is suppressed because of the inhibitory connections. This end result occurs because the inspiratory center is the dominant one. This "spontaneous" inspiration (actually an apneusis) does not involve maximal response of the inspiratory center, however, for stimulation during an apneusis can increase the magnitude of the inspiration (obviously this might represent recruitment of previously quiescent neurons, increased rate of neuron firing, or both). This analysis is of basic importance to the theory of tonic inspiratory drive, and it is clear that it predicts that an animal having a brain stem section which isolates the medullary components from all rostral influence must demonstrate an apneusis. It may be mentioned here that several authors who have subscribed to this theory have actually demonstrated that this does not occur. Lumsden (23) explained this by assuming that the true inspiratory center (his apneustic center) is located

in the pons, with only a vestigial "gasping center" in the medulla. Pitts and associates, as far as I have been able to discover, never made a brain section which would actually test the point, though they assumed that the postulated response would occur.

When a section is made in the midpontine region, it is clear that this does not actually isolate the medulla from rostral influences, for pontine neural structures are included with the medulla. Nevertheless, in some cases it would appear that investigators have assumed that pontine section essentially isolates the medulla. One of the important contributions of Hoff & Breckenridge has been the demonstration of the fallacy of this assumption.

In a vagotomized animal, a midpontine section immediately produces apneusis, while if the vagi are left intact, no inspiratory cramp ensues. The theory of tonic inspiratory drive offers a reasonable explanation. It is known that inflation of the chest stimulates receptors in the lung which are inhibitory to respiration. Thus, according to the theory, spontaneous activity of the inspiratory center leads to ever-increasing expansion of the chest, which in turn causes a mounting number of impulses over the vagi. These impulses discharge into the nucleus solitarius and then are relayed to the expiratory center, stimulating it. When the lung inflation has

proceeded sufficiently, the resulting vagal activity excites the expiratory center (thus over-riding the inhibition of the center caused by the active inspiratory center), and this suddenly inhibits the inspiratory center, abruptly terminating inspiration and initiating expiration. In eupnea, the intensity of the inspiratory effort is comparatively low (thus less inhibition is required to stop it), the inflation of the chest is moderate in amount and rate (thus stimulation of the Hering Breuer reflex is sub-maximal), and the inherent excitability of the expiratory center is low (minimal stimulation by CO_2 or chemoreceptors). These factors summate to account for the fact that expiration in eupnea involves only inhibition of inspiration with elastic recoil of the chest. In hyperventilation, alterations in the factors mentioned leads to active participation of the expiratory muscles. It may be mentioned that Pitts et al (7) felt that the vagal influence is mediated through the expiratory center because the respiratory responses to central vagal stimulation and direct stimulation of the expiratory center were markedly similar. It is seen that according to this analysis the vagi are capable of exerting a breath-by-breath control over respiration. In fact Pitts et al concluded that in the intact animal under most circumstances the vagal mechanism outlined is

the one which is responsible for periodically inhibiting the tonic inspiratory drive and producing rhythmic breathing (2,5,7).

From the time of Marckwald, however, it has been apparent that the vagal mechanism is not the only one, for vagotomy in a midcollicular preparation does not produce apneusis but only slows the respiratory rate and increases the depth (the same effect occurs with vagotomy in the intact animal). Marckwald (3,4) concluded that the center responsible for periodic interruption of inspiration was located in the inferior colliculi, but subsequent investigations have shown that the center, called the pneumotaxic center, is actually located in the anterior 2-3 mm of the pontine tegmentum (7,8,23,33). The most accurate localization (24) has shown that the neurons which prevent apneusis in the decerebrate, vagotomized animal (i.e. the pneumotaxic center) are located in the extreme dorsolateral portion of the anterior pontine tegmentum, and the fiber paths connecting these cells with the medullary centers run in the lateral ventral pontine tegmentum (8).

Lumsden (23,28) concluded that this pneumotaxic center was the only one responsible for the production of rhythmic breathing, for in his experiments vagotomy was not necessary to produce apneusis after appropriate

brain section. All others, however, have shown that vagotomy is a necessary procedure (see Hoff & Breckenridge, 13), and Pitts et al (2,5,7,8) considered that the pneumotaxic mechanism is a separate one which is usually subsidiary to the feed-back system involving the vagi. While it was admitted that no cogent evidence was available, Pitts et al (7) suggested that the operation might be as follows: collaterals from the active inspiratory center stimulate the pneumotaxic center, which in turn stimulates the expiratory center. In the absence of the Hering Breuer reflex (i.e. after vagotomy), which is a more sensitive mechanism and which therefore ordinarily is the controlling one, increasing activity of the spontaneously firing inspiratory neurons leads to increasing stimulation of the pneumotaxic center. When the threshold of these neurons has been reached, they become active and stimulate the expiratory center. When this cycle of events becomes strong enough, the inhibition of the expiratory neurons (due to activity of the inspiratory center) is oversome, they become active, inspiration is terminated, and expiration occurs. By assuming that this mechanism is rather sluggish, it was possible to account for the slow, deep respiration seen after vagotomy. When the brain section is made almost anywhere in the pons, the pneumotaxic center is severed from the medulla, for the center

is in the extreme rostral portion of the pons. After such a section, coupled with vagotomy, the medullary respiratory centers are isolated from all significant rostral influences (according to the theory; recall that this may not be true). Then the natural dominance of the inspiratory center exerts itself, and lasting apneusis is produced (actually proponents of this theory (e.g. Marckwald, 3,4; Lumsden, 23; Pitts et al, 7,8) have observed that apneusis is <u>not</u> always permanent, but they have not apparently regarded the observation of any great significance).

It was mentioned that Marckwald thought the pneumotaxic center was in the inferior colliculi and that the trigeminal nerves had a special respiratory significance. Henderson & Sweet (30) were of the opinion that the red nucleus was vital in the production of apneusis (at that time the red nucleus was thought to be responsible for decerebrate rigidity). All three of these structures have been shown since then to be without special respiratory function in the sense under discussion (8,33). In addition, it has been suggested (2,8) that the pneumotaxic center may extend farther down into the pons than described above and that this might be of significance in explaining some discrepancies between theory and fact with low brain section. Actually, the anatomic evidence

of Tang (24) makes this very unlikely, and the possibility seems altogether ruled out by the reasoning presented by Hoff & Breckenridge (1,14,19), to be presented later.

THEORY OF INHERENT MEDULLARY PERIODICITY. There have long been investigators who have believed that the basic respiratory rhythm is generated within the medulla and is only modified by externally arising influences. Evidence compatible with such a view, for instance the continuing breathing in a decerebrate animal after vagotomy or the slow potential swings showing respiratory rhythmicity in the isolated brain stem of the goldfish (51) was criticized by Pitts (2) on the grounds that the pneumotaxic mechanism was still present in the experiments and was actually generating the respiration. As mentioned earlier, several pieces of evidence of greater (in retrospect) decisiveness have been available since Lumsden's contributions, but these items were either disregarded entirely or thought of minor importance compared to the evidence considered as strong support for the theory of tonic inspiratory drive. The short abstract by Nicholson & Hong (38), which reported results definitely not in accord with the theory of inspiratory drive, was mentioned by Pitts in his 1946 review (2) but was not considered to be a serious threat to the theory. In 1949 Hoff & Breckenridge published a paper (12) reporting

experiments which were in the main merely a confirmation of previous bits of evidence scattered in the literature, but the points were considered in toto to constitute strong evidence that the theory of tonic inspiratory drive is incorrect and that the medullary centers are inherently rhythmic. These men have followed up the original work with a series of papers, all of which are consistent with the expressed view, and which form the basis for a new concept of respiration and the factors which modify it. Some of these points may now be discussed. Many of them were brought out in the first paper presented in 1949, and the others have come up in more recent publications (13,14,15,16,17,18,19,20).

Apneusis is not permanent. That it should be so is implicit in the theory of inspiratory drive, but several authors pointed out (or their published records show) that apneusis is maintained for only a few minutes, then a forceful expiration occurs, a few phasic breaths are taken, and another inspiratory cramp develops (3,4,7,8, 12,23,38). Pitts et al (7) pointed out that this occurs especially in animals subjected to prolonged artificial respiration, and they therefore considered that this apneustic breathing (in contrast to a simple, maintained apneusis) might be largely artefactual in nature. Most work prior to Hoff & Breckenridge was done using cats,

for the actual brain sections are more easily accomplished in this animal, and the bleeding is more easily controlled. Hoff & Breckenridge pointed out that while apneustic breathing is not always prominent in cats, it always takes place in dogs (which they have used in most of their experiments). Furthermore, after apneusis has been established in an animal, with occasional expirations fractionating it to produce apneustic breathing, the periods of breath holding shorten progressively with time, so that the breathing aspects of the apneusis become more pronounced and the inspiratory cramp less. Hoff & Breckenridge (13) subsequently showed that apneustic breathing does take place in the cat as well as the dog, and therefore the statement that apneusis is never permanent, which is contrary to the theory of tonic inspiratory drive, is justified. While in the absence of artificial respiration there are marked changes in blood pressure during apneustic breathing (23), periodic interruption of the inspiratory cramp still occurs even if asphyxia is prevented by adequate artificial ventilation. Also, Hoff & Breckenridge have stated that the blood pressure variations during apneusis are more responsible for death of the animal (due to increased bleeding around the site of transection)than the accompanying asphyxia (19).

Apneusis is not complete. According to the theory of tonic inspiratory drive apneusis represents uninhibited activity of the inspiratory center. Although this activity need not be maximal (8), the theory requires that it be maintained and steady (i.e. that there be no vestige of respiratory rhythmicity). Actually, this is not the case. During an apneusis there is unmistakable evidence of persisting respiratory rhythmicity. This takes the form, first, of a "respiratory fringe" superimposed on the apneusis. This is a series of slow, phasic breaths, whose amplitude varies inversely with the completeness of the apneusis (see later discussion of periodic breathing). Of equal significance is the persisting activity of the accessory muscles of respiration (12,13,19). It is stated that this always occurs even when the apneusis is so vigorous that all trace of phasic breathing is eliminated from the respiratory record. The respiratory fringe has been known to exist for a long time (23), and it has been demonstrated in the dog (12, 38), cat (7,13,23), and rabbit (47).

Anatomic or physiological isolation of the medulla will abolish apneusis and replace it with eupnea. This would appear to be perhaps the most crucial point of the whole discussion. By the theory of inspiratory drive, the isolated medulla should produce steady, maintained

inspiration without trace of rhythmicity until death from asphyxia occurs. The only modification of the theory which could explain any deviation from this expected behaviour is that of Lumsden (23), who believed that the true inspiratory center was located in the pons and that a vestigial gasping center was located in the medulla. The preponderance of work, particularly the precise investigations of Pitts, Magoun & Ranson (6) have demonstrated that the basic inspiratory and expiratory centers are located in the medulla. Thus the demonstration of rhythmic breathing in the vagotomized medullary preparation would seem to be a very serious blow to the theory of tonic inspiratory drive. That this is the true situation seems beyond any question, for it has been demonstrated by quite a number of independent investigators (12,13,23,24,30,33,38,47,49). All of the mentioned workers performed surgical section of the brain stem at the medullary level in animals breathing apneustically and observed that the apneusis was abolished immediately and in its stead there appeared irregular phasic breathing. As Hoff & Breckenridge have pointed out, the irregularity of the breathing, both in terms of timing and amplitude, allow it to be classified nicely as "ataxic", but the fact that it is not labored plus the demonstration that it is adequate to maintain the life of the animal for

hours requires that it also be classed as a form of eupnea. This latter concept is in contrast to Lumsden (23), who thought that the phasic breathing seen under these circumstances represented the final respiratory effort in a dying animal. As mentioned before, it appears that Pitts et al never performed section of the brain stem at the medullary level to see if the postulated apneusis would develop, although such omission is in marked contrast to the meticulous precision of other aspects of their work.

It seems that the action of many depressants on the nervous system is to attack the most vulnerable centers first, which centers appear to be the most recently acquired phylogenetically, thus leaving the most vital and "firmly established" ones to last. Following this concept, one may assume that impending death, progressive asphyxia, deepening anesthesia, and several drugs may perform functional "serial section" of the brain stem. Thus one might expect that anesthesia etc. could convert apneusis into a "medullary preparation". This seems to be the case (7,12,13,23,24). In these cases anoxia or anesthesia abolished apneusis and replaced it with the ataxic eupnea described above. Thus again the last act of the isolated medulla was not apneusis but phasic breathing. Further pharmacological "sectioning" of the

brain stem, as done by Hoff & Breckenridge, will be described below.

As was mentioned earlier, it has been suggested (2, 33) that perhaps the pneumotaxic center extends down into the medulla. This might explain why breathing in the medullary preparation is not apneustic. As stated, however, the localization by Tang (24) showed that the pneumotaxic center occupies a small, discrete area in the anterior pons. More important refutation comes from the respiratory behaviour of vagotomized animals subjected to brain section at various levels. When the cut is made at the extreme rostral end of the pons, no apneusis results. As the section is performed more caudally, apneusis appears, and reaches a maximum intensity when the transection is made at the junction between the upper third and the rest of the pons. As the cut is made increasingly caudad, the degree of breath holding progressively decreases, until all trace of it is gone in the medullary preparation. This makes it appear very likely that during apneusis the medullary centers are being "driven" by neural structures diff-usely arranged within the substance of the pons, and as the brain sections exclude more and more of these areas the intensity of the inspiratory cramp decreases. Certainly if the pneumotaxic center extended from the anterior pons into the medulla,

and there were no other important respiratory structures within the pons (as assumed in the theory of inspiratory drive), then it would be difficult to explain how progressive removal of the pneumotaxic center (by serial brain section) could cause first increasing apneusis, then diminishing inspiratory cramp, and finally, with medullary section (which should by this reasoning leave the least pneumotaxic center and therefore the greatest net inspiratory drive) complete loss of apneusis and replacement by eupnea.

At this point it may be convenient to present the essentials of Hoff & Breckenridge's concepts of respiration. They conclude that the basic respiratory rhythm is generated within the neural structures of the medulla, a view fully consistent with the finding that the medullary preparation continues to show a kind of eupnea. All other influences, chemical and reflex, merely modify this medullary rhythmicity, although to be sure in some cases (e.g. apneusis) the modification may almost entirely obscure the medulla's fundamental activity. Thus the vagus nerves, for instance, are thought to mediate in general an inhibition of respiration, principally by modulating supramedullary mechanisms, although this inhibition is not manifest in the breath-by-breath control of respiration as previously thought. Other specific fac-

tors will be discussed later. The dramatic inspiratory cramp found in the vagotomized midpontine preparation, which constituted the main evidence for the existence of the pneumotaxic center in the sense postulated by the theory of inspiratory drive, must be explained satisfactorily by any reasonable concept of this subject. It appears that the mechanism may be similar to that proposed in 1929 by Henderson & Sweet (30), namely that apneusis basically represents a decerebrate rigidity of the respiratory muscles. Earlier it was pointed out that Stella (33) made some valid observations on certain dissimilarities between apneusis and decerebrate rigidity, and therefore he rejected this explanation. However, Hoff & Breckenridge (19) have observed that all breathing is superimposed as a phasic act upon a postural background and that there are actually a number of important correlates between the properties of decerebrate rigidity and apneusis (for example in the decerebrate animal the rigidity does sometimes increase when the vagus nerves are sectioned). Thus, while there are undoubtedly at least some quantitative differences between the two states, apneusis may well represent a form of decerebrate rigidity. It should be realized, however, that while the basic mechanisms may be quite similar, it is not necessary that the same neurons be involved in both phe-

nomena, and if they are not identical this could easily account for some differences between the two states. Following this reasoning, the pneumotaxic center, as named by Lumsden (23), and best localized by Tang, corresponds to a semi-specialized portion of the bulbar suppressor system; and exclusion of this region of the reticular substance allows the more generalized facilitatory areas to become dominant, fully analogous to the production of decerebrate rigidity by the exclusion of inhibitory influences from (especially) the basal ganglia. When the suppressor areas (pneumotaxic center) are removed from a vagotomized animal, the pontine reinforcing system drives the medullary respiratory centers into prolonged inspiration, and this spasm occludes major phasic respiration. That the medulla continues to generate a respiratory rhythm despite these events is shown by the respiratory fringe superimposed on the apneusis and the persisting activity of the accessory respiratory muscles throughout apneusis (they do not go into any inspiratory spasm). This explanation fits the presented facts quite well. In the vagotomized animal with brain section at the anterior limit of the pons, both the suppressor and facilitatory areas of the reticular substance are intact, and normal phasic breathing continues. With slightly more caudal sections, the suppressor system is rapidly cut

off (recall it lies in the anterior 2-3 mm of the pons), the reinforcing areas gain dominance, and apneusis supervenes, the degree being maximal when all of the pneumotaxic center has been excluded but almost all of the reinforcing system remains. As the section is made still farther posterior, more and more of the facilitatory areas are removed, and, as would be expected, the apneusis lessens, disappearing in the medullary animal.

Further confirmation for these views was obtained by Hoff & Breckenridge using techniques which apparently accomplish pharmacological section of the brain stem. One method of doing this (17) involves controlled hypoxia. If a midcollicular animal is vagohomized, it continues to breathe phasically. If it is now subjected to hypoxia, it first develops apneusis and later the pattern of medullary ataxic eupnea. Readmission of oxygen often reverses the sequence. In one favorable preparation carefully controlled hypoxia first caused apneusis with a respiratory fringe, then diminished inspiratory spasm with coincident increase in phasic breathing, later fully developed Biot's respiration (see later discussion on periodic breathing), and lastly medullary eupnea. Assuming the correctness of the opinion that hypoxia in general tends to attack first the most vulnerable and also most rostral structures, these experiments are obviously

in full harmony with the analysis of apneusis and medullary rhythmicity presented above.

It is clear that decerebrate rigidity involves pleurisynaptic pathways. Myanesin is a drug which is thought to be rather specific in depressing polysynaptic reflexes (18), and, as might be anticipated from this, Myanesin is capable of abolishing decerebrate rigidity. If apneusis basically represents a form of decerebrate rigidity, it must depend on polysynaptic pathways, and it should be affected by Myanesin. This is, in fact, true. In every case, the drug either abolished or diminished apneusis, with concomitant increase in the phasic breathing component which is superimposed. In some cases Biot's respiration was produced. Morphine also appears to depress polysynaptic reflexes, and morphine affects respiration in a way basically similar to Myanesin (15). However, morphine also causes marked deleterious changes in the cardiovascular system. D-l Dromoran, a synthetic narcotic, was found to be a superior tool in this research (16). This drug also appears to cause the equivalent of serial brain stem sections in dogs. In the intact dog it facilitates the appearance of "all-or-none" breathing (see below), as it also does in the midcollicular animal with intact vagi. In the latter preparation, however, if the vagi are cut, Dromoran produces apneusis; and if the

dosage is increased the inspiratory spasm diminishes with simultaneous increase in phasic breathing. In some cases a pharmacological section at the medullary level can be produced, giving rise to the usual ataxic eupnea. If Dromoran is administered to an animal breathing apneustically, the inspiratory cramp is always reduced or abolished, and often Biot's respiration appears. In the medullary animal (produced surgically), the drug always produces apnea in expiration.

It may be noted that respiratory mechanisms appear to be similar in the cat, dog, rabbit, and monkey (the only animals studied to date), and all reports containing comparable experiments have confirmed the points made by Hoff & Breckenridge relating to generation of a respiratory rhythm by the medulla and to the production of apneusis (3,4,6,7,8,23,24,30,33,38,43,47,49,50). It appears justified, therefore, to conclude that the currently av-ailable evidence definitely favors the theory of inherent medullary rhythmicity in the generation of respiration and casts great doubt on the existence of any tonic inspiratory drive in the medullary respiratory centers. In the next section the different components in the respiratory assemblage will be discussed in the light of what is presently known. It must be emphasized that knowledge is limited to the extent that we are in the des-

criptive rather than analytic stage, and generalizations which are made now mainly as a matter of convenience may soon have to be revised or rejected.

FUNCTIONS OF THE RESPIRATORY COMPONENTS. Medullary Centers. The basic respiratory act of the isolated medulla is ataxic eupnea. This type of breathing differs from normal eupnea in that the inspiration is very sharp and jet-like, with a duration shorter than normal. Furthermore the amplitude of each breath bears no predictable relation to adjacent ones (the variation from the smallest to the largest is about 1:2), and the timing of the breaths is very irregular (the whole phenomenon is similar t_o cardiac auricular fibrillation, where the force and timing of the ventricular beats is irregular). This type of breathing can maintain life for many hours under favorable circumstances. Thus this "gasping" respiration does not appear to be a vestigial accomplishment performed by a dying animal, and without physiological significance, as claimed by Lumsden, but on the contrary probably represents the basic respiratory rhythm which may be modified by the several well-known mechanisms, reflex and chemical.

The medulla is capable of producing another type of respiratory act (19). This is characterized by an apparently maximal inspiration which is accompanied by intense

activity of the accessory respiratory muscles. It has been called sighing respiration or "all-or-none" respiration (40). This latter appellation, which is the one preferred by Hoff & Breckenridge, should not imply the same physiological behaviour as "all-or-none" when applied to conduction of the action potential in nerve or muscle, but rather is used only to indicate that the breath is about as forceful as the animal is capable of producing. It is often seen as the final respiratory effort in a dying animal. The rate of all-or-none breathing is slow and irregular in the medullary animal (up to perhaps 2/minute) and is mingled with medullary eupnea. Rarely the all-or-none rhythm may be the only one seen in the medullary animal.

One other type of rhythm may occasionally be seen in the medullary preparation. This is Biot's breathing, which consists of clusters of breaths, all of about the same amplitude, separated by apneic intervals. Biot's respiration will be discussed more later.

Pontine Centers. The true anatomical and physiological makeup of the pontine reticular system as it relates to the respiratory act is not yet known. Thus at the present time it is necessary, to some degree, to make broad interpretations based on somewhat fragmentary and indirect evidence. For instance, in the present discus-

sion "centers" subserving certain "functions" will be assigned to the pons, the reasoning being largely based on the form of the respiratory record as influenced by brain stem sections and other experimental procedures. Actually, it is very possible (perhaps even probable) that no such centers exist in any anatomical sense, and the physiological demonstration of them depends on a certain pattern of procedures. Realizing then that the following description is mainly for convenience and is highly tentative, essentially 4 pontine centers may be described (12,14,16,18,19).

The facilitatory centers probably are part of the brain stem reticular system described by Magoun (25) which regulates the inherent excitability of motoneurones. Activity of these centers tends to discharge the respiratory neurons. The system is widely distributed in the pontine tegmentum, and it is the unopposed effect of it which is responsible for apneusis.

The inhibitory centers likewise appear to have a function similar to the reticular suppressor areas which regulate motor irritability, but the respiratory center (the pneumotaxic center) may be semi-specialized in function and partially separated anatomically from the general suppressor system (7,24,25,33).

The inspiratory breath holding center is probably

entirely a descriptive convenience. Operationally it controls the tonus of the respiratory muscles, and as such likely represents the balance between the facilitatory and inhibitory centers mentioned. It is described as an entity here because it is this balance which is actually apparent in the breathing record of an animal.

The center for apneustic rhythm also could quite possibly have no real existence but rather represent a functional combination of neuronal activity. This center is responsible for generating the peculiar, slow respiratory rhythm seen classically in apneusis, with equal or (usually) more time in inspiration than expiration. As will be discussed below, the action of this center is strongly reminiscent of that of an electronic switch, which alternately allows a signal to pass or blocks it.

The patterns of breathing seen in the pontine animal may now be described. When a brain section is made in the lower reaches of the pons, the irregularity which characterizes medullary eupnea is not seen. In the classical decerebrate animal the eupnea is indistinguishable from that in the intact animal. The only obvious change caused by decerebration is a marked facilitation of the all-or-none rhythm with definite post-sigh inhibition of eupnea. This is demonstrable on the respiratory record by the increased rate of the deep sighs and by the

decreased amplitude of the eupneic breaths immediately following each all-or-none breath. Often several eupneic breaths are required to bring the amplitude gradually back to the normal level. This makes it appear that eupnea and all-or-none breathing represent fundamentally different rhythms which are mutually inhibitory. Post-sigh inhibition may be seen sometimes in the medullary preparation, but it is much less developed. Administration of Dromoran facilitates the sighing respiration in the decerebrate animal and produces very prominent post-sigh inhibition, and it is under these circumstances that the phenomenon is best developed.

When the vagus nerves are cut in the midcollicular animal, there is such facilitation of all-or-none breathing that eupnea is entirely suppressed. Thus the slow, deep respiration seen under these conditions (or after vagotomy in the intact animal) is due entirely to the sighing respiration. When the midpontine animal is vagotomized, the breath holding center becomes active, as does the apneustic rhythm center. The result is the usual apneusis, which represents periodic shutting-off of inspiratory cramp (produced by the unopposed activity of the reinforcing centers) by the pontine "electronic switch". The respiratory fringe seen during apneusis is that all-or-none respiration which has not been occluded

by the apneusis. Usually the breath holding and apneustic rhythm centers become demonstrably active together, producing apneusis; but occasionally it is possible to have function of the "electronic switch", with minimal or no activity of the inspiratory cramp system. Under these conditions the sighing breathing comes in clusters, which constitutes Biot's respiration. This differentiation has been produced by lucky brain section (19), by administration of Myanesin (18) and Dromoran (16), and by controlled hypoxia (17).

It seems possible that the inspiratory breath holding center may, under some conditions, be active alone. The effect would be to increase the respiratory midposition of the chest. This commonly occurs in hypoxia, emphysema, and Cheyne-Stokes breathing, for example (19).

Since surgical brain section at the caudal end of the pons occasionally removes all of the breath holding center but leaves some of the apneustic rhythm center (producing Biot's respiration), it would seem that the latter has some medullary representation, while the former does not.

Since the all-or-none rhythm is markedly facilitated in the decerebrate animal while this type of breathing is almost never found in the intact animal, it appears that the midbrain and forebrain contain structures which

normally inhibit the sighing respiration, leaving eupnea as the form which is seen. Also, panting probably depends on influences from these areas, particularly the heat regulating centers in the hypothalamus (2), for panting is never seen in the medullary or decerebrate preparation. Detailed discussion of what little evidence is available on these subjects is beyond the scope of this Thesis.

Vagal Reflexes. That the vagus nerves have important respiratory function has been known for many years, and since the researches of Hering in the middle nineteenth century it has been realized that the main vagal effect is inhibitory to respiration, though there are reflexes originating in the lungs which may stimulate respiration (5). It was basic to the theory of tonic inspiratory drive that the inhibitory fibers in the vagus were instrumental in interrupting inspiration to produce breathing. The evidence cited above has shown that the theory is not adequate, and instead it is now felt that the vagus exerts a more steady inhibitory bias to the respiratory centers, despite the fact that stimulation of the receptors occurs only during inspiration. A strong piece of evidence for this view is the observation by Kerr et al (47) that sustained high frequency stimulation of the central end of the vagus can change apneusis into rhythmic breathing. Lumsden (28) and Pitts et al (8) made

similar observations but did not emphasize them. Obviously the vagus nerves cannot exert breath-by-breath control over respiration under these circumstances; while the results are in full accord with the view that afferent vagal impulses act similarly to the bulbar suppressor system. Hoff & Breckenridge (1) suggest that the vagal impulses become so diffused in time and space in the brain stem that much of the inspiratory timing becomes lost. The action is somewhat analogous to that of a half wave rectifier and filter in an electronic power supply. While the evidence seems to rule out the participation of the vagi in the actual generation of the respiratory rhythm, it should be realized that it is still possible that in the intact animal the mounting vagal inhibition during inspiration might "tip the scales" and thereby be instrumental in determining the exact moment at which the inherent respiratory rhythm switches from inspiration to expiration. For descriptive purposes it is possible to consider vagal action in relation to the various respiratory centers already outlined.

The vagus appears to exert little direct control on the medullary centers, for vagotomy is usually without dramatic effect in the medullary animal showing ataxic eupnea (12,14, 23). Whenever an effect is seen, it consists of a moderate increase in respiratory rate and depth after vagotomy. This suggests that at this level

the vagus exerts only a general depressant action on the respiratory centers, a concept confirmed by the finding that vagotomy increases the apneustic response to electrical stimulation of the inspiratory center (6,43).

The most important vagal influences seem to be channeled through the pontine reticular substance. Here the vagus inhibits the breath holding center, as shown by the fact that apneusis never occurs when the vagi are functioning. Also, the vagus inhibits the all-or-none rhythm, as shown by the great increase in this type of breathing after vagotomy. All-in-all it is clear that the vagi exert an important source of drive to the reticular suppressor system and/or they directly inhibit the facilitatory centers. The general problem of vagal action cannot by any means be considered as solved, however, and much further investigation needs to be done.

Chemoreceptors. These peripheral receptors respond particularly to decreased arterial oxygen tension and somewhat to increased acid and CO_2. From the facts that hypoxia increases apneusis (12,33) and denervation of the carotid bodies decreases apneusis with concomitant increase in phasic breathing (12), it is clear that the carotid and aortic bodies constitute a source of afferent drive similar to the reticular reinforcing centers. Perhaps the chemoreceptors act through the reticular sub-

stance. Stimulation of the chemoreceptors has not been shown to cause any increase in phasic breathing. This topic has clinical importance in relation to the breathing in hypoxia conditions, and the mechanism outlined explains nicely the increased respiratory midposition with hypoxia (e.g. emphysema, Cheyne-Stokes breathing, acute asthma, anoxic anoxia).

Direct Effects of Carbon Dioxide. Whether this gas acts directly on the respiratory neurons (44) or through chemosensitive receptors in the medulla (26), and whether molecular carbon dioxide as well as increased acidity (52,53) is effective are not well established points, but fortunately they are not crucial to this discussion. Certainly excess carbonic acid applied to the medulla is an exceedingly potent respiratory stimulant. In the intact animal very minute increases in the concentration increase the rate and (especially) the depth of breathing. Stella (34) believed that increased CO_2 increased apneusis, while Hoff & Breckenridge (19) have not found this to be the case, although an excess of the substance increases sensitivity of the medullary structures to chemoreceptor influence. Chatfield & Purpura (43) showed that when the inspiratory center was stimulated electrically with just sufficient current to cause a maintained apneusis without respiratory fringe, ventilation

with 5% CO_2 caused a definite respiratory fringe to be superimposed on the inspiration-- that is, the CO_2 apparently increased the basic rhythmicity of the respiratory centers. The same conclusion is suggested by the more dramatic experiment of von Euler & Soderberg (50), who found that ventilation of the completely deafferented medullary preparation with 6% CO_2 caused it to produce a burst of action potentials, whose crescendo form resembled strikingly the record seen during a normal inspiration. That is to say, it would seem that the CO_2 induced a medulla which had previously been perfectly quiescent (full apnea) to generate a respiratory cycle. This important observation was not emphasized by the original authors, nor has it been confirmed, but it deserves further study. On the basis of present fragmentary information it is therefore possible to assume that CO_2 in some unknown way causes the respiratory complex in the medulla to be more vigorous in generating a respiratory rhythm. This action of the gas in stimulating oscillatory behaviour is especially interesting since CO_2 is a very powerful agent in stabilizing peripheral nerve and decreasing spontaneous oscillations of membrane potential (54,55,56).

PERIODIC BREATHING. This term is used to describe those types of respiration in which the individual breaths

vary periodically in amplitude or in which the breaths come in clusters with apneic intervals between. The first is the well known Cheyne-Stokes respiration, often seen in desperately ill patients, and sometimes seen under less unfavorable circumstances (e.g. after voluntary hyperventilation). The second type is Biot's respiration, which is uncommon in humans, but is frequently seen in dogs.

In Cheyne-Stokes respiration the breaths gradually build up to a maximum amplitude, then die away again, only to repeat the entire cycle with or without an intervening period of apnea. This behaviour has usually been explained on the basis of a depressed respiratory center and its reactions to CO_2 and chemoreceptor stimulation, as follows: the depressed medullary centers do not respond to the usual concentrations of CO_2; therefore there is apnea, and asphyxia develops. The falling oxygen tension stimulates the chemoreceptors, contributing to respiratory drive; and the rising CO_2 also increases the excitability of the respiratory centers. The net result is that phasic breathing begins, and gradually builds up in vigor. However, this ventilation rapidly eliminates the asphyxia and therewith also the effective respiratory stimulus. Therefore, the breathing dies away, and the cycle is repeated. It is clear that varying gas

tensions during the cycle play a vital role in this explanation.

Hoff & Breckenridge (19) have suggested another explanation, according to which periodic breathing is a result of physiological mechanisms within the brain stem, and changes in gas tensions, while doubtless of importance in modifying the respiratory pattern, are not vital in the production of it. They point out that Cheyne-Stokes breathing is really a whole syndrome, involving important changes in consciousness, blood pressure, heart rate, eye reactions, and other bodily functions. Furthermore, the characteristic sequence of events is not essentially changed in humans or experimental animals by adequate artificial ventilation, which naturally removes the major portion of asphyxia during the apneic intervals. This fact, which was first demonstrated many years ago, would seem to be a strong point for the thesis that periodic breathing is generated within the central nervous system. Their argument is deemed cogent enough to warrant a brief summary of some experimental findings.

Biot's Breathing. This type of respiration is seen frequently in dogs and cats, and the evidence that it represents a certain level of intrinsic respiratory organization seems quite good. From the description already given of apneustic breathing, with the superimposed respir-

ation, it is clear that if the breath holding component be removed, the phasic breaths remaining will occur in clusters, and this constitutes Biot's respiration. Conversely, if Biot's respiration has occurred, addition of inspiratory cramp will change it into apneustic breathing. Experimentally, Hoff & Breckenridge Have accomplished both changes. Sometimes Biot's respiration has developed spontaneosuly in their animals, and in a few such dogs vagotomy has produced apneusis, consistent with the proposed vagal function of inhibiting the breath holding center. The conversion of apneusis into Biot's breathing has been done more often. It can be accomplished by controlled hypoxia (17), Myanesin (18), and Dromoran (16). Under these conditions, there may be a progressive decrease in the inspiratory cramp during apneustic breathing, with coincident increase in the superimposed phasic breaths, until in many cases all trace of apneusis disappears, leaving pure Biot's respiration. Further treatment with the experimental agent will produce medullary eupnea, sometimes with Cheyne-Stokes respiration as an intermediate form. In a few cases, a lucky surgical brain section has produced Biot's respiration. In terms of the respiratory components described earlier, Biot's breathing corresponds to exclusion of the breath holding center with retention of the apneustic rhythm center. Putting

it another way, the "electronic switch" function is operating with very crisp turn-off and turn-on, so that the basic medullary rhythmicity is alternately allowed to be fully manifest and then shut off completely. These experiments seem to constitute convincing evidence that Biot's respiration is indeed a product of brain stem activity, representing only partial integration of the various central nervous system respiratory complexes, and that no external influences are vital to its production.

Cheyne-Stokes Respiration. Unfortunately, this phenomenon does not appear often in dogs, and this must detract from an theory based on experience using dogs as the experimental object. In some dogs, however, during progressive deterioration, or as a result of increasing dosage of Myanesin or Dromoran, Biot's respiration was observed by Hoff & Breckenridge to change over gradually into Cheyne-Stokes breathing. Thus, after a period of apnea, the first breaths were small in amplitude, gradually building up to a maximum, and then fading away again. Further deterioration of the animal always produced medullary eupnea. It would seem that Cheyne-Stokes respiration appeared when the apneustic rhythm center was in the process of failing, so that the "electronic switch" function was not sharp, and a few breaths at the beginning and end of a cycle were allowed to be manifest

in an attenuated form rather than being either completely suppressed or entirely unhindered. Thus Cheyne-Stokes respiration would represent even less respiratory organization than Biot's and would be only one step removed from the simple medullary preparation. Such a concept is compatible with the clinical fact that Cheyne-Stokes respiration often occurs in patients near death and that it carries in general a bad prognosis. The explanation is not completely satisfactory when it is considered that (1) a patient may breathe in this manner for many hours without a tendency for medullary breathing to appear as the terminal act, (2) Cheyne-Stokes breathing does not often appear in dogs, and the records of Hoff & Breckenridge do not seem to match completely the picture seen in patients, and (3) Cheyne-Stokes breathing may be seen in sleep and after voluntary hyperventilation, when it does not seem that the person is only one step removed from medullary breathing and two steps removed from death. Hoff & Breckenridge point out that during sleep important changes in function of the brain stem reticular systems occur, as evidenced by alterations in many bodily functions, but intuitively it seems unlikely that they could be potent enough to produce essentially a low decerebration. And it seems very unlikely that Cheyne-Stokes breathing in the conscious person after

hyperventilation could represent almost complete loss of respiratory coordination, as suggested by the analysis presented above.

Thus there is very important evidence that the mechanisms of periodic breathing must in large part be sought within the confines of the central nervous system, but it does not seem that present knowledge is sufficient to elucidate the problem completely.

THE MECHANISM OF MEDULLARY RHYTHMICITY. No definite statements can be made on the mechanisms by which the medulla is capable of generating the respiratory rhythm, but a few observations in the literature are pertinent to this problem of neurophysiology.

The problem essentially is how neurons of the inspiratory center are caused to fire at an increasing rate until, at the peak of inspiration, they suddenly become quiescent, remaining so throughout expiration (which may be active or passive), and then becoming active again as the next inspiration commences. The subject may be considered under two main headings: the repetetive firing of single neurons, and the alternating periods of activity and silence.

Recent findings in the field of electrophysiology have shed considerable light on the manner in which single cells may discharge trains of impulses. In general, excitable cells (nerve, skeletal muscle, and cardiac

muscle are the tissues most extensively studied) are stimulated when their membrane potential is reduced to a critical value, which is in the neighborhood of 50-60 millivolts for all cells studied (57,58,59,60,61), compared to the resting membrane potential which ranges from 70 to 100 millivolts. Within certain limits, the critical membrane potential is quite independent of the rate at which it is approached (58). Thus if a very we-ak constant current be applied to a cell, its membrane potential will fall slowly until, when the critical membrane potential is reached, it generates an action potential. If the stimulating current is strong, however, the threshold potential will be reached much more quickly. A second principle of utmost importance is that many cells will discharge repetitively when a sustained constant stimulating current is applied. This is contrary to the commonly accepted (but incorrect) statement that a cell will fire only once in response to a constant stimulus. This misconception is based on the fact that a myelinated nerve usually (but not always) responds in this fashion. Actually most tissues behave in the opposite way-- they fire trains of action potentials when stimulated by a constant current. This has been shown in vertebrate muscle (62), unmyelinated nerve (63-- a classical paper), motoneurons (64), and several types of peripheral re-

ceptors (65,66,67). Furthermore, the rate of firing in all cases is smoothly graded with the intensity of the stimulating current. It appears possible that under these conditions the membrane potential is driven close to the resting level by membrane processes following each action potential, and then the stimulating current again causes a depolarization which leads to another action potential when the critical membrane potential has been reached. Thus sustained firing is produced. It may be noted that, in all probability, it makes little difference how the membrane potential is reduced to the threshold level; that is, an electrical catelectrotonus or chemical mediation may work equally well. Certainly at the neuromuscular junction and in the heart depolarization processes are normally intimately associated with chemical processes but may be influenced readily by electrical polarization (68,69). These concepts apply readily to the physiology of the respiratory centers. Thus if, during inspiration, some stimulating process gradually built up in intensity, the inspiratory neurons would respond with a smoothly graded increase in rate of firing. That the membrane potential of neurons within the central nervous system does actually undergo slow variation is strongly indicated by the alpha rhythm of the EEG, the microelectrode studies of Li & Jasper (70) in the brain stem of

the cat, the potential records of Adrian & Buytendyk (51) taken from the isolated brain stem of the goldfish, and the striking strychnine potentials in the mammalian spinal cord (71). Furthermore, in several of these instances, it was found that brief bursts of action potential occurred during the waves of depolarization, the rate in general increasing with the magnitude of the depolarization. These findings are all in excellent agreement with the concept that some smoothly progressing process drives the respiratory neurons. Since neuromuscular transmission is almost certainly chemical (69), and evidence is accruing that central synaptic transmission likewise is chemical as opposed to electrical (61,72,73), it seems quite possible that it is the accumulation of some depolarizing substance which drives the inspiratory neurons in their crescendo pattern.

Concerning the second topic-- the alternating periods of activity and quiescence of the respiratory neurons, there is less satisfactory information. There is not even good evidence to delimit the areas necessary for the generation of breathing. As Hoff & Breckenridge have pointed out, in the medullary preparation there is still the possibility that important ascending impulses from the spinal cord could be affecting the medulla. That this might be the case is indicated by one of their

experiments in which postural movements occurred synchronously with respiration. Section of the brain stem just caudal to the obex (thereby removing the medullary respiratory centers) immediately produced permanent apnea, but the postural changes continued as before with the same rhythm, suggesting that spinal cord centers are of generalized significance. On the other hand, Adrian & Buytendyk (51) isolated the brain stem of the goldfish (thus spinal centers removed) and found slow potential variations (proved actually to represent waves of negativity of the vagal lobes)(thus the general area of the respiratory centers) having a striking resemblance to the respiratory rhythm. Also, von Euler & Soderberg (50) found that increased CO_2 was able to produce a burst of action potentials suggestive of respiratory activity from the completely deafferented cat medulla. Clearly, available evidence shows that structures rostral to the medulla are not essential to a respiratory rhythm, but further work must be done to determine if ascending impulses are dispensible.

If the crescendo activity of the respiratory neurons involves an accumulation of some excitatory neurohumoral agent, as proposed above, there is presumably some local positive feedback circuit. One simple mechanism would involve some "pacemaker" area, whose influence is made

widespread by rich synaptic connections. Such a mechanism would be somewhat analogous to the known pacemaker region in the heart (60). The question at once arises concerning the physiology of a limited aggregate of neurons-- must each neuron in such a pacemaker pool be unstable in order that the whole be unstable? According to Ashby (74) the answer is "no", for a random network of neurons will tend to become unstable (that is oscillatory) as the network becomes larger, regardless of whether the individual cells are stable or not. From this point of view, any stable network of neurons becomes the unusal case, and one which requires explanation in terms of neurophysiological mechanisms.

Thus one might imagine that a pacemaker area may be stimulated to initial firing in a non-specific way (e.g. by the direct action of CO_2), and then its own intrinsic capabilities generate two opposing tendencies: first positive feedback, so that increasing inspiratory activity leads to augmented firing of the driving pacemaker, and second a threshold negative feedback, so that at a certain level of inspiratory activity central inhibition (75) becomes manifest, and the inspiratory tide is arrested. Some recent findings have suggested one possibility for this inhibition. It has been confirmed (72) that the firing of spinal motoneurons generates, with

very brief latency, an inhibition of the neurons of the same motor pool. This has been shown to be due to stimulation, by collaterals of the motoneuron, of special small neurons, called Renshaw cells after the man who discovered the phenomenon. The Renshaw cells are cholinergic (i.e. are stimulated by acetylcholine), and they in turn presumably liberate an inhibitor substance at the motoneuron soma. (Recent work from Eccles' laboratory--76-- has suggested that direct central inhibition--75-- may involve an interneuron, contrary to previous concepts. Thus its mechanism would be entirely analogous to Renshaw cell physiology.) In terms of the respiratory mechanism, one might imagine that pacemaker activity with its positive feedback leads to increasing inspiratory neuron discharge, and collaterals from these axons impinge on neurons of the Renshaw type. However, their threshold is high enough that considerable excitation is needed to fire them. When this level of excitation has been reached, they become active, liberating an inhibitory substance on neurons of the pacemaker, and thus suddenly the pacemaker function is quenched, the positive feedback chain is broken, and inspiration is suddenly halted. After a period of time the whole cycle is repeated. Such an action brings to mind the mechanism of an electronic relaxation oscillator, for instance

of the type used in generating time bases in cathode ray oscilloscopes.

By such a formulation, apneusis could result when excitatory influences other than through the pacemaker mechanism continue to discharge neurons of the inspiratory center despite inhibition of the type mentioned. Under conditions of respiratory stimulation, the depth of breathing could be increased if the excitability of the neurons were raised so that greater inhibition through the Renshaw-type system was required to stop them.

Admittedly, the suggestion offered is entirely speculative. However, at least one instance can be pointed out in which the central nervous system can generate an alternating rhythm with a frequency in the same range as that of respiration. In Sherrington's studies (77), it was found that simultaneous equal stimulation of sensory nerves to both legs gave rise to alternating movements of the legs. Thus a perfectly symmetrical stimulation was fractionated by mechanisms entirely within the spinal cord into an oscillatory response. It will be recalled that it has been demonstrated (47) that maintained stimulation of the vagus can transform apneusis into phasic breathing.

SUMMARY

A review of the literature has been presented of research on the mechanisms by which the respiratory rhythm is generated. The results may be summarized as follows:

1. The basic respiratory centers are located in the medulla, and an inspiratory center is to a certain extent separated from an expiratory center. In the pontine region there are neurons in the reticular substance which have important respiratory functions. In general these nerve cells either facilitate or inhibit the medullary respiratory centers. The other influences of greatest respiratory significance are the vagal reflexes from the lungs, the chemoreceptors, and the direct action of carbon dioxide on the medullary centers.

2. Based largely on the demonstration of a prolonged inspiratory cramp, called an apneusis, in the vagotomized animal aften midpontine brain section, the theory of tonic inspiratory drive has been developed. It proposes that the medullary inspiratory center is inherently tonically active and would, if unrestrained, cause an apneusis prolonged until death from asphyxia occurs. Normally, this apneusis is segmented into rhythmic breathing by the Hering Breuer vagal reflex or (after vagotomy) by a structure called the pneumotaxic center, which has been accurately localized in the extreme dorsolateral

anterior pontine tegmentum.

3. There have long been experimental findings which are not in agreement with the theory of tonic inspiratory drive, but until the researches of Hoff & Breckenridge, beginning in 1949, they have not attracted wide attention. Now there is such an array of evidence that it appears that the theory of tonic inspiratory drive must be abandoned, in favor of the theory of inherent medullary rhythmicity. This hypothesis contends that the medullary respiratory centers are capable of generating a rhythmic discharge, and all of the other central nervous system structures and peripheral mechanisms only modify this basic rhythm. The evidence for this theory appears to be very convincing, and therefore it is felt that at the present time this theory should be adopted as the working hypothesis.

4. In the past, periodic breathing, particularly Cheyne-Stokes respiration, has been considered to be dependent on varying tensions of carbon dioxide and oxygen during the cycles of activity. According to a new explanation by Hoff & Breckenridge, periodic breathing represents a degree of respiratory organization inherent within the central nervous system and is not dependent upon fluctuating gas tensions.

5. Recent researches in the field of electrophysiol-

ogy are pertinent to the subject of respiratory rhythmicity. Some of these findings suggest possible mechanisms for certain respiratory phenomena in terms of the physiology of cell excitation and repetitive responses.

REFERENCES

1. Hoff, H.E., & Breckenridge, C.G. 1955. Chapters 42 and 43 in Textbook of Physiology, 17th Edition. Edited by J.F. Fulton. W.B. Saunders Co. Philadelphia.

2. Pitts, R.F. 1946. Organization of the respiratory center. Physiol. Rev. 26:609-630.

3. Marckwald, M. 1887. Die Athembewegungen und deren Innervation beim Kaninchen. Ztschr. Biol. 23:149-283.

4. Marckwald, M. 1890. Die Bedeutung des Mittelhirns fur die Athmung. Ztschr. Biol. 26:259-289.

5. Pitts, R.F. 1950. Chapters 41 and 42 in Textbook of Physiology, 16th Edition. Edited by J.F. Fulton. W.B. Saunders Co. Philadelphia.

6. Pitts, R.F., Magoun, H.W., & Ranson, S.W. 1939. Localization of the medullary respiratory centers in the cat. Am. J. Physiol. 126:673-688.

7. Pitts, R.F., Magoun, H.W., & Ranson, S.W. 1939a. Interrelations of the respiratory centers in the cat. Am. J. Physiol. 126:689-707.

8. Pitts, R.F., Magoun, H.W., & Ranson, S.W. 1939b. The origin of respiratory rhythmicity. Am. J. Physiol. 127:654-670.

9. Pitts, R.F. 1940. The respiratory center and its descending pathways. J. Comp. Neurol. 72:605-625.

10. Pitts, R.F. 1941. The differentiation of the respiratory centers. Am. J. Physiol. 134:192-201.

11. Pitts, R.F. 1942. The function of the components of the respiratory complex. J. Neurophysiol. 5:403-413.

12. Hoff, H.E., & Breckenridge, C.G. 1949. Medullary origin of respiratory periodicity in the dog. Am. J. Physiol. 158:157-172.

13. Breckenridge, C.G., & Hoff, H.E. 1950. Pontine and medullary regulation of respiration in the cat. Am. J. Physiol. 160:385-394.

14. Hoff, H.E., & Breckenridge, C.G. 1952. Levels of integration of respiratory pattern. J. Neurophysiol. 15:47-56.

15. Breckenridge, C.G., & Hoff, H.E. 1952. Influence of morphine on respiratory patterns. J. Neurophysiol. 15:57-74.

16. Breckenridge, C.G., & Hoff, H.E. 1953. Pharmacological analysis of the nervous control of respiration by d-l Dromoran. Arch. internat. Pharmacodyn. 93:1-32.

17. Breckenridge, C.G., & Hoff, H.E. 1953a. Ischemic and anoxic dissolution of the supramedullary control of respiration. Am. J. Physiol. 175:449-457.

18. Breckenridge, C.G., Hoff, H.E., & Smith, H.T. 1950. Effect on respiration in midpontine animal of chemical inhibition of facilitatory system. Am. J. Physiol. 162:74-79.

19. Hoff, H.E., & Breckenridge, C.G. 1954. Intrinsic mechanisms in periodic breathing. Arch. Neurol. Psychiatr. 72:11-42.

20. Hoff, H.E., Breckenridhe, C.G., & Cunningham, J.E. 1950. Adrenaline apnea in medullary animal. Am. J. Physiol. 160:485-489.

21. Ranson, S.W., & Clark, S.L. 1947. The Anatomy of the Nervous System, 8th Edition. W.B. Saunders Co. Philadelphia. Chapters 10, 11, and 12.

22. Gesell, R., Bricker, J., & Magee, C. 1936. Structural and functional organization of the central mechanism controlling breathing. Am. J. Physiol. 117:423-452.

23. Lumsden, T. 1923. Observations on the respiratory centers in the cat. J. Physiol. 57:153-160.

24. Tang, P.C. 1953. Localization of the pneumotaxic center in the cat. Am. J. Physiol. 172:645-652.

25. Magoun, H.W. 1950. Caudal and cephalic influences of brain stem reticular formation. Physiol. Rev. 30: 459-474.

26. von Euler, C., & Soderberg, U. 1952. Medullary chemosensitive receptors. J. Physiol. 118:545-554.

27. Lumsden, T. 1923a. Observations on the respiratory centers. J. Physiol. 57:354-367.

28. Lumsden, T. 1923b. The regulation of respiration. J. Physiol. 58:81-91.

29. Lumsden, T. 1923c. The regulation of respiration. II. Normal type. J. Physiol. 58:111-126.

30. Henderson, V.E., & Sweet, T.A. 1929. On the respiratory center. Am. J. Physiol. 91:94-102.

31. Teregulow, A.G. 1929. Zur Frage der Existenz von Atmungszentren in des vorderen Abschnitten der Medulla oblongata. Arch. ges. Physiol. 221:486-498.

32. Hess, W.R. 1931. Die Regulierung der Atmung gleichzeitig ein Beitrag zur Physiologie des Vegitativen Nervensystems. Leipzig, G. Thieme.

33. Stella, G. 1938. On the mechanism of production, and the physiological significance of "apneusis". J. Physiol. 93:10-23.

34. Stella, G. 1938a. The dependence of the activity of the "apneustic centre" on the carbon dioxide of the arterial blood. J. Physiol. 93:263-275.

35. Stella, G. 1939. Apnea from transverse section of the pons in the dog. Arch. internat. Pharmacodyn. 62:135-145.

36. Stella, G. 1939a. The reflex response of the "apneustic centre" to stimulation of the chemoreceptors of the carotid sinus. J. Physiol. 95:365-372.

37. Beaton, L.E., & Magoun, H.W. 1941. Localization of the medullary respiratory center in the monkey. Am. J. Physiol. 134:177-185.

37a. Magoun, H.W., & Beaton, L.E. 1941. Respiratory responses from stimulation of the medulla in the cat. Am. J. Physiol. 134:186-191.

38. Nicholson, H.C., & Hong, J. 1942. Respiration effects of brain stem transections. Fed. Proc. 1:63.

39. Adrian, E.D. 1933. Afferent impulses in the vagus and their effect on respiration. J. Physiol. 79: 332-358.

40. Barcroft, J. 1934. Features in the Architecture of Physiological Function. Cambridge University Press. Cambridge, England.

41. Bernthal, T. 1944. Respiration. Ann. Rev. Physiol. 6:155-194.

42. Borison, H.L. 1948. Electrical stimulation of the neural mechanism regulating spasmodic respiratory acts in the cat. Am. J. Physiol. 154:55-62.

43. Chatfield, P.O., & Purpura, D.P. 1953. Factors affecting responses of the inspiratory center to electrical stimulation. Am. J. Physiol. 172:632-638.

44. Gesell, R. 1940. A neurophysiological interpretation of the respiratory act. Ergebn. Physiol. 43:477

45. Harris, T.D., & Borison, H.L. 1954. Effect of pentobarbital on electrical excitability of respiratory center in the cat. Am. J. Physiol. 176:77-82.

46. Kerr, D.I.B., & Dunlop, C.W. 1954. Vagal respiratory responses during chemically induced apnea. Am. J. Physiol. 177:496-500.

47. Kerr, D.I.B., Dunlop, C.W., & Best, Effie, & Mullner, Judith. 1954. Modification of apneusis by afferent vagal stimulation. Am. J. Physiol. 176:508-512.

48. Meier, R., & Bucher, K. 1941. Uber atmungsregulierende Systeme in der Pons. Arch. ges. Physiol. 245: 412-419.

49. Ngai, S.H., Frumin, M.J., & Wang, S.C. 1952. Organization of the central respiratory mechanism in cats. Fed. Proc. 11:112.

50. von Euler, C., & Soderberg, U. 1952a. Slow potentials in the respiratory centers. J. Physiol. 118:555-564.

51. Adrian, E.D., & Buytendyk, F.J.J. 1931. Potential changes in the isolated brain stem of the goldfish. J. Physiol. 71:121-135.

52. Leusen, I.R. 1954. Chemosensitivity of the respiratory center: influence of CO_2 in the cerebral ventricles on respiration. Am. J. Physiol. 176:39-44.

53. Leusen, I.R. 1954a. Chemosensitivity of the respiratory center: influence of changes in the H^+ and total buffer concentration in the cerebral ventricles on respiration. Am. J. Physiol. 176:45-51.

54. Monnier, A.M. 1952. The damping factor as a functional criterion in nerve physiology. Symp. Quant. Biol. 17:69-95.

55. Lorente de No, R. 1947. A Study in Nerve Physiology. Studies from the Rockefeller Institute for Medical Research, vol 131 & 132.

56. Brink, F. 1954. The role of calcium ions in neural processes. Pharmacol. Rev. 6:243-298.

57. Hodgkin, A.L., Huxley, A.F., & Katz, B. 1952. Measurement of current-voltage relations in the membrane of the giant axon of Loligo. J. Physiol. 116:424-449.

58. Hagiwara, S., & Watanabe, A. 1955. The effect of tetraethylammonium chloride on the muscle membrane examined with an intracellular electrode. J. Physiol. 129:513-527.

59. Jenerick, H.P., & Gerard, R.W. 1953. Membrane potential and threshold of single muscle fibers. J. Cell. Comp. Physiol. 42:79-102.

60. Weidmann, S. 1955. Effects of calcium ions and local anesthetics on electrical properties of Purkinje fibers. J. Physiol. 129:568-582.

61. Brock, L.G., Coombs, J.S., & Eccles, J.C. 1952. The recording of potentials from motoneurones with an intracellular electrode. J. Physiol. 117:431-461.

62. Hamilton, C.A. 1951. Electrical polarization effects related to fibrillation in skeletal muscle. Master of Science Thesis. University of Nebraska College of Medicine. Omaha.

63. Hodgkin, A.L. 1948. The local electrical changes associated with repetitive action in a non-medullated axon. J. Physiol. 107:165-181.

64. Fuortes, M.G.F. 1954. Direct current stimulation of motoneurones. J. Physiol. 126:494-506.

65. Hartline, H.K., Wagner, H.G., & MacNichol, E.F. Jr. 1952. The peripheral origin of nervous activity in the visual system. Symp. Quant. Biol. 17:125-141.

66. Katz, B. 1950. Depolarization of sensory terminals and the initiation of impulses in the muscle spindles. J. Physiol. 111:261-282.

67. Gray, J.A.B., & Sato, M. 1953. Properties of the receptor potential in Pacinian corpuscles. J. Physiol. 122:610-636.

68. del Castillo, J., & Katz, B. 1955. Effects of vagal and sympathetic nerve impulses on the membrane potential of the frog heart. J. Physiol. 129:48-49P.

69. Fatt, P. 1954. Biophysics of junctional transmission. Physiol. Rev. 34:674-710.

70. Li, C.L., & Jasper, H. 1953. Microelectrode studies of the electrical activity of the cerebral cortex in the cat. J. Physiol. 121:117-140.

71. Frank, K., & Fuortes, M.G.F. 1955. Potentials recorded from the spinal cord with microelectrodes. J. Physiol. 130:625-654.

72. Eccles, J.C., Fatt, P., & Koketsu, K. 1954. Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. J. Physiol. 126: 524-562.

73. Eccles, J.C., Eccles, Rosamond, & Fatt, P. 1956. Pharmacological investigations on a central synapse operated by acetycholine. J. Physiol. 131:154-169.

74. Ashby, W.R. 1950. The stability of a randomly assembled nerve-network. EEG & Clin. Neurophysiol. 2: 471-482.

75. Lloyd, D.P.C. 1941. A direct central inhibitory action of dromically conducted impulses. J. Neurophysiol. 4:184-190.

76. Eccles, J.C., Fatt, P., & Landgren, S. 1956. Central pathway for direct inhibitory action of impulses in largest afferent nerve fibers from muscle. J. Neurophysiol. 19:75-98.

77. Fulton, J.F. 1955. Textbook of Physiology. W.B. Saunders Co. Philadelphia. p. 116.