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Differential function of costal and crural diaphragm in the awake canine

by

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December 9, 1992

A thesis submitted to
The Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy.

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Differential function of costal and crural diaphragm.
ABSTRACT

These investigations examined the relative function of costal and crural diaphragm segments. This work produced the first direct measurements of length and electromyogram (EMG) of the diaphragm in an awake, intact animal. Examination of diaphragm function following laparotomy revealed a consistent pattern of postoperative segmental recovery, and showed the inadequacy of EMG alone as an indicator of diaphragm activity. Segmental contraction during airway occlusion was confirmed to be non-isometric and different per segment. The basic relation between segmental velocity of shortening and mean inspiratory flow, was confirmed for diaphragm but not for intercostal musculature. Anesthesia produced a distinctive alteration in the resting length of crural compared to costal segment, suggesting a difference in inherent segmental tonic activity. Costal and crural activity during hypoxic and hypercapnic stimulated breathing revealed different, stimulant-specific activities of the segments; hypoxia elicited prominent crural post inspiratory inspiratory activity (PIIA). During thermal panting, peak crural shortening was out of phase with costal shortening and inspiratory airflow. This unique segmental asynchrony may represent a natural analog to high frequency ventilation.

We conclude that costal and crural diaphragm segments can function as individual segment-muscles, exhibiting distinctive, differential activities under certain conditions of respiration.
RÉSUMÉ

Ce document consiste d'études portant sur la fonction des segments diaphragmatiques costaux et cruraux. On documente pour la première fois par mesures directes, la longueur ainsi que l'électromyographie de segments diaphragmatiques chez le chien non anesthésié.

L'examen de la fonction diaphragmatique suivant la laparatomie a démontré un profil de récupération segmentaire typique et a illustré l'insuffisance de l'électromyographie comme seul indicateur d'activité diaphragmatique. Nous avons confirmé une contraction segmentaire non isométrique et différente d'un segment à l'autre lors d'une occlusion de la trachée. Il existe une relation fondamentale entre la vitesse de contraction segmentaire et le débit inspiratoire moyen pour le diaphragme mais non pour les muscles intercostaux. L'anesthésie induit des changements caractéristiques dans la longueur des segments cruraux comparés aux segments costaux diaphragmatiques. Ceci suggère qu'il existe une différence primitive dans l'activité tonique segmentaire de ces deux régions. Le profil de l'activité costale et crurale diaphragmatique résultant d'une respiration forcée par un stimulus hypoxique est différent de celui induit par une stimulation hypercapnique. Par exemple, lors d'un stimulus hypoxique, on décèle une activité inspiratoire post-inspiratoire importante dans le segment crural. Lorsque que le chien pantèle, le raccourcissement maximal crural n'est pas en phase avec le raccourcissement costal et le débit gazeux inspiratoire. Cet asynchronisme unique de l'activité segmentaire représente en quelque sorte l'analogue naturel de la ventilation à haute fréquence.

Nous concluons que les segments diaphragmatiques costaux et cruraux peuvent fonctionner comme des muscles distincts lors de la respiration insuite par des stimuli différents.
ACKNOWLEDGMENTS

This work was possible only because of assistance and support from many individuals. To all who participated, I am indebted. I must mention at least a few by name, in approximately chronological order...

Dr. N. R. Anthonisen, now Dean Anthonisen, of the University of Manitoba, was the trusted mentor who inspired this endeavor. Thank you Nick.

Dr. Alejandro E. Grassino, my supervisor in all these projects, provided the opportunity for the chronic canine preparation. From you Alex, I learned a great deal about science, and your conceptual style of data analysis fundamentally altered my investigative approach. I continue to benefit from it each day.

In the development of the chronically implanted canine, experimentation, and computer analysis, I was greatly assisted by a cadre of research fellows, graduate students, colleagues, and friends - my fellow soldiers. Although the pioneering efforts of Dr. Stephen Newman and Dr. Jean-William Fitting with the canines did not quite succeed, those early efforts were critical to my eventual success. Over the first couple of years, Dr. Fitting was my faithful partner and co-worker; without his fine Swiss resolve, good humour, and friendship, the project could not have been undertaken. Merci, Jean-Will. Your co-authorship in most of these manuscripts is not thanks enough.

Later, I relied upon another special scientific partner, Dr. Tadashi Abe, from Kitasato, Sagamihara, Japan. We too shared the honor and travail of nursing and worrying over our furry patients, and the satisfaction of our results. The projects could not have been completed without your support Tadashi. Thank you my dear friend.

Several other colleagues provided faithful service to allow the projects to succeed. Dr. John Smith leant a helping hand in many experiments, and many strip charts bare testimony to his piloting of the recorder. Mr. Richard N. Young's enthusiasm and generous willingness to help out, were crucial in the final stages of the projects. To the wisest young man, and most illustrious athlete and friend that I know, Richard, thank you again. Throughout the project, assistance, friendship, and wise counsel were provided whenever needed by a terrific scientist. Thank you Dr. Alain Comtois.
Of course, before any experimentation was possible, skillful surgical implantation was a prerequisite. In this we were ably assisted by Dr. Raymond Arnoux in several canines. My deepest gratitude is reserved for Dr. A. Guerraty. To a great surgeon, with technique so slick that it was magic, and to one of the finest gentlemen I will meet, thank you.

In a project which succeeded only by religious adherence to every detail, even the custom tailoring of canine jackets by a retired artisan named Gordie at a Montreal Tent and Canvas shop, and the able animal husbandry at Notre Dame Hospital by Serge and Francois, deserve a note of gratitude. Finally, two other individuals made crucial contributions to the project in areas other than physiology. Mr. Bob Thomson provided technical assistance and outright artistry in the design and construction of our sonomicrometry implants. In addition, as a fine Christian of Baptist persuasion, he provided a reassurance that this project too, must turn out as it should. Inspired work Bob! Computerization and programming to utilize the fledgling IBM PC was fundamental to acquisition and analysis of all data with this project. While many co-workers assisted in the biology of this project, a single individual served as professor, programming wizard, and mentor for all computer aspects. Mr. Pierre Goyette, of the McGill Computing Centre, contributed immeasurably to this success. Yes, Pierre, finally I can properly XOR or logical AND in assembly code! Thank you for all your help, my great friend.

A special word of gratitude is reserved for the Head of Experimental Medicine at McGill, Dr. Harry Goldsmith, who is both distinguished scientist and venerable shepherd of graduate students. Without your imaginative and good humored prodding I wouldn't be able to write any of these acknowledgments. Thank you.

A few words of acknowledgment must be directed towards an inanimate object and its deceased creator. Unbelievable as it now seems, my original IBM PC, wheezing along on its 8088, gathered all of this data and analyzed most of it. To the late Don Estridge, you did a wonderful thing, wherever you are. And to the four footed ones, although the scars in my hand confirm that some are remembered more fondly than others, I express my appreciation by resolving always to treat a sick animal exactly like a patient.

I've noticed that theses are often dedicated to family, and now I understand why. To my parents who engendered the desire to do such things, and helped out steadfastly: thank you, Mom and Dad, this one's for you. And to my fellow alumnus and best friend, who also happens to be my wife, a final thank you. Hurray, we did it!
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PREFACE: THESIS STYLE AND CONTENT

Thesis Format

This thesis is composed of a series of related original manuscripts which are published, in press, or under review. This thesis format has been selected with the approval of the Department of Experimental Medicine and in accordance with the Guidelines Concerning Thesis Preparation of the Faculty of Graduate Studies, McGill University. As required by those regulations, the complete text of section #2 of the Guidelines, pertaining to Manuscripts and Authorship, is reproduced here.

McGill University
Faculty of Graduate Studies and Research
GUIDELINES CONCERNING THESIS PREPARATION
Revised 1991

B. THESIS FORMAT:

2/ Manuscripts and Authorship

The candidate has the option, subject to the approval of their Department, of including as part of the thesis the text, or duplicated published text, of an original paper or papers.

- Manuscript-style theses must still conform to all other requirements explained in the Guidelines Concerning Thesis Preparation.
- Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g., in appendices) to allow clear and precise judgement to be made of the importance and originality of the research reported.
- The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interest of cohesion.
It is acceptable for theses to include, as chapters, authentic copies of papers already published, provided these are duplicated clearly and bound as an integral part of the thesis. In such instances, connecting texts are mandatory and supplementary explanatory material is always necessary.

-Photographs or other materials which do not duplicate well must be included in their original form.

-While the inclusion of manuscripts co-authored by the candidate and others is acceptable, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent, and supervisors must attest to the accuracy of the claims at the Ph.D. Oral Defense. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make the responsibilities of authors perfectly clear.

THE COMPLETE TEXT OF THE ABOVE (#2) MUST BE CITED IN FULL IN THE INTRODUCTORY SECTIONS OF ANY THESES TO WHICH IT APPLIES.

Thesis Format: Published Manuscripts and Co-Authorship

The manuscripts contained in this thesis are grouped together in the central section entitled RESULTS. This section begins with a brief overview, and then each individual manuscript is prefaced by a brief statement entitled Thematic Overview which highlights insights derived from the individual manuscript in support of the general theme of this work. Titles of the manuscripts are:


7) Activity of costal and crural diaphragm during progressive hypoxia or hypercapnia, by Paul A. Easton, Tadashi Abe, John Smith, Jean-William Fitting, Albert Guerraty and Alejandro E. Grassino. J. Appl. Physiol. manuscript under review.


These manuscripts relied upon measurement of respiratory muscle length and electromyogram (EMG) by implantation of, and data acquisition and analysis from, canines. Although primary overall responsibility for all activities pertaining to the chronically implanted canines belonged to this candidate, obviously the execution of these studies required teamwork and the participation of other individuals. The worthy co-authors for each manuscript are listed above and on the introductory page for each manuscript. In accordance with the Thesis Preparation Guidelines, the relative contributions of the co-authors are noted here briefly. For all projects, overall supervision was provided by Dr. Alejandro Grassino. Chronic surgical implantation of sonomicrometry transducers and EMG wires into the canine diaphragm was carried out or supervised by Dr. Albert Guerraty, a Cardiovascular-Thoracic Surgeon from Royal Victoria Hospital, McGill University, with assistance by Dr. Raymond Arnoux, a Research Fellow in General Surgery at Notre Dame Hospital, University de Montreal. The other co-authors of this series were research fellows or graduate students, from the Meakins-Christie Laboratories at McGill University, also working under the supervision of Dr. A. Grassino. These individuals assisted with experimental data collection and animal care in the execution of the individual studies. They are accorded listing in the authorship record of each manuscript commensurate with their relative contribution to each project. In the early phases of the development and use of the chronically implanted canine, Dr. J.W. Fitting, a Research Fellow under the supervision of Dr. A. Grassino from Lausanne, Switzerland, worked most closely with me. For those specific projects in which Dr. Fitting was primarily responsible for analysis of the data we gathered together, and for preparation of the manuscript, he is the first author and this candidate is the second author. In later projects Dr. Tadashi Abe, a Research Fellow from Kitasato University, Japan, worked most closely with me, and
he is cited as second author in two manuscripts. Dr. John Smith, another Research Fellow, and Mr. Richard N. Young, a Graduate Student, assisted in data collection for the manuscripts for which they are cited as co-authors.

Thesis Format: No: Published Thesis Chapters

To avoid a manuscript catalogue style of thesis, the RESULTS:MANUSCRIPTS are preceded by a brief INTRODUCTION, then a chapter of GENERAL BACKGROUND summarizing aspects of respiratory control and respiratory muscle function which underpin this work. Then a chapter summarizes the RATIONALE for this series of investigations, including the general hypothesis and a series of specific questions which were derived from this hypothesis. And, just before the series of manuscripts, a chapter of GENERAL METHODS summarizes key aspects of methods and techniques for all the projects in more detail than can be found in individual manuscripts.

After the RESULTS:MANUSCRIPTS section, the thesis concludes with a brief summary of CONCLUSIONS, the CLAIMS OF ORIGINAL RESEARCH required by the university, and an Appendix entitled FUTURE STUDIES. This line of investigation has been carried on by this candidate, supported by Scholarships and Operating Grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research, and this final chapter summarizes some current investigations arising directly from work in this thesis.
INTRODUCTION

This thesis attests to a series of related studies focused upon the relative function of the costal and crural segments of the diaphragm. Successful execution of these investigations required the development of a chronically implanted canine preparation, which provided the first known direct measurements of diaphragm length in any awake intact animal.

Experimentation with the chronically implanted preparation was a fruitful endeavor. This thesis is organized as a series of related manuscripts based upon the costal and crural theme. Altogether, this course of study has generated original physiological knowledge sufficient for nine published abstracts and eight manuscripts in print or under review. These endeavors were undertaken with the scientific collaboration of other individuals in an active laboratory; the relative responsibility for co-authored papers is commensurate with the listing of authors per each manuscript.

Production from this course of study extends beyond the scientific manuscripts encompassed within the thesis. This line of investigation has been continued as the primary research focus of this candidate. In the interim since the studies within this treatise, additional original information regarding differential activities of the respiratory muscles, following on this work, has been gleaned. This later material is appended under the guise of future studies; it represents an additional fourteen published abstracts and a dozen manuscripts in review or preparation.
BACKGROUND

Overview
Mechanical coordination of the respiratory muscles
Control of respiratory muscles
  central control and respiratory motor output
Differential respiratory response to hypercapnia or hypoxia
  breathing pattern
  pulmonary mechanics and chemical stimulation
  hypoxia, hypercapnia, and muscles of the upper airway
  chemical stimulation and braking
  hypoxia, hypercapnia, and lower respiratory muscles
Costal and crural diaphragm
  diaphragm innervation and architecture
  mechanics of costal and crural diaphragm
  hypoxia and hypercapnia effects on costal and crural diaphragm
  sonomicrometry and intact animals
Examination of diaphragm segmental function
  limitations of EMG
  avoidance of anesthesia
  post operative respiratory muscle dysfunction
  intact and awake: confounding effects of sleep state
References
Overview

An investigation of costal and crural diaphragm function in awake intact mammals rests upon several reasonable presuppositions. This begins with the assumption that by deductive study of the complete respiratory system that the function of individual components can be elucidated. This assumption is explored in Mechanical coordination of the respiratory muscles. Recognition of distinctive actions of individual respiratory muscles and coordinated interactions, implies that specific muscles must be capable of unique individual control and activation, which is considered in Control of respiratory muscles. Stimulation of ventilation by increasing hypercapnia or hypoxia provides a common clinical expression of the system capability for differential activation of individual respiratory muscles. The relative respiratory muscle effects of these chemical stimulants is reviewed in Differential respiratory muscle activity: hypercapnia or hypoxia. Since the diaphragm is approached not as a single functional entity, but as two diaphragm segments, each capable of differential activity, some of the evidence supporting the distinctive character of these two segment-muscles is reviewed in Costal and crural diaphragm. Finally, optimal study of the function of the diaphragm segments should proceed according to several technologic and physiologic constraints; this background is reviewed in Examination of costal and crural function.

Mechanical Coordination of the Respiratory Muscles

Insight into the actions of the diaphragm segments, or any respiratory muscles, begins with, a now classic, deductive analysis of the mechanical output of the respiratory system. Beginning in 1916, Rohrer espoused the view that analysis of mechanical properties of the respiratory system could only succeed by relating the motions of the system with the forces producing the motions (143,144). Arising from the fundamental length-tension relationship of contracting muscle, the study of respiratory mechanics evolved through thoughtful analysis of the pressure-volume relationships of the system (145).

Beginning primarily from the work of Mead and Agostoni, almost two decades of investigation have concentrated on defining the properties of key components of the system and examining the manner in which they interact to generate the relationship in the pressure-volume diagrams (146). Konno and Mead demonstrated that with body posture relatively fixed, the chest wall enjoys two degrees of freedom: displacement of rib cage and displacement of the abdominal wall (138,147). By partitioning the pressure-volume relationships between rib cage
and abdomen-diaphragm, using transthoracic pressure versus rib cage volume displacement, and transabdominal pressure versus abdominal volume displacement, they were able to infer net mechanical activity of the muscles within the two portions of the respiratory system. This approach is summarized schematically in Figure 1.

![Figure 1](image)

**Figure 1.** Two dimensional graphic representation of chest wall configuration in the upright posture based on 2 degree of freedom model. Changes in chest wall configuration are described by anteroposterior (A-P) displacements (A) and by volumetric displacements (B) of rib cage and abdominal wall. Volume displacements are derived from measurements of anteroposterior displacements. Displacements are expressed as a percentage of the total displacement over the vital capacity (VC) relative to the active state at residual volume (RV). Solid lines and open points indicate configurations in relaxed states. Applied muscle forces or other externally imposed forces deform chest wall surfaces from the relaxed configurations. Surfaces enclosed by dashed line illustrate a range of possible configurations produced by submaximal contraction of rib cage and abdominal musculature. Constant volume isopleths (solid lines and closed points) define displacements at lung volumes indicated. Adapted from Konno and Mead. [from (136,138)].

Following from this introduction, the general concept of mechanical interaction between diaphragm and rib cage was advanced by Goldman and Mead (148), who attempted to further partition the rib cage pressure-volume relation between rib cage musculature (rib cage volume displacements versus transabdominal pressure) and diaphragm (rib cage volume displacements versus...
transdiaphragmatic pressure). Such analyses, including voluntary relaxation tracings and resting breathing, are illustrated in Figure 2.

Evidence of this type could be interpreted to indicate that during resting breathing, diaphragm contraction and its effect on abdominal pressure drove the rib cage along its relaxed pressure-volume curve, as well as the abdominal wall along its respective curve. To this discussion, Macklem et al. (139,137) added an alternate point of view, by their attempts to evaluate the contributions of the rib cage muscles...
relative to the diaphragm by noting the pressure deviations on a diagram of pleural versus abdominal pressure. This approach is shown in Figure 3.

Figure 3. Abdominal pressure-pleural pressure diagram. The heavy solid line represents the relationships between transpulmonary pressure and abdominal pressure during relaxation, where the units for transpulmonary pressure are on the ordinant but are positive in sign. The dashed lines are isopleths, giving the locus of points for constant PDI. The loop starting at FRC depicts the relationships obtained in a normal subject breathing quietly in the seated position. [from Macklem et al (137,139)]

This type of analysis presumes a fully relaxed abdominal musculature. In this figure, the solid line is intended to represent inspiration where only the diaphragm is thought to contract from FRC, while AC represents primarily intercostal/accessory inspiration where transdiaphragmatic pressure remains zero. This approach was supplanted by later analysis by Loring and Mead. Besides a reevaluation of some of the assumptions of the Macklem style pressure analysis, these workers attempted a "force-balance" analysis of the chest wall movements, invoked the area of apposition in the functional character of the diaphragm, and set about calculating the relative contribution of the diaphragm and other muscles, to inspiratory tidal volume (153,154). It is not the aim of this brief review to consider the subtleties or controversies of this elegant approach. We only need to note the extent to which movements in respiratory system components and resultant
pressures have been evaluated to infer changes in the pattern of recruitment and coordination of the muscles of the respiratory system.

Although the studies mentioned thus far concentrated on resting breathing in the sitting position, these same methods were employed profitably to deduce changes in differential action of the muscles of the respiratory system under other circumstances of respiration. For example, the effect of a transition to the supine position has been the subject of study (135,140,155). In the supine position, FRC decreases significantly, and tonic abdominal activity is decreased (140). In the supine posture, thoracoabdominal movements are distinct from their corresponding movements during normal resting breathing in the upright posture. The usual upright predominance of rib cage displacement with lesser abdomen displacement, is reversed in the supine position. This is illustrated in Figure 4.

![Figure 4. Rib cage and abdominal anteroposterior (AP) diameter changes during breathing at low (50-55 Torr; loops originating from open circles) and high (65-70 Torr; loops originating from closed circles) levels of CO\textsubscript{2}. Isovolume lines separated by 1 liter. [from Grassino et al (140)].](image)

Finally, this same analytic approach has been employed to deduce the changes in relative recruitment and activity of the respiratory muscles as ventilation
is increased under conditions of chemical stimulation (135,141,149,150,151,152). The degree by which movements of the rib cage and abdomen deviate from their relaxed pressure volume traces, permits some inference into the strategy of muscle recruitment that is operative during stimulated ventilation. This is shown in Figure 5. This figure illustrates clearly the departure from the relaxation characteristic, with phasic contraction, during expiration of the abdominal muscles, and during inspiration of the intercostal muscles.

The investigations reported in this thesis follow logically from the classic studies of respiratory mechanics just discussed. Modern studies of respiratory mechanics began with a model that enjoyed a single, and then two degrees of freedom, and exploited these limited observations even to insights about function of the major muscles by sophisticated modelling and study of a limited number of parameters. This work, moves to the direct measurement of the mechanical and electrical function of individual muscles. This approach pursues the specific neuromechanics of individual muscles, and offers a model with multiple "degrees of freedom".

Figure 5. Rib cage and abdominal musculature pressure volume diagrams during resting breathing and stimulated breathing. A: mild increases in ventilation. B: moderate to severe increases in ventilation. C: increases in ventilation associated with exercise of CO2 stimulation. , abdominal pressure. [from (135)].
Control of Respiratory Muscles.

The preceding discussion of respiratory muscle coordination derives from fundamental observations of the mechanical interactions of the components of the respiratory system. From that foundation of mechanical deductions, the actions of the major respiratory muscles were recognized. From that background, our emphasis shifts now to the unique character and function of individual respiratory muscles. Distinctive actions of individual respiratory muscles and coordinated interactions, requires that specific muscles be capable of differential control and activation. But what capability can we expect for differential activation of specific respiratory muscles? Does the respiratory control system offer such potential?

Central control and respiratory motor output.

The regular alternating sequence of inspiration and expiration probably derives from a bistable system of reciprocally active interconnected neurons, located primarily in the medulla with additional significant rhythmic capability from the pons (64,65). In close anatomic proximity, and thoroughly interconnected with the "central pattern generator" neurons, are the diffuse collections of medullo-pontine respiratory neurons, extensively interconnected, about which some generalizations can be made: influence from the dorsal respiratory group (DRG) projects predominantly in inspiration and more generally to the diaphragm, while the ventral respiratory group (VRG) neurons have a more mixed, pharyngeal, intercostal and expiratory character of their projection (66). Clear redundancy exists; several diverse cell groups can, under certain circumstances, influence diaphragm or intercostal muscles. Afferent chemical and reflexic influences contribute to the pool of neuronal information, but regardless of the sophisticated coordination of respiratory motor control in the bulbo-pontine area, the final common integration of respiratory motor control is at the level of the anterior horn cell in the spinal cord (67). Supraspinal inputs from the bulbo-pontine neurons, as well as inputs from segmental afferents, converge on the respiratory motor neurons with resultant firing and respiratory muscle contraction, but the complete process is not well worked out (68,69). From the minimal muscle participation of resting eupnea, which depends primarily on diaphragm and parasternal intercostals, increasing levels of stimulated ventilation recruit other respiratory muscles which are inactive at rest (70). However, we do not understand the complex sequence and processing of input information which results in a pattern of muscle recruitment. Nonetheless, it is apparent that the respiratory pattern can be quickly adjusted and that respiration can be regulated in order to optimize the efficiency of total
respiratory muscle output (71). We may gain some insights regarding the processing of respiratory motor output by analogy with the organization and control of limb skeletal muscle, including its anatomy, central localization of motor functions, and control of motor neurons.

The classic mapping of skeletal muscle across the cell bodies of the motor cortex began conceptually as early as 1870 and remains a fundamental tenet of skeletal muscle motor output and control. Localization of the cortex can be viewed as representing "movements", in that the motor area functions to coordinate the localized muscles in a myriad of movement combinations (72,73). Additionally, for more elaborate acts, larger numbers of related neurons over a larger area are required to control neural output. Peripherally at the level of the individual motor neuron and respective motor units, we can glean additional analogies regarding respiration since the motor neuron pools again must function to translate heterogeneous inflow signal to a simpler output that will precisely control tensions in a particular muscle or groups of muscles. Evolving from the original insight by Henneman (74), suggesting that motor neuron recruitment and function was related in orderly fashion to size, recent investigations provide insight into the potential for alternative patterns of recruitment, where motor neurons are recruited outside of the usual orderly pattern (75). Some evidence of a capability for specific, alternative, ordered recruitment even at the level of motor units within a muscle exists; experiments by Basmajian may suggest that for some muscles, human volunteers can control the activity of a single motor unit within a muscle (76).

We have no reason to believe that respiratory motor control is primitive and simplistic compared to the control of the skeletal limb muscle counterparts, so the aforementioned facts raise certain expectations. We expect that within the bulbo-pontine area, there may be discrete areas that project motor activity predominantly to a single respiratory muscles. We may also expect, for a complex act such as breathing, that there will be large groups of obviously related and interconnected neurons which participate, in some as yet undefined way, in the total motion and coordination of the respiratory act. We expect that, despite the complicated higher organization and convergence at the spinal motor neurons, differential and preferential recruitment of, not only individual respiratory muscles, but even sections of individual respiratory muscles under specific circumstances, will be possible.
Evidence accumulates to support these expectations. The overlap of the neuronal functions of the DRG and VRG group may provide a degree of differential recruitment of diaphragm versus inspiratory intercostal muscles (66.77). Additional supporting evidence is provided by investigations showing that for the diaphragm, recruitment of motor units generally obeys Henneman's size principle, as usually applied to skeletal muscles (78). However, many of the same complexities in the study of muscle function during hindlimb locomotion also constrain our efforts to examine respiratory muscle function and recruitment. For example, caution is necessary in attempts to correlate individual muscle recruitment and coordination with summed events such as limb flexion or extension, and the same caution must be extended to interpreting respiratory muscle actions in terms of inspiratory and expiratory events. Paradoxically, our inquiries into the motor control of individual respiratory muscles brings us back to mechanical characterization of the actions and interactions of key respiratory muscles. Indeed, improved understanding of respiratory muscle motor control is delayed "until more precise information is gathered on the mechanical events of specific respiratory muscles" (79).

Differential Respiratory Response to Hypercapnia or Hypoxia.

The preceding background regarding neural control of the respiratory muscles implies that there is a stereotyped pattern of activation of individual respiratory muscles which can be specifically altered by various stimulants to ventilation. The chemical stimulants hypoxia and hypercapnia are the stimulants of most medical interest. As ventilation is stimulated from resting levels by increasing hypercapnia or hypoxia, minute ventilation (VI) increases and additional respiratory muscles are recruited. The question is, exactly which respiratory muscles are recruited with this chemically stimulated hyperventilation? Or more precisely, are all respiratory muscles recruited in an identical fashion when ventilatory stimulation is predominantly by either hypoxia or hypercapnia?

Breathing pattern.

Compared to hypercapnia, there is a traditional and controversial claim that ventilatory stimulation by hypoxia is characterized by disproportionately greater increases in respiratory rate than tidal volume. Hypercapnia is believed to produce an alternate breathing pattern which is more tidal volume and less frequency
dependent (1,2,3). However, a number of investigators have failed to note such differences in respiratory pattern response to hypoxemia and hypercapnia (4,5).

Some investigators have viewed any alterations in breathing pattern as expressions of fundamental differences in respiratory control relating to hypoxemia or hypercapnia. Some evidence suggests that even at a fundamental level of integration with rhythm generation, increasing levels of CO$_2$ tend to cause a tonic expiratory activation, while hypoxia induces an "inspiratory shift" in activation of respiratory motor neurons (6). Using the paralyzed cat, Fitzgerald (7) examined the phrenic nerve activity and respiratory timing in response to hyperoxic hypercapnia or isocapnic hypoxia. For the same phrenic nerve activity, peak phrenic nerve activity (which the authors interpreted as representative of tidal volume), TI, and TE were all less with hypoxia than with hypercapnia. Other investigations in anesthetized cats suggested that a greater increase in the rate of integrated phrenic nerve activity was required during hypoxia than during hypercapnia to produce equivalent increases in measured tidal volume (2). And, in the study by Ledlie et al. (2) the changes in respiratory timing with hypoxia were significantly less after carotid sinus denervation. Altogether, this basic information seems to suggest that a fundamentally different pattern of neural output during hypercapnia and hypoxia underlies the traditionally described changes in breathing pattern.

**Pulmonary mechanics and chemical stimulation.**

The differing effect of chemical stimuli on pulmonary mechanics has been controversial. An initial investigation by Rebuck et al. (9) regarding acute hypoxia in humans, suggested after a few minutes of isocapnic hypoxia, that there were small increases in total pulmonary resistance without change in vital capacity, but an increase TLC measurable by plethysmography. However, in the follow up study by the same group with a similar hypoxic stimulation, tests of "airway function" (including helium effect on maximal expiratory curves at 50% VC, slope of phase III, and changes in TLC measured non-plethysmographically) were studied, and no changes in mechanics were found with hypoxia (8,9).

This pair of studies is representative of the relative contradictions regarding the effects on pulmonary mechanics of the chemical stimulants in adults. Compared to normocapnia or hypercapnia, it is not certain whether hypoxia changes airway resistance (10,11). At least during hypoxia of altitude, hypoxia as a chemical stimulant apparently induces a small but measurable increase in RV, small decrease in TLC, small drop in VC, and significant increase in FRC initially
with gradual return to sea level values; a decrease in lung elastic recoil, increase in most parameters of expiratory and inspiratory flow, and decrease in both airway and pulmonary flow resistance (12).

However, even if such measurements are definitive and there really is a quantifiable difference in ventilation and mechanics with hypoxia or hypercapnia, this only resolves one controversy. All such studies of potential differences in respiratory neural output, breathing pattern, or pulmonary mechanics with hypoxia or hypercapnia, raise a larger question. Based upon a presumed difference in central output, and underlying differences in breathing pattern and mechanics, what are the fundamental patterns of neuromuscular activation of hypoxia or hypercapnia? That is, what is the expression among the respiratory muscles of the pattern of neural output related to the chemical stimulus, and how is that output transformed as differential muscle activation during hypoxia or hypercapnia?

Hypoxia, hypercapnia, and muscles of the upper airway.

The effects of hypercapnia and hypoxia on the respiratory muscles of the upper airway have been studied more extensively than effects on muscles of the "lower" respiratory system including diaphragm, intercostals, and accessories. Generally, CO₂ inhalation causes increased EMG activity in various inspiratory laryngeal motor neurons and a decrease in expiratory laryngeal motor neuron activity, at least in anesthetized animals (13). In humans, there is a small increase in inspiratory widening of the glottis during both hypoxia and hypercapnia, associated with increased posterior cricoarytenoid (PCA) activity (14,15). By contrast, during expiration, the usual expiratory narrowing of the glottis is decreased substantially during hypercapnia but not during hypoxia. With hypoxia, the vocal cords actually move closer to midline than with the equivalent ventilatory stimulation by hypercapnia (14). After vagotomy in anesthetized cats, hypoxic inhibition of PCA activity during expiration has been shown to cause an increase in expiratory laryngeal resistance; an hypoxic effect which is obliterated following carotid sinus nerve section (16). Assuming that conscious humans are relatively insensitive to vagally mediated stretch receptor feedback (17), then in humans, as in the anesthetized animal, hypoxia presumably stimulates chemoreceptors, inhibits PCA activity, and increases laryngeal resistance during expiration. Consistent with this, is a recent observation that at comparable tidal volumes with hypoxia, there is a relatively greater decrease in phasic activity of the thyroarytenoid (TA) muscle, a vocal cord adductor, compared to hypercapnia (18).
Several investigators have tried to integrate the relative effects of chemoreceptor stimulation on the respiratory neurons controlling either muscles of the upper airway or of diaphragm and intercostals. With chemical stimulation, is there evidence of preferential distribution between upper and lower respiratory system muscles? At least in anesthetized animals, such a chemostimulation related distribution seems evident. There is evidence suggesting that the threshold of the genioglossus muscle response to hypoxic and hypercapnic stimuli is greater than that of diaphragm (19). In anesthetized canines, hypercapnia produces a greater increase in phrenic than in alae nasi EMG activity (20), and even with central brain hypoxia in anesthetized animals, there is a disproportionate augmentation of genioglossus EMG relative to diaphragm EMG during CO₂ rebreathing (21).

These findings are not as clear when the cumulative results of many studies of awake animals are reviewed (13). However, in at least one experiment in awake, intact, chronically implanted cats, Haxhiu, et al. (22) demonstrated that hypercapnia preferentially increased EMG of genioglossus compared to posterior cricoarytenoid (PCA) or diaphragm.

**Chemical stimulation and braking.**

As implied by several studies cited in preceding paragraphs, by affecting laryngeal activity and upper airway resistance, both hypoxia and hypercapnia can have a measurable effect upon "braking" and the post inspiratory inspiratory (PIA) activity of certain inspiratory muscles. The influence of normal upper airway resistance compared to tracheostomy on braking has been noted (23). It has been suggested that hypercapnia decreases the post inspiratory activity and inhibits the braking effects on inspiratory muscles (24). Some studies seem to suggest that hypoxia may have the opposite effect, to actually prolong post inspiratory activity (PIA) (25,26). In anesthetized cats, isocapnic hypoxia was found to increase PIA of diaphragm, whereas hypercapnia shortened the duration of PIA (29).

**Hypoxia, hypercapnia, and lower respiratory system muscles.**

The two chemical stimulants have also been shown to have differential effects on activity of other respiratory muscle below the upper airway. In anesthetized canines, hypercapnia was associated with an increase in phasic expiratory abdominal activity (27). Measurements of efferent activity from the cranial ilohypogastric nerve in decerebrate, paralyzed, vagotomized, and ventilated cats showed that hypercapnia enhanced abdominal expiratory activity while
isocapnic hypoxia caused inhibition of abdominal nerve discharge (28). One report has suggested that in awake humans, there is a greater peak diaphragmatic EMG activity for a given minute ventilation for hypoxia than for hypercapnia, and that hypercapnia causes a more consistent recruitment of abdominal expiratory muscles at lower minute ventilation than does hypoxia (30). It is worth mention that the preceding study measured diaphragm EMG using an esophageal electrode, and presumably this reflects mainly the crural diaphragm segment. In awake ponies, Brice et al measured EMG from implanted wires in crural diaphragm and transversus abdominis, and showed that hypoxia increased diaphragm, and decreased transversus abdominis EMG (31). In awake canines, implanted EMG wires in crural diaphragm and transversus abdominis recorded a sustained increase in crural and transversus EMG activity with hypercapnia, but with hypoxia, crural EMG activity was augmented and sustained while transversus activity diminished with time (32,33).

Costal and Crural Diaphragm.

Throughout this treatise, the diaphragm is presented not as a conventional, single functional entity, but as two diaphragm segments each capable of differential activity. Existing evidence of the distinctive character of these two diaphragm segment-muscles is reviewed briefly here.

Diaphragm innervation and architecture.

In the canine, the phrenic nerve has 3 divisions, ventral, lateral, and dorsal, which roughly innervate the anterior portion of the costal, lateral portion of the costal, and crural diaphragm, respectively (39,38). Occasionally, the lateral division of the phrenic also innervates the lateral aspect of the crural segment (39). If the innervation of the diaphragm is examined from cervical roots, then the approximate origin of the phrenic nerve for human, cat, and canine is C3-5, C4-6, and C5-7, respectively (39,40). In the canine, Landau et al. (41) stimulated individual left phrenic cervical roots and noted that when stimulated: C5 activated the sternal portion of the costal and medial portion of crural diaphragm, C6 activated the entire left diaphragm except the sternal costal region, and C7 activated the dorsal portion of the costal and lateral portion of the crural (41). More recently, De Troyer et al. stimulated the C5, C6, and C7 roots individually while recording EMG activity from approximately the posterior portion of the costal and midline of the crural diaphragm segments. They concluded that in the canine, the costal segment was
innervated predominantly by C5 and C6, while the crural segment by innervated by C6 and C7 (43).

Although most workers do not report cross innervation, Ogawa et al. reported that the medial portion of the right crural diaphragm was innervated by the dorsal division of the left phrenic nerve in 30% of the canines they studied (42). Recently, using both EMG and glycogen depletion methods, the organization of motor units supplied by the primary branches of the phrenic nerve were evaluated in the cat. These four branches consisted of three branches, SC1, SC2, SC3 to the costal, and a single branch to the crural hemidiaphragm. Stimulation of the crural branch only in the ipsilateral crus, whereas each of the remaining costal branches caused activity in overlapping areas of the Sternocostal diaphragm (42).

Regardless of the exact regional innervation, the costal and crural segments of the diaphragm differ in terms of fibre type composition and embryologic development. Embryologically, the costal segment rises from myoblasts originating in the lateral body walls, while the crural segment arises from the dorsal mesentery of the oesophagus (44). Although there is not complete agreement, a general consensus based upon current investigations is that the crural segment is comprised of a significantly larger proportion of slow-twitch, oxidative fibres and fewer fast-twitch, glycolic fiber types (45,46,38) than the costal.

Mechanics of costal and crural diaphragm.

Based upon the observed differences in innervation and fiber content of the diaphragm segments, it has been hypothesized that the segments have distinctive mechanical effects on the abdomen and chest wall, and have the potential for differential neural activation in various respiratory maneuvers. Recently, this hypothesis of two diaphragm functionality has gained wide acceptance. However, the original hypothesis of segmental functionality was proposed in 1904 by Keith (47), expanded and expounded again in 1920 (48,49), and then popularized again after 1980 (50,51).

In a series of experiments in the canine involving direct individual stimulation of costal and crural diaphragm and stimulation of cervical nerve roots, it was apparent that costal and crural segments of the diaphragm had quite different effects on the lower rib cage. The costal segment had a direct inspiratory action thus increasing the diameter of the lower cage, whereas the crural segment, with the
abdomen open, had an "expiratory" action on a lower rib cage, that is the crural pulled the lower rib cage inward (43). Data from this study (43) is illustrated in Figure 6.

Figure 6. Effect of increase in abdominal pressure on changes in lower rib cage dimensions during separate stimulation of costal and crural parts of the diaphragm.

Calibration of the abdomen and rib cage signal gains was done so that the isovolume lines at functional residual capacity had a slope of -1. Relaxation curve of the chest wall (dashed line) was obtained during mechanical ventilation while the animal was deeply anesthetized. [from 43].

Based upon these results, a mechanical model of costal and crural diaphragm function with other inspiratory muscles has been proposed (50) (Figure 7). This model suggests that costal and crural diaphragm are arranged mechanically partly in parallel and partly in series. The costal segment is presented as mechanically in parallel with the inspiratory intercostal and neck accessory muscles in acting on the rib cage. Conversely, the crural segment is proposed to be in series mechanically with inspiratory intercostal and neck accessory muscles in acting on the rib cage (50,51).
Elucidation of the mechanical arrangement of the costal and crural portions of the diaphragm was advanced by the use of ultrasonic piezoelectric transducers for direct measurement of segmental length. Decramer et al. (52), using acutely anesthetized canines, examined the respective changes in length of the segments with selective stimulation of crural or costal diaphragm. Clearly the two segments did not appear to be arranged in series since stimulation of an individual segment resulted in shortening of that same segment but resulted in lengthening, as measured directly by sonomicrometry, of the opposite segment (52).
Information regarding the relative characteristics of costal and crural segments continues to accumulate. In anesthetized cats, EMG activities of costal and crural segments were not found to be equivalent with changes to a head up posture. Crural segmental EMG was noted to be significantly greater than costal, both with change in posture and increasing CO$_2$ stimulation (53). Of course not all investigations have come to identical conclusions. In one notable investigation, utilizing decerebrate paralyzed cats, activities of the costal and crural branches of the phrenic nerve were recorded as well as costal and crural EMGs. Recording in this study, failed to confirm any difference between costal and crural segmental activity during either hypercapnia or hypoxia (54). The authors speculated that the differential responses of costal and crural diaphragmatic EMG activity seen in other anesthetized preparations or spontaneously breathing animals, might have arisen because of reflex modification of the respiratory pattern because of afferent information arising from chest wall or from the diaphragm (54).

Hypoxia and hypercapnia effects on costal and crural diaphragm.
Since costal and crural diaphragm segments are functionally distinct and apparently capable of separate, differential activity in some circumstances, then there is the potential for the chemical stimulants to induce differential segmental activation. Even in the first measurements of costal and crural diaphragm length by sonomicrometry in the acute anesthetized preparation, Newman et al. suggested that costal and crural shortening was not identical during normocapnia (34). Subsequently, Van Lunteren, et al. (35), suggested that ventilatory stimulation elicited by CO₂ rebreathing had a nonuniform effect upon the two diaphragm segments. Crural diaphragm was noted to have a greater relative increase in EMG activity with hypercapnia than was noted for costal diaphragm EMG with hypercapnia. Measurements of costal and crural segmental length and shortening by sonomicrometry, during hypoxia and hypercapnia in the anesthetized canine provided further information that the segments were not affected identically during chemical stimulation. Crural diaphragm shortened more than costal with both types of chemostimulation and the velocity of shortening of both costal and crural diaphragm segments was greater during hypoxia than hypercapnia. In addition, increasing degrees of hypoxic stimulation resulted in a reduction in resting length of both diaphragm segments, and a slight relative increase in recruitment and shortening of costal diaphragm (36).

Measurements in acutely anesthetized canines by other investigators have shown similar results. Anesthetized canines with bipolar EMG wires demonstrated a greater peak and rate of rise of crural EMG compared to costal, at all levels of hypoxia (37), as well as an earlier onset of electrical activity of the crural diaphragm over much of the range of hypoxia.

**Sonomicrometry and intact animals.**

Since 1984, the distinctive capabilities of the costal and crural diaphragm segments have been elucidated by two major types of study: the first investigative format has involved the application of sonomicrometry ultrasound for direct measurement of muscle length, and the second type of experimentation has utilized the awake, chronically implanted animal for the study of intact segmental function.

The initial direct measurements of costal and crural segmental length were performed first by Newman et al. (34) in acute, implanted, anesthetized canines, utilizing sonomicrometry as it had been developed commercially for cardiovascular investigation. In this seminal study, the investigators demonstrated that existing sonomicrometry techniques could be applied reliably to measurement of the
diaphragm, and that the measurements were stable and valid compared in vitro calibration of the instrument. Even in the initial study, costal and crural function was distinctive. Resting tidal shortening, velocity of shortening, and shortening during occlusion, all differed significantly between costal and crural segments (34). As part of the calibration process, the authors compared length measurements derived from ultrasound sonomicrometry transducers which were implanted within the diaphragm, to those which were attached to the muscle surface, and did not note any significant difference in recorded length. Similarly, in a small number of animals, the influence of regional variability of the diaphragm segments was examined and it was concluded that shortening of rostral costal compared to caudal costal regions of the diaphragm segment were not different.

Figure 9. Costal tidal diaphragmatic shortening in 3 dogs comparing implanted (sewn) with nonimplanted (glued) transducers. Each dog, mean of 10 breaths. Bars, ± SE. P = NS by paired t test. LFRC, change in length from resting length. [from 34]

Subsequent investigations employing the sonomicrometer examined the in vivo length-force relationship of costal and crural diaphragm segments (55). The crural segment was noted to develop a lesser pressure per length than the costal over the length range of 80% of optimal muscle length (Lo) to Lo. With the animal supine and anesthetized, at functional residual capacity (FRC) the resting length (titled LFRC) of the costal and crural segments were not found to be at Lo. At FRC, the costal segment was noted to be distended to approximately 105% Lo, while the
crural was shortened to 90% Lo (55). In a later study by Farkas and Rochester, sonomicrometry crystals were implanted in canines to relate in situ segmental lengths with measured force-frequency and length-tension properties evaluated from excised costal and crural diaphragm bundles. At functional residual capacity (FRC), costal diaphragm was thought to be at 95% and crural diaphragm at 84% of optimal length (Lo) (63).

Insight regarding the differential function of the diaphragm segments has been advanced by recent investigations utilizing intact, awake, chronically implanted mammals.

Interesting data has been generated from 6 canines chronically implanted with bipolar EMG wires in costal and crural diaphragm, transversus abdominis, and triangularis sterni (32,33) and studied in the standing position two weeks post implantation. Studies with these animals suggested that, costal PIIA was more extensive than crural, and that with hypercapnia or normocapnic hypoxia, PIIA of crural and especially costal tended to remain unchanged or decrease. An unexpected finding was the observation of a significant delay in the initiation of inspiratory EMG of both costal and crural segments compared to inspiratory airflow, by as much as 23% of TI during hypercapnic stimulated breathing, suggesting that mechanical inspiration and expiration began before there was any electrical activity of the respective muscles including diaphragm. The observation that PIIA was usually not different between isocapnic hypoxia and hypercapnia is novel, and not in complete agreement with results from some other investigations (25,26,29,37).

Recently, some unique information about costal and crural segmental function has been generated by chronic attachment of small radiopaque lead spheres to the surface of costal and crural segment in canines (56). After recovery from implantation, the position and shortening of the row of spheres extending along muscle bundles from near the origin to the insertion of the central tendon was calculated using biplane videofluoroscopy (56). From these radiographic determinations of shortening, it was deduced that there were significant regional variations within the same diaphragm segment and that the typical contraction along the length of a row of costal lead markers ranged from a low of 4-6% to shortening values as high as 18% of baseline, costal, pre-inspiratory resting length (56). Both the generous shortening of the marker tags along the rows and the large heterogeneity of shortening within regions of the same segment differed from results
of previous investigations. Recently, preliminary results from an acute, anesthetized canine preparation which measured shortening simultaneously within multiple regions of costal and crural diaphragm segment by sonomicrometry, also showed significant variations in regional shortening within the costal segment (62). These differences contrast with the initial findings of Newman et al. (34). A plausible explanation to synthesize these differing observations is that Newman’s original measurements in a small number of animals compared shortening at only 2 points on each diaphragm segment. It may have happened that the two locations which were chosen on the segment both showed equivalent, intermediate amounts of shortening, so that an existing gradient of shortening over the entire length of the segmental fibre from origin to insertion was not suspected.

An investigation of awake, intact, sheep, utilizing chronic sonomicrometry and EMG implants to characterize the diaphragm segments, has appeared during the course of this work. The ovine data are of special interest since the implantation protocol is similar to that which will be described here, except for the species and implantation via thoracotomy. In the reported ovine study, seven sheep were implanted in right costal and crural segments with bipolar EMG electrodes and piezoelectric, ultrasonic, sonomicrometry transducers. Electrical activity and shortening were measured sequentially for up to 6-7 weeks after implantation. These results suggested that, after thoracotomy and implantation at least four weeks were required before stabilization of shortening of costal and crural segments. In the interim, segmental EMG was not different from day to day even as measured segmental shortening gradually increased and eventually stabilized at approximately 28 days postop. Differences in both baseline length and segmental shortening were noted between costal and crural segments, both at rest and during CO₂ stimulation (57).

A few final insights into the relative function of costal and crural diaphragm can be gleaned from investigations of non-inspiratory segmental activities.

The act of coughing is associated with persistence of EMG activity beyond the cough into subsequent expiration for parasternal and crural segments, as well as for the costal diaphragm. But, the increase in crural segmental EMG with coughing was significantly greater than the proportional increase recorded for costal (60). The crural segment has been shown to have an effect different than the costal at the high pressure zone (HPZ) of the gastroesophageal junction (59).
And in another non-respiratory gastroesophageal activity, crural segmental activity diverges markedly from costal function: that is during the act of emesis. Monges et al. (58), showed that crural segmental EMG activity diminished at the moment of emetic expulsion to a greater extent that was noted for costal segmental EMG. Of particular note was the apparent intrasegmental difference. With the act of emesis, crural EMG abated in the medial, inner hiatal fibers of the segment, while a sustained increase in the EMG was still recorded from both the outer hiatal fibers of the crural segment and costal fibers of the diaphragmatic dome. These findings imply that the crural segment is capable of intrasegmental differential activity. The costal segment may also be capable of more specific, regional differential function, beyond whole segmental activity. In normal cats during spontaneous sleep, different activity has been measured from EMG electrodes implanted in two separate regions of the costal diaphragm during REM sleep. This suggests that differential activation of intrasegmental regions of the costal diaphragm is a normal event in REM sleep, with the potential to contribute to the disordered breathing characteristic of that sleep state (61).

Examination of Diaphragm Segmental Function.

Limitations of EMG.

In the current drive to characterize the function of the major individual respiratory muscles, an insight from some classic studies of skeletal limb muscle function is helpful. It is helpful to recognize the limitations of specific measurements of muscle function, and avoid unwarranted substitution of one measurement of muscle function for another. Every type of measurement imposes some selection and modification of information, and respiratory muscle EMG remains subject to such limitations.

Although adequate for many applications, typical, respiratory muscle, moving average EMG must be interpreted with circumspection. Early studies demonstrated a relationship between tension developed in contracting skeletal muscles and integrated EMG (97). And for the human quadriceps, integrated EMG and force output retained a linear relationship when studied under relatively limited conditions of shortening, tension, and velocity of shortening (98,99). Certainly as the intensity of muscular contraction increases, so too does the peak-to-peak amplitude of EMG mass discharge (100).
But, even if EMG provides a valuable indicator of muscle activity, correlation of EMG changes with most respiratory output variables is empiric (101). Moving average, or slope of moving average, phrenic ENG or diaphragm EMG during inspiration, correlates with increasing levels of CO\textsubscript{2} during rebreathing (102). Instantaneous moving average EMG correlates well with dynamically changing mouth pressure during an occluded inspiration (103). Both the characteristics of the respiratory EMG signal and the moving average conditioning of the signal demand careful consideration.

Initial efforts to quantify diaphragm EMG attempted to integrate the rectified EMG signal over the complete inspiratory cycle (104). Then the integrated EMG signal was examined over shorter intervals, evolving to the present Paynter filter treatment to approximate a continuous moving average (105). Elegant modelling studies by several authors suggest that each EMG represents a gross myoelectric signal arising from a complex temporal and spatial summation of many action potentials, from multiple motor units firing asynchronously, with modifications by various filtering properties within the system and added electrical noise (106,107). Thus, the typical recorded respiratory EMG reflects a splendid mix of information about both the total electrical signal over a region of the muscle, as well as significant amounts of information about individual motor unit action potentials (MUAPs). In steady state or slowly changing circumstances, an empiric moving average treatment of this complex signal probably works well up to moderate levels of contraction, because each additional MUAP adds proportionately to the rectified signal. Unfortunately, at higher levels of muscle activation, the resulting interference pattern of many superimposed action potentials becomes increasingly complex with a significant amount of cancellation (108,111). Generally, there is sufficient nonlinearity of MUAP behavior compared to the viscoelastic properties of muscle tissue, including force output, to prompt one writer to exclaim that "A linear relationship should be considered as accidental" (109). Practical illustrations of the limitation of typical moving average EMG are not uncommon. Kim et al (110,31) noted that for the same level of phrenic nerve stimulation, changes in length of strips of canine diaphragm affected the measured moving average EMG. A more subtle reminder of the limitation of the typical EMG is the recorded difference of the rate of increase of integrated EMG of parasternal intercostal compared to diaphragm during increasing hypercapnia (112). When additional problems of surface and esophageal electrodes are included, some authors are wary of the results of many traditional studies of human diaphragm function (113). And for the diaphragm with
its large surface area and defined segments and segmental regions, there is additional concern about the validity of sampling a handful of motor units with the expected mixture and diversity of fibre types.

Clearly a moving average EMG is most useful as an approximate indicator of myoelectric activity of the diaphragm. Satisfactory characterization of the function of individual respiratory muscles, especially the diaphragm segments, thus requires, in addition to EMG, some other reliable and independent indicator of mechanical output. Ideally, this can be provided as an independent, direct measurement of length, shortening, and velocity of shortening, utilizing the ultrasonic sonomicrometer technology described later in this treatise. This need to supplement EMG in the study of respiratory muscles is not new; to quote from W.O. Fenn in his review of pulmonary mechanics in the 1964 edition of the Handbook of Physiology, "There may be muscles in the body that never do shorten significantly when they are activated, and there is perhaps no more likely place to search for such an anomaly than in the respiratory apparatus with its peculiar mechanical arrangements. It is not enough to know from electromyographic records just when a muscle is active; it is very important to know also the tension developed and the amount and rate of shortening and lengthening" (142).

Avoidance of anesthesia.

In the field of pulmonary mechanics, general anesthesia is usually associated with a decrease in spontaneous ventilation, reduced CO₂ responsiveness, a minor decrease in arterial oxygenation, and an interesting fall in functional residual capacity (FRC) (114,115,116,117). Superficially, these changes can appear to be a modest response to a homogeneous influence of anesthesia on the respiratory system and its control. Investigations of respiratory muscles and their interactions in acutely anesthetized animal preparations are accorded wide citation in our knowledge of differential function of individual respiratory muscles. This carries the potential for serious misinterpretation.

The aforementioned "modest" changes in mechanics may be reexamined in the context of respiratory muscle dysfunction. The generally dose dependent depressant effects on CO₂ responsiveness and hypoxic response have been known for almost 50 years (118,119,117). This attenuation may seem homogenous. But, the other effects belie a more significant confounding effect of anesthesia on respiratory muscle function. As early as 1847, John Snow's classic observations of
altered patterns of chest wall motion with different depths of anesthesia indicated that the effect of anesthesia on respiratory muscles was nonuniform (120). The well known decrease in FRC with anesthesia is associated with a relative cranial shift in the position of the diaphragm and a change in the overall shape of the thorax (114). This has profound implications for differential function of the respiratory muscles. Diaphragm tone probably decreases and in supine humans there is a relatively dose dependent decrease in the relative contribution to tidal volume of the muscles of the rib cage versus abdomen-diaphragm (121). Coincident with this is a disappearance of the normal phasic activity of the parasternal intercostals (121,122). The relative decrease in the contribution of the intercostals compared to the diaphragm may reflect different sensitivities of phrenic and intercostal motor neuron pools, or even a nonuniform, direct effect, on brain stem respiratory control (123). And, in contrast to the effects on the intercostals, anesthetics seem to increase the phasic electrical activity of the lateral abdominal muscles active in respiration (124). Thus, the important confounding effect of anesthesia is not its obvious ventilatory depression, but in the insidious alterations in pattern and timing of electromechanical output. Anesthesia has the potential to affect the outcome of interest in most studies of individual respiratory muscle function.

If the results of these investigations are to represent honestly the differential function of the costal and crural segment-muscles, and are to be widely applicable in the intact mammal, avoidance of anesthesia is a necessity.

Postoperative respiratory muscle dysfunction.

Clinically, postoperative diaphragm dysfunction is associated with both thoracic and abdominal interventions. Recently, the most publicized post surgical diaphragm dysfunction has occurred following coronary artery bypass surgery (125,126,127). Post coronary bypass atelectasis is ubiquitous, but the etiology is multifactorial (125). However, a major atelectasis contributor is undoubtedly the topical cold cardioplegia which is used during open heart surgery. This "phrenic frostbite" has been recognized clinically since 1973 (126), and corroborated in many clinical reports (127,128).

There is convincing clinical and experimental evidence, albeit indirect, that diaphragm function is impaired after upper abdominal surgery (129,130,131,132). Ford et al. (130) showed that after upper abdominal surgery, patients exhibited decreases in tidal volume, tidal swings in transdiaphragmatic pressure, and changes in the ratios of gastric to esophageal pressures and of abdominal to rib cage.
diameters. These postoperative indicators of decreased diaphragm activity all reverted to near-normal values within 24-48 h after surgery. In a second study by the same group (131), the abnormalities in pressure and magnetometer evidence of a shift from abdominal to rib cage breathing were confirmed by controlled, pre- and postoperative measurements in anesthetized canines undergoing cholecystectomy. These findings have been confirmed by other investigators (129,132). Other evidence suggests that the observed diaphragm dysfunction is related to the upper abdominal location of the procedure, and unrelated to anesthesia (131), postoperative analgesic (132), or postoperative pain (133). In a recent human investigation, percutaneous phrenic stimulation confirmed that intrinsic diaphragm contractility as reflected in maximum transdiaphragmatic pressure generation, was not different before and after upper abdominal surgery (129). Cumulatively, this evidence supports an hypothesis (134) suggested as long ago as 1910, that postoperative complications including atelectasis are related to decreased excursion and reflexic dysfunction of the diaphragm.

The investigations which are proposed herein envisage upper abdominal surgery, without cholecystectomy or significant visceral organ trauma, but including laparotomy with manipulation of upper abdominal contents and sensory stimulation of the peri-diaphragm region and diaphragm abdominal surface. From existing knowledge of postoperative diaphragm function after upper abdominal surgery, we must anticipate some diaphragm dysfunction after any upper abdominal implantation, and expect dysfunction to persist for some time after surgery.

Intact and awake: confounding effects of sleep stage.

State-dependent alterations in CNS activity profoundly alter the expression of respiratory motor output. Presumably this occurs through the intimate anatomic relationship between ponto-medullary pattern generating and respiratory motor nuclei, and the brainstem reticular formation, although the neuro-anatomical interconnections are not known. In nonREM sleep, probably a general reticular deactivation is associated with a concurrent decrease in activity of many bulbopontine respiratory neurons (80). This general de-excitation produces a wakefulness-like, "automatic", breathing; a relatively pure reflex control respiration. By contrast, REM sleep presents a reticular activation and a complex CNS alteration of control of breathing where some respiratory neurons actually increase activity, and where profound phasic irregularities of respiratory pattern can be unrelated to gas exchange and uniquely derived from the CNS status of the sleep state (81).
our quest to discern the differential activities of the diaphragm segments in the intact, non-anesthetized mammal, we must recognize that additional, potentially confounding, changes in breathing are evident in the CNS state changes associated with nonREM and REM sleep.

Compared to awake ventilation, global ventilation seems to decrease progressively from stage 1-4 in nonREM, by an average of about 15% of awake baseline (82,83). Globally, REM sleep is less regular, and despite variability and discordant reports from different investigations, average tidal volume and respiratory rate through REM sleep probably differs very little from values in nonREM sleep (83). Despite this superficial similarity in average total ventilation between REM and nonREM, considerable evidence suggests fundamental alterations in the recruitment of respiratory muscles in the generation of tidal volume in REM and nonREM sleep compared to the awake state. At the most superficial level, relative thoracoabdominal movements vary with sleep stage. In nonREM sleep, a synchronous, relative increase in rib cage and a relative decrease in abdominal contribution to tidal volume is usually reported (84). In REM sleep, rib cage movement is frequently paradoxical (85), and rib cage contribution to tidal volume is generally found to be decreased. But besides global observations of relative rib cage and abdominal motion, what of phasic activity and tonic tone of individual respiratory muscles?

Corresponding to the observed changes in rib cage contribution to tidal volume, phasic external intercostal EMG activity has been found to increase during nonREM sleep(30), and both tonic and phasic intercostal EMG activity is thought to be globally reduced during REM sleep (86). In nonREM sleep there are sufficient changes in interactions of the respiratory muscles to alter functional residual capacity (87). Generally, in REM sleep abdominal muscle activation also decreases.

Classically, it has been claimed that diaphragm activity during REM sleep is preserved, perhaps by being spared the inhibition which affects other respiratory muscles. It has been suggested that the rib cage and abdominal muscles are rich in proprioceptive organs including muscle spindles and tendons, compared to the diaphragm which is relatively spindle-poor and is apparently spared the supraspinal inhibition during REM sleep (88). But this notion that REM can be characterized as a global inhibition which spares the diaphragm is undoubtedly a gross over-simplification. Within the diaphragm, changes associated with REM sleep are not homogeneous. There is preliminary evidence in lambs of a costal-
crural segmental difference in the degree of post-inspiratory and inspiratory activity (PIIA); during REM there was the addition of PIIA in the crural diaphragm to accompany the PIIA that was already seen in the costal diaphragm during nonREM sleep (86,89). Careful scrutiny of diaphragm EMG suggests recurrent "inhibitions" or other changes in frequency content which are particularly related to phasic REM (90). Even within regions of the costal diaphragm segment, it has been suggested that during REM sleep there is differential EMG activity (91).

Finally, an essential component of the respiratory muscle changes of sleep, is the influence upon activity of individual "lower" respiratory muscles, of increasing upper airway resistance to inspiratory airflow (92). EMG recordings of genioglossal muscle activity and laryngeal muscle activity during sleep suggest that both tonic and phasic inspiratory activity decreases from awake to nonREM sleep, and further decreases with REM sleep (93,94). Since aspects of muscle function in sleep revolve about the state changes in resistance in the upper airway, then diaphragm function in sleep could be considered within the context of diaphragm response to respiratory loading (95,96).

Based upon this brief review of respiratory muscle activities during the sleep process, it is clear that state changes of sleep could severely confound any distinctive actions of the diaphragm segments in the intact awake state. But this confounding effect of sleep is not a problem. Our studies of the diaphragm segments in the intact canine must proceed under conditions of strict wakefulness. And, the effects of sleep state change on the costal and crural segments are available for future study.
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Costal and crural diaphragm: Background


Costal and crural diaphragm: Background


Costal and crural diaphragm: Background


RATIONALE

General hypothesis
Conditions of measurement
Specific questions: individual projects/manuscripts
General hypothesis.

Costal and crural diaphragm segments are respiratory muscles capable of distinctive actions and differential function. For all respiratory muscles, including costal and crural diaphragm, we expect that there is a consistent, stereotyped pattern of activation and coordination of individual muscles, which can be specifically altered by the chemical stimulants hypoxia and hypercapnia, adjusted by normal respiratory system mechanical loads such as posture change, and subject to involuntary central revision during reflexic events such as panting.

Conditions of measurement.

Optimal examination of individual respiratory muscle function in the canine or other mammal requires simultaneous study of electrical activity plus another direct measurement of muscle mechanical action, such as length. The animal under study must be intact and normal, that is, free of any confounding effect of any instrument of measurement or anesthetic, and in an awake state.

During the series of original investigations presented here, these conditions of measurement were achieved within a chronically implanted canine preparation which provided direct, computerized, simultaneous measurement of length and EMG of the costal and crural diaphragm segments.

Specific questions: individual projects.

Based upon the stated hypothesis, and within these conditions of measurement, a series of inter-related questions were posed and addressed in a series of investigations. The questions for study, and the title of the corresponding investigations are summarized here.

By direct measurement in the intact canine, what is the relative change in function after abdominal surgery of the diaphragm segments? What is the time course and degree of synchrony of the recovery of the two segments? Is direct length measurement of the diaphragm possible in a chronic preparation, and is this equivalent to EMG? These questions are addressed in Recovery of diaphragm function after laparotomy and chronic sonomicrometer implantation, the first manuscript.

By direct measurement of the intact diaphragm, is contraction against an occlusion truly isometric? What is the relative performance of the individual
segments during contraction against occlusion? Can we evaluate segmental performance over specified portions of the respiratory cycle, rather than average segmental performance over an entire tidal breath? These questions are addressed in the second manuscript entitled Costal and crural diaphragm in early inspiration: free breathing and occlusion.

Exploiting the simultaneous EMG and length data, we evaluated a basic mechanical relation of the diaphragm with measured inspiratory airflow. We also evaluated the response of the segments to common mechanical loads imposed by posture change and anesthetic. What is the relationship between segmental shortening and velocity of shortening, and mean inspiratory airflow? What are the changes in length and relative activity of the segments during general anesthesia? What change in activity of the segments is induced by posture change in the awake intact canine, especially when compared to the unconscious state? These mechanically related questions are addressed in manuscripts three, four and five, entitled Diaphragm length adjustments with body position changes in the awake dog, Effect of anesthesia on canine diaphragm length, and Velocity of shortening of inspiratory muscles and inspiratory flow.

What is the differential response of costal and crural diaphragm segments when ventilation is stimulated by hypercapnia? Within the respiratory cycle, what are the relative segmental actions in early inspiration? Does the differential action of the diaphragm segments extend to end inspiration, or post inspiration activity (PIA), and respiratory system braking? These queries are addressed in Costal and crural diaphragm function during CO₂ rebreathing in awake canines.

From the traditional expectation that hypoxia is characterized by a relatively greater change in respiratory rate with increasing ventilation, is there evidence of differing patterns of respiratory muscle recruitment, between hypoxia and hypercapnia? Does any such chemical stimulant-specific muscle activation extend to costal and crural segments? Are the costal and crural segments activated differentially by these chemical stimulants in the awake intact mammal? We examine these questions in the seventh script Activity of costal and crural diaphragm during progressive hypoxia or hypercapnia.

What is the role of the diaphragm segments in a typical automatic reflexic event such as thermal panting? Is there evidence of differential activity of the
segments in the high frequency ventilation-style breathing movements that characterize this respiratory assisted temperature control? These questions are addressed in the final manuscript Costal and crural diaphragm function during panting in awake canines.
GENERAL METHODS

Overview
Length measurement by sonomicrometry
EMG techniques
  limitations of the recording electrode
  additional EMG considerations with this preparation
Surgical implantation
  a practical addendum to sonomicrometry implantation and recovery
Data acquisition and analysis by microcomputer
References
Overview

These investigations relied upon development and function of a chronically implanted canine preparation for computerized, simultaneous direct measurement of length and EMG of the diaphragm segments. This preparation encompassed several essential measurement technologies and analytic techniques. These are fundamental to all the projects in this work and are reviewed briefly here.

Application of a technique commonly used in cardiac physiology, direct measurement of dynamic length change by transmission of ultrasound, is central to these studies, so we include a summary of length measurement by sonomicrometry. Since respiratory muscle EMG was measured simultaneously for continuous correlation with changing length, the characteristics and assumptions of the EMG deserved careful scrutiny; some aspects of the EMG techniques are reviewed. Beyond these technologies of measurement, the application of this theory required skillful attachment of the transducers to the animals and a recovery to full normal function of the diaphragm. Some of the critical, practical, aspects of this surgical implantation are reviewed. Even when the implantation was a success, the implanted animal only represented the live half of the preparation. The other half was the comprehensive computerization of all aspects of collection and analysis of data from the animal. This is reviewed in Data acquisition and analysis by microcomputer.

Length measurement by sonomicrometry.

The use of ultrasound to measure the distance between pairs of small transducers implanted in muscle was described first in 1956 by Rushmer and Franklin (12), and improved subsequently by Franklin, in particular (13). Subsequent use in cardiac physiology resulted in development of various transducer designs (21), including the biconvex spherical shape which was adopted in the first studies of respiratory muscles with sonomicrometry (14). Other cardiac investigations also indicated that chronic implantation and measurement was feasible in cats and canines (15), at least in cardiac muscle. Recently, chronic implantation and measurement by sonomicrometry has been extended successfully to limb muscles (16).

Until sonomicrometry measurements were introduced for the exact determination of respiratory muscle length, previous in vivo direct measurements of diaphragm length were possible only with isolated muscle strips (17).
As used in these studies, each transducer in a pair consisted of a central piezoelectric ceramic plate surrounded on both surfaces by a biconvex epoxy lens. A typical transducer pair is shown in Figure 1.

Figure 1. Spherical sonomicrometry transducers. These are representative of the basic transducers used for measurement of diaphragm length.
Briefly, when electrically excited, the emitter piezoelectric transducer resonates, radiating ultrasound waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a measurable voltage. A quartz crystal clock oscillator measures the transit time of the waves, and because the conduction velocity in muscle is known, the sonomicrometer provides the intertransducer distance. Typically the emitter crystal is excited by voltage pulses occurring at a rate of 1537 Hz. Generally, a 1.58 MHz quartz crystal oscillator measures the transit time, which is convenient since the conduction velocity in muscle of the ultrasound is assumed to be 1.58 mm/microsecond, although this latter specification can be altered to adjust the resonant frequency of the system. For these studies, dynamic measurement of the changing distance between the sonomicrometry transducers of each pair was provided by the sonomicrometer (model 120, Triton Technology, San Diego, CA) (18).

![Diagram of sonomicrometry setup](image)

**Figure 2.** Measurement of length by sonomicrometry ultrasound. A schematic; see text for details.

Essential questions of accuracy of measured length within the range required for diaphragm movement have been answered adequately by careful in vitro
calculation of the sonomicrometer in the diaphragm (14) and other organ tissues (19). Theoretically, an error is introduced by contraction of a muscle being measured as tissue density slightly increases conduction velocity (14,16). This has been estimated to result in a underestimation of length change by about 1%. Indeed, two investigators have reported an increase in longitudinal velocity of transmission of sound of about 1.5% in isometrically contracted frog muscle, although isometric cat muscle has not been noted to alter velocity (20,16).

An innovation in sonomicrometry transducer design was used to advantage in these studies. The standard biconvex spherical transducer head was specifically modified with the addition of a projection in epoxy over one lens, giving the revised transducer a "tail", with a tear drop shaped silhouette. In preliminary studies, this modification was noted to improve directional stability in the diaphragm after implantation. Throughout these investigations, the major disturbance of measurement was in triggering. If inter-transducer alignment gradually deteriorated causing signal strength to decline, then there was a tendency for the sonomicrometry to falsely trigger off a part of the signal other than the first wavefront (16). Typically this potential error presented as slippage or loss of triggering stability and was easily detected by monitoring oscilloscopic or computer images of the continuous length signal.

**EMG techniques.**

The validity of the electromyographic (EMG) signal as a representation of the neural activity per each respiratory muscle depends on the adequacy of the EMG measurement technique and our own fidelity in interpretation of the EMG signal. For these experiments, we utilized the "traditional" moving average EMG measurement of a respiratory muscle. That is the EMG signals from the chronically implanted bipolar wire electrodes were amplified (MkIII, TECA, White Plains, NY) and band-pass filtered (16 Hz-1.6 kHZ). The output signals were then rectified and processed by passage through resistance-capacitance leaky "integrators" with a time constant of 100 ms, to provide moving averages of the electromyograms of costal and crural diaphragm.

Often, the bipolar wires were "recycled" sonomicrometry transducers, minus the "crystals". That is, after removal of the sonomicrometry lens, the 36 gauge Teflon-coated monel lead-in wire from Kooner was stripped for implantation. Initially, the wires were attached by careful suture onto the face of the muscle. However, shortly after the projects commenced, EMG attachment was modified after
a discussion with Dr. Sandra J. England. A short portion of the wire was bared (approx. 5-8 mm), at a location back from the wire tip. Then the wire was passed through the diaphragm with a surgical needle, and the extruding end which was still insulated was knotted back onto the EMG wire, leaving the only bared section imbedded within the diaphragm. A similar technique has been employed recently by other investigators, also apparently with practical suggestions by Dr. England (see reference 1, note acknowledgements).

Signal quality of the raw EMG was evaluated by careful initial observation on an oscilloscope, plus confirmatory auditory screening (10). After this initial review, the raw EMG signal quality was reviewed frequently during each experiment. And, the quality of the moving average signal was monitored continuously throughout each experiment using the strip chart record.

**Limitations of the recording electrode.**

As modelled by Lindstrom and Magnusson (5) essential parameters which effect the electromyogram include electrode size, geometry, and distance separating electrodes from active fibres, as well as position of the electrodes relative to innervation zones, muscle size, MUAP firing duration, the number of fibres per unit, action potential conduction velocity, and the total amount of cross-talk. Space does not permit exhaustive discussion, but several of these influences are especially applicable and have practical implications for the EMG recordings in these investigations.

Because of the presumed interdigitation of muscle fibres from several MUAPs with influence within the approximately 2mm diameter "sweet spot" surrounding each electrode (6,7), then practically we would wish to orient the bipolar electrode parallel to the voltage gradient to be measured, i.e., along the axis of the muscle fibre bundles, with an electrode separation about equal to the estimated wavelength of the action potential (up to about 10mm for most mammalian muscles) (8). This corresponds well with a practical placement and separation of sonomicrometry crystals along the axis of the muscle bundle separated by 10-12mm. Fortuitously, this was within the range of separation for the sonomicrometry transducers which provided optimal signal output. So, both measurement transducers could be implanted at approximately equivalent separation distances in the segments. In general, the actual recording contact of each electrode should be relatively large for bipolar electrodes; noise and single fibre sampling bias is probably minimized with each electrode contact approaching half the distance between the bipolar pair. In our instance, this would recommend approximately 5mm of bare wire intramuscle
contact for each of the bipolar electrode pairs (8). Besides the obvious averaging effect of the electrode contact area, we know that interelectrode distance will impose an element of "tissue filtering" (3,4,5). Assuming that the EMG signal emanates from a single muscle fibre modelled as an infinitely long cylinder, the interelectrode muscle behaves as a type of comb filter with attenuation of particular frequencies dependent on the conduction velocity and the interelectrode distance (9). However, the practical influence of such effects is of no practical consequence when the EMG signal is to be moving averaged after recording.

Although proximity to motor end plates and individual fibres will affect both MUAP shape and the whole EMG spectrum, for these respiratory muscles this should be a random effect for the group of fibres affecting the electrode. Unlike some other mammals, the canine does not offer a regular row of end plate bands on the diaphragm; instead the endplates are interdigitated over a wide area of the diaphragm. Therefore proximity to end plates is a theoretic limitation of any EMG recording from the canine diaphragm, which cannot be controlled or much defined, and should not have a significant practical effect given the moving average treatment of our EMG (2,3).

Additional EMG considerations with this preparation.

Of particular importance for these studies was the matter of electrical noise and the EMG. Bipolar recordings such as these, in the vicinity of sonomicrometry transducers, are subject to high frequency interference emanating from the "pinger" transducer at a base frequency and harmonics which are dependent upon the actual oscillatory frequency of the transducer. Exclusion of this artifactual frequency component from the EMG spectrum was an initial concern. However, even after preliminary studies, it was apparent that this interference was easily manageable in practice. With a few exceptions, the ultrasonic interference was minimal. It was evaluated empirically by visual examination of the signal, and comparison of the moving average signal with and without the presence of the sonomicrometer.

As with esophageal EMG recordings, ECG contamination posed a significant concern. At the outset it was feared that because of the proximity of the diaphragm measurement source that ECG artifact would be extreme. In fact this did not prove to be problem. With very few exceptions, we were able to use the available EMG signal for purposes of moving average EMG analysis. ECG contamination was modest on visual inspection of the signal, and was considered to present only a minimal source of random noise to our calculations which were computer averaged.
over many breaths. We did not attempt to gate the contaminating QRS interference in this series of studies (11).

A unique theoretic concern with these animals was that the exact distance between sonomicrometer crystals and between bipolar EMG electrodes was known but not identical, and the exact bundle of muscle fibres shortening about the crystals was not necessarily being sampled by the bipolar wires. Although we judged this to be acceptable for moving average EMG measurements, clearly this is not an ideal technique for resolving the relationship between muscle length and EMG signal content, especially frequency content.

Figure 3. Chronically implanted canine preparation for simultaneous measurement of length and EMG of costal and crural diaphragm segments.
Surgical implantation.

General implantation technique.

Mongrel dogs which had previously been tracheostomized, were chronically implanted for postoperative study. Instrumentation and implantation was performed under general anesthesia induced with thiopental sodium (15 mg/kg), and maintained with halothane. The left hemidiaphragm was exposed through a midline abdominal incision. A pair of ultrasonic transducers, with fine (36 gauge) Teflon coated lead-in wires, were implanted between muscle fibers on a flat portion of each of the costal and crural segments of the left hemidiaphragm (for complete discussion of relative location see Background). The transducers were implanted consistently, in approximately the same regions of the two respective segments in each animal. The crural was located in the most posterior, perivertebral region of the segment and the costal was placed lateral and approximately midway between central tendon and chest wall insertion; both were located approximately midway between origin and central tendinous insertion. Opposing transducers in each pair were inserted approximately 10-15 mm apart. On each segment, immediately adjacent to each pair of transducers, a fine wire, stainless steel (molybdenum alloy), bipolar, electromyogram (EMG) electrode was attached. In a few animals early in the series of implantations, a flexible, plastic catheter tipped with a compliant, latex balloon was placed in the abdomen in the left upper quadrant; the balloon catheter was carefully positioned to ensure that it could not abut on the undersurface of the diaphragm. After implantation, the abdominal incision was tightly sutured. A second balloon catheter was inserted into the left pleural space and positioned well above the diaphragm through a small, lateral intercostal incision. Continuous pleural suction was maintained on an accompanying chest tube to thoroughly evacuate any pneumothorax, and after suction the intercostal incision was tightly sutured. All wires and catheters were externalized via a subcutaneous skin tunnel, and the animals were recovered. After anesthesia and implantation, animals which successfully recovered normal diaphragm contractility, had stable measurements of diaphragm shortening and EMG, by 7-10 days post implantation. Most details of this implantation process are recorded in the first manuscript. Post mortem examinations were conducted on all implanted animals. A typical photomicrograph of the implanted diaphragm is shown in Figure 4.
Figure 4. Photomicrograph of canine diaphragm after removal of sonomicrometry transducer. Tissue specimen collected at 26 days postimplantation. Silhouette of the pair of transducer lead-in wires visible to the left, providing absolute scale since external wire diameter was 0.35 mm. The large cavity to the right was occupied by the sonomicrometer transducer ("crystal"). Around the transducer cavity, the edge of a thin (1-1.5 mm) fibrotic capsule encasing the transducer can be noted.
A practical addendum to sonomicrometry implantation and recovery.

Initial attempts to achieve a chronically implanted canine for sonomicrometry measurement were disappointing. The first attempts at implantation pre-date my arrival in the laboratory. These attempts were made via thoracotomy. As described elsewhere in this thesis, these early thoracic implants were not a success. After undertaking an abdominal approach to diaphragm implantation, the project did not produce a canine that we judged to be in possession of a fully and normally contracting diaphragm, prior to loss of implanted transducer signal or clinical complication affecting the entire animal, for approximately 18 months. During this time, sequential modifications were made in implant design, implantation techniques, and postoperative care specific for the implanted animal.

The evolution of "tailed" crystals to provide additional directional stability during healing, and the revision of the style of attachment of the EMG electrode have been described above. Overall, these changes gradually improved the reliability and durability of the implants. Despite these improvements, two essential features of the sonomicrometry implant became apparent. First, an implant could never be reimplanted. And, secondly, many perfectly aligned crystals could still fail abruptly, even many weeks after implantation, without preceding signal deterioration. The cause of sudden failure was eventually traced to simple fatigue and rupture of the transducer wire, usually at the soldered junction to the ceramic piezoelectric plate. This was not a failure of soldering technique, but a physical fatigue of the wire or adjacent components apparently precipitated by innumerable mildly stressed flexions which occurred with each inspiration.

In this format, with lead-in wires, even canines with flawless implantation and ideal transducer alignment were destined to lose some signals over time. Therefore, the preparation can properly be described as "semi-chronic" or "recovered". Recovery and study for an indefinite period extending over several years is not possible with this technology. A typical "successful" chronically implanted canine was not usable for 7-14 days postimplantation, then optimally functional for 2-3 weeks, and then available with decreasing rigor of signal quality and number of signals for an additional period, typically of 2-8 weeks. Thus the cycle of a successfully implanted canine was 1-3 months.

Besides improved implants, successful recovery of an implanted canine depended on extreme devotion to apparently trivial aspects of post implantation care, plus "luck". In time it became apparent that "luck" was a collection of factors
which could adversely influence contractility of the portion of the diaphragm segment that was under study. And since the lifespan of the total set of implants was limited, delayed recovery of a segment for many weeks amounted to a failed implantation.

We became accustomed to diaphragm segmental behavior that was impervious to certain types of rough, direct treatment, yet exquisitely sensitive to other seemingly insignificant irritants. As cited in the data chapter on recovery after implantation, the segment could continue to shorten robustly despite significant direct cautery injury. Yet the slightest hint of peridiaphragmatic inflammation, any overzealous chest or abdominal retraction during surgery, a slightly misplaced exit site, or even a history of pregnancy in an apparently normal animal, was associated with a persistent segmental dysfunction. The later animals were implanted without catheters for measurement of pleural or abdominal pressure; the catheters slightly increased the postoperative incidence of superficial wound infection and inflammation, but that was enough to dramatically decrease the proportion of successful recoveries. Fastidious postoperative care and generous use of antibiotics were inordinately important. The overall time of surgical duration of implantation was important, even with a complete absence of recognizable postoperative complications. The diaphragm reacted like a "powerful wimp".

The degree of post implantation dysfunction was related in some fashion to the route of implantation onto the diaphragm. As noted in our recovery chapter, earlier efforts in the laboratory by some of my peers to chronically implant the canine diaphragm via thoracotomy resulted in subtle abnormalities of function that persisted for many weeks. Subsequent investigations have reinforced this observation. Torres et al (22), implanted adult sheep by thoracotomy and observed that functional recovery often required 3-4 weeks or longer after implantation. It is of interest that even sonomicrometry implantation of periperal limb muscles require a post implantation recovery of 7-10 days to allow encapsulation and bonding and ensure validity of the measured signal (16).

Data acquisition and analysis by microcomputer

An essential, complementary component of the chronically instrumented preparation was a dedicated system of microcomputer programs for real time acquisition and analysis of the respiratory waveforms including airflow, muscle length and shortening, moving average EMG and CO₂ or O₂. This suite of programs allowed complete assimilation of multiple simultaneous measurements from several
individual muscles, continuously gathered over long experiments, and fully exploited the precision of the sonomicrometer.

The first obstacle to satisfactory, paperless data acquisition was an inability to gather and observe on screen in real time, continuous incoming signals to permanent storage on the fixed disk drive of the microcomputer, at moderate sampling rates over eight channels. No commercial product at the time offered this capability. This limitation was partially overcome by the appearance of a rudimentary set of acquisition routines which had been coded in Assembly language for the IBM PC and a common A/D board for the PC, from the University of Michigan. Unfortunately, these routines were not fully functional and required additional programming, so the candidate was obliged to gain familiarity with Assembler coding to proceed. But, that only provided data acquisition capabilities of adequate speed; there was still no real time display of incoming signals. The data acquisition was "blind". All incoming signals had to be monitored simultaneously a paper chart recorder. This analysis power was not adequate for this application.

This prompted an intensive period of programming in Assembly language, by the candidate, in pursuit of at least a minimally acceptable real time display. This effort was almost a success. That is, a real time display was worked out but it retained several serious and apparently insoluble "bugs". These problems led to a fortuitous acquaintance with Mr. Pierre Goyette, a Senior Computing System Analyst at McGill University. His extraordinary expertise in an area of programming that was, at that time, relatively new and uncharted, was crucial. With his cooperative efforts in the project, a continuous real time display and playback of several key data channels was achieved.

Two representative screens showing the real time display during experimentation are shown in Figures 5 and 6. The first is presented exactly as seen in the laboratory; the second has been annotated for explanation. The merit of real time display can be noted by comparing the signals showing costal and crural length between the two Figures. Crural length is indicated by the red trace. In Figure 5, crural shortening is normal and phasic with inspiratory airflow. By comparison, review of the real time display in Figure 6 during the experiment would have shown clearly that crural shortening was still biphasic; the canine had not yet recovered normal shortening post-operatively.
Figure 5. Photograph of typical real time, computer monitor image of costal and crural length and inspiratory airflow during an experiment. Note shape of segmental shortening; see text for details.
Completion of data acquisition programs with the prerequisite power and display occurred just as the chronically implanted canine began to provide unique measurements of diaphragm shortening and EMG, in copious quantity. Unfortunately, microcomputer analysis of such continuous, respiratory event based signals could not be accomplished using software available commercially, at that
time. Indeed, even today after the conclusion of these projects, generic commercial software suitable for such a task is not available. So, a suite of programs capable of breath-by-breath analysis of respiratory muscle shortening referenced to the cycle of respiratory airflow, had to be written by the candidate.

Although the task was undertaken with some serendipity, the choice of programming language was deliberate. At the time, both Fortran and Pascal for the microcomputer suffered from significant practical limitations. Available dialects of microcomputer Fortran were severely limited in I/O and graphics capabilities, and existing dialects of Pascal enforced a severe syntax for such an innovative programming project. And, at that time several dialects of Basic were evolving a logical structured Pascal-like syntax and acquiring enviable speed in compiled form. So the project was undertaken in Basic; the first set of programs were coded in IBM Compiled Basic and recompiled/reassembled utilizing Microway Inline Basic assembler routines to achieve maximum speed of computation utilizing the Intel 8087 math co-processor.

The suite of programs started with a series of numeric utilities to make the necessary conversions of A/D binary integers to calibrated floating point real numbers. Thereafter, several continuous signals required some digital numeric preprocessing, e.g. differentiation of the segmental length to derive velocity of shortening, and integration of the raw flow signal to generate minute volume. These first programs are listed schematically in Figure 7.
Figure 7. Schematic outline of original data acquisition and analysis programs. Part I: raw signal input to calibrated real numbers for each recorded signal. Variable and file names listed on the left, executable file names listed on the right.

Subsequent programs made the necessary calculations to identify the timing of the respiratory events of interest, and then based upon this timing, a series of breath-by-breath analysis programs were devised. These were capable of exhaustive analysis of aspects of breath-by-breath segmental EMG and shortening.
Figure 8. Schematic outline of original data acquisition and analysis programs. Part II: conditioning of real numbers values, and selection of breath events. Variable and file names listed on the left, executable file names listed on the right.
The digital analysis of the segmental length signal was a significant challenge. To that time, the character of the signal was not known. Each new observation or question, each system crashing-exception to the recognized behavior of the signal until that moment, required extensive reprogramming.

The remainder of the analysis programs are listed schematically in the subsequent figures. Final analysis and review was restricted by the availability of commercial software for graphical and statistical analysis. Details of the digital analysis of data is provided in subsequent manuscript chapters; for the sake of brevity it is not repeated here.
The final Figures 11-13 outline the more comprehensive analysis scheme that had evolved by the completion of the projects. This scheme had begun to incorporate sections of C code for improved performance and flexibility. And, the capability of the commercial and scientific end user software to which the data was ported had improved immeasurably. It can be seen from figure 13, that by the time these projects had concluded, that the microcomputer that had acquired the original data was able to finish with complete statistical analysis in SAS, without any reference to mini or mainframe resources. A review of current information via Medline was available, presentation graphics of the final summarized data could be plotted, and the manuscript synthesized without any additional resources or personnel. The microcomputer system for analysis was complete - but not quick. Speed of computation had to wait for later generations of Intel 80x86 machines, such as the current 80386 models.
Figure 11. Schematic outline of final process for data acquisition and analysis. Part 1: raw signal input to conditioning of real number values.
Figure 12. Schematic outline of final process for data acquisition and analysis. Part II: calculation of breath-by-breath values, spreadsheet summary and graphic review.
Figure 13. Schematic outline of final process for data acquisition and analysis. Part III: export of summarized data and interface to commercial software for statistical analysis, generation of summary presentation graphics, literature review and manuscript presentation.
REFERENCES


RESULTS

Overview

Segmental function postoperatively: a mode of study.

1 Recovery of diaphragm function after laparotomy and chronic sonomicrometer implantation.

Intrabreath segmental distinctions

2 Costal and crural diaphragm in early inspiration: free breathing and occlusion.

Segmental effects of mechanical stress

3 Diaphragm length adjustments with body position changes in the awake dog.

4 Effect of anesthesia on canine diaphragm length.

5 Velocity of shortening of inspiratory muscles and inspiratory flow.

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6 Costal and crural diaphragm function during CO2 rebreathing in awake canines.

7 Activity of costal and crural diaphragm during progressive hypoxia or hypercapnia.

Segmental participation in reflexic events

8 Costal and crural diaphragm function during panting in awake canines.
Overview.

This body of results is comprised of a series of manuscripts, reprinted together. Each manuscript is introduced by a brief synopsis to highlight insights pertinent to the overall theme of the work. After the synopsis, each manuscript is reprinted in the style of original publication, with permission by the owners of copyright of the respective manuscripts.
Segmental function postoperatively: a mode of study.

Recovery of diaphragm function after laparotomy and chronic sonomicrometer implantation. Manuscript 0

Thematic overview.

This series of investigations began with this first successful measurement of length and shortening, with concurrent EMG, of the costal and crural diaphragm in an awake intact canine. The outcome was much more than a tactical success in overcoming technological obstacles.

En route to a reliable chronic implant, the study delivered the first direct measurement of diaphragm dysfunction after abdominal surgery and characterized objectively in terms of muscle mechanical output, the sequence of postoperative diaphragm recovery. Although not emphasized in the manuscript, the divergence of recovery between segments provided preliminary evidence of the distinctive character of the segments. And, clear evidence of dysfunction as revealed by length and shortening in the face of apparently normal phasic EMG, strongly repudiated EMG as a valid solo indicator of diaphragm function.
Recovery of diaphragm function after laparotomy and chronic sonomicrometer implantation

PAUL A. EASTON, JEAN-WILLIAM FITTING, RAYMOND ARNOUX, ALBERT GUERRATY, AND ALEJANDRO E. GRASSINO.

Notre-Dame Hospital, University of Montreal, and Meakins-Christie Laboratories,
McGill University, Montreal, Quebec H3A 2B4 Canada

EASTON, PAUL A., JEAN-WILLIAM FITTING, RAYMOND ARNOUX, ALBERT GUERRATY, AND ALEJANDRO E. GRASSINO. Recovery of diaphragm function after laparotomy and chronic sonomicrometer implantation. J. Appl. Physiol. 66(2): 613-621, 1989.--If sonomicrometry transducers could be implanted permanently into the diaphragm, direct measurements of costal and crural length and shortening could be made during recovery from the laparotomy and then indefinitely in an awake, nonanesthetized mammal. We report results from six canines in whom we successfully implanted transducers onto the left hemidiaphragm through a mid-line laparotomy and measured segmental shortening and ventilation at intervals through 22 days of postoperative recovery. After laparotomy, breathing pattern, including tidal volume, respiratory rate and mean inspiratory flow, stabilized by the 4th postoperative day (POD). Tidal shortening of costal and crural segments increased from 1.82 and 1.45% of end-expiratory length (%LFrC), on the 2nd POD to 5.32 and 8.56 %LFrC, respectively, after a mean of 22 POD. Segmental shortening did not stabilize until 10 POD, and the recovery process displayed a sequence of segmental motions: lengthening, biphasic inspiratory lengthening-shortening, and increasing simple shortening. Three weeks after implantation, costal and crural segments were stable and shortening 5.32 and 8.56 %LFrC, respectively, and capable of shortening 49 %LFrC with maximal phrenic stimulation. In a pair of recovered animals, the initial postoperative dysfunction did not recur after a subsequent, simple laparotomy. At postmortem examination, the chronically implanted sonomicrometer transducers were found to have evoked only a thin fibrotic capsule within the diaphragm.

costal; crural; shortening; canine; upper abdominal surgery; recovery; awake; nonanesthetized
LENGTH AND SHORTENING of the costal and crural diaphragm have not been measured directly in any intact, awake, and spontaneously breathing mammal. Recent investigations employing sonomicrometry (8, 16, 26) have provided direct measurements of diaphragm motion during ventilation, but under restrictive conditions. These measurements always occurred under the cover of some type and depth of general anesthetic shortly after laparotomy or thoracotomy for sonomicrometer implantation, in mammals that were usually (17) restrained in a surgical, supine position. Novel information has been gleaned from these experiments but reliable delineation of spontaneous, resting segmental ventilatory shortening in a stable, reproducible laboratory preparation is a necessary precondition for future investigations involving control of respiratory motor output, reflexes, and coordination among respiratory muscles.

Cardiac muscle measurements (13) suggest that chronic implantation of sonomicrometer transducers into the diaphragm may be possible. Recognition of the clinical and physiologic disturbance of diaphragm function (10, 21, 24) that accompanies laparotomy and peri-diaphragmatic manipulation, raises doubts about the stability of diaphragm function if such sonomicrometer implantation are attempted. This concern evokes a second question of respiratory physiology about which we have no direct measurements. The length and shortening performance of diaphragm segments during recovery from such common events as laparotomy and minor diaphragm trauma is not known.

To address these questions, we implanted sonomicrometer transducers chronically onto the left hemidiaphragm of canines at laparotomy and directly measured costal and crural performance during spontaneous ventilation throughout an extended period of postoperative recovery.

METHODS

Surgical preparation. Mongrel dogs (mean wt 26.7 kg; range 20 to 30 kg), which had previously been tracheostomized, were chronically instrumented and studied postoperatively over 3-4 wk. Instrumentation and implantation was performed under general anesthesia induced with thiopental sodium (15 mg/kg), and maintained with halothane. The left hemidiaphragm was exposed through a midline abdominal incision. A pair of spherical (28), ultrasonic transducers, with fine Teflon coated lead-in wires, was implanted between muscle fibers on a flat portion of each of the costal and crural segments of the left hemidiaphragm (16). The transducers were implanted consistently in approximately the same region of the respective segment in each animal. The crural was located in the most
posterior, perivertebral region of the segment and the costal was placed lateral and approximately midway between central tendon and chest wall insertion. Opposing transducers in each pair were inserted ~15 mm apart. On each segment, immediately adjacent to each pair of transducers, a fine wire, stainless steel, bipolar electromyogram (EMG) electrode was attached. A thin, flexible, plastic catheter tipped with a compliant, latex balloon was placed in the abdomen in the left upper quadrant; the balloon catheter was carefully positioned to ensure that it could not abut on the undersurface of the diaphragm. After implantation, the abdominal incision was tightly sutured. A second balloon catheter was inserted into the left pleural space and positioned well above the diaphragm through a small, lateral intercostal incision. Continuous pleural suction was maintained on an accompanying chest tube to thoroughly evacuate any pneumothorax, and after suction the intercostal incision was tightly sutured. All wires and catheters were externalized via a subcutaneous skin tunnel, and the animals were recovered.

Measurement techniques. Sequential measurements were made over the 3-4 wk after implantation. During the measurements, the animals breathed spontaneously through a cuffed endotracheal tube. The endotracheal tube was attached through a unidirectional valve to a low-resistance open breathing circuit (<1.0 cmH2O/L/s), connected to a pneumotachograph (Fleisch no. 2) for measurement of airflow. Pleural and abdominal balloon catheters were connected to pressure transducers (model MP45, Validyne, Northbridge, CA). Dynamic measurement of the changing distance between the sonomicrometry transducers of each pair was provided by the sonomicrometer (model 120, Triton Technology, San Diego, CA). Measurement of diaphragm length by sonomicrometry has been described in detail (16). Briefly, when electrically excited, the emitter piezoelectric transducer resonates, radiating ultrasound waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a measurable voltage. A quartz crystal clock oscillator measures the transit time of the waves, and because the conduction velocity in muscle is known, the sonomicrometer provides the intertransducer distance. EMG signals from the wire electrodes were amplified (MkIII, TECA, White Plains, NY) and band-pass filtered (16 Hz-1.6 kHz). The output signals were then rectified and processed by passage through resistance-capacitance leaky "integrators" with a time constant of 100 ms, to provide moving averages of the electromyograms of costal and crural diaphragm.

Experimental protocol. Six animals that maintained sonomicrometer signals from both costal and crural diaphragm implants were each studied on at least six
occasions at similar intervals after implantation. Specifically, each animal was studied within each range of postoperative days (POD): A) 1-2, B) 3-5, C) 6-9, D) 10-11, E) 13-16, and F) 18-24, respectively. These periodic measurements were performed in the right lateral decubitus position, placing the instrumented hemidiaphragm in the nondependent posture. For each recording, the animals were awake, relaxed, comfortable, undistracted, and breathing quietly. The animals were completely familiar with the location, routine, and personnel of the recordings; all studies were performed by the same junior authors to whom the animals were bonded through provision of daily nursing and animal husbandry.

In addition to the preceding recordings, each animal was examined by chest radiograph during recovery, and reanesthetized after all recovery recordings were complete for a measurement of diaphragm length change during transcutaneous supramaximal phrenic nerve stimulation (0.2 ms, 100 Hz). The sonomicrometry transducers were recovered posthumously; the diaphragm was inspected and examined by microscopic section at postmortem. The diaphragm was sampled at regular intervals between pairs of crystals, and compared to undisturbed areas of the segment. The diaphragm samples were fixed, set as paraffin-plastic blocks, sliced in 5 μm sections, trichrome stained with hematoxylin-phloxene-safran, and mounted for microscopic examination.

Case study. In two animals an additional procedure was appended to the original protocol during the course of the study. After conclusion of the final recovery recording, two animals were subjected to a second laparotomy. Anesthetic, operative duration, incision, and abdominal retraction simulated the original implantation procedure but the diaphragm surface and upper abdominal contents abutting on the diaphragm were not disturbed. Another recovery recording was performed on the 2nd postoperative day.

Analysis of ventilation. All signals were recorded on a strip chart recorder. Simultaneously the data were gathered via single board analog-to-digital system (model 2801-A, Data Translation, Marlboro, MA) directly to hard disk on a microcomputer (PC, IBM, Boca Raton, FL) for subsequent examination using a series of dedicated analysis programs written by one of the authors (PE). The flow signal was evaluated for respiratory timing and digitally integrated; minute ventilation (VI), respiratory frequency (f), tidal volume (VT), inspiratory time (TI), mean inspiratory flow (VT/TI), and inspiratory fraction of respiration (TI/TT), were calculated breath-by-breath.

From the sonomicrometry data from each diaphragm segment, the computer algorithm identified muscle length at end expiration of each breath which was titled
"length at FRC" (L_{FRC}), whereas muscle shortening per breath was expressed as a percentage of change from the resting length or %L_{FRC}. Mean velocity of shortening was also calculated. The percentage of shortening for each animal per postoperative day was calculated from the resting length measured on that corresponding day. In addition to this traditional calculation of length change based on inspiratory flow, the algorithm incorporated an additional set of calculations. After identifying the muscle length corresponding to the onset of inspiratory flow defined above as L_{FRC}, in each breath the computer compared this value to the muscle length within the final third of the preceding expiration. The maximum length identified was titled X_{L_{FRC}}. Shortening per breath was then expressed as %X_{L_{FRC}}, from this less flow-dependent indicator of diaphragm length change.

EMG activity was quantified arbitrarily per breath as the maximum difference in volts between end expiratory base line and the peak height of the moving average signal. Pressure recordings from abdomen and pleura were evaluated only qualitatively for evidence of a waveform in phase with inspiration which was judged to be either positive or negative with reference to atmospheric pressure.

Statistical analysis. Mean values from each animal were ported directly to a spreadsheet program for examination, then exported to graphics software to output Figs. 1, 2, and 5 and to the PC version of SAS (23) for statistical analysis. Mean values for parameters of breathing pattern, shortening, and EMG were tested across the six recovery periods by two-way analysis of variance with repeated measures on one factor (14, 25). Multiple comparison testing of the mean values of the individual periods was performed using Tukey's test (23, 25). Maximum shortening of costal and crural segments during supramaximal stimulation was compared by Student's paired t test. The degree of association between the measurement of diaphragm shortening and EMG activity for each segment was evaluated by calculation of the product-moment correlation.

RESULTS

Laparotomy and instrumentation for the six animals reported was achieved without operative or postoperative complication. The canines were awake and ambulatory within 3-6 h of the operation, and were freely active and feeding normally within 1-2 days, prior to the first recovery measurement. The rigorous procedure to prevent or extract pneumothorax was successful; physical
examination and chest X-ray confirmed that during recovery, pleural air was either undetectable or present early only in trivial amounts.

Several animals instrumented at the outset of the experiment could not be studied because of postoperative complications or electronic malfunction. Subsequent animals were excluded only if they failed to retain sonomicrometer signals from both diaphragm segments throughout the recovery and study period. The major causes of sonomicrometer implant failure were postoperative displacement, loss of alignment, or late breakage of lead-in wires.

Each of the six animals was studied sequentially during postoperative recovery, with each measurement occurring within a range of postoperative days. Because of this design, for the group the measurements A-F occurred on mean postoperative days 1.8, 3.8, 7.0, 10.0, 15.0, and 21.5, respectively. Because the actual time of day for the surgery was not controlled, we report our POD of measurement rounded to the next integer.

**Breathing pattern.** Indexes of breathing pattern, including minute ventilation, tidal volume, and respiratory rate, reverted promptly by the second recovery measurement (B; POD 4), to values which then remained unchanged throughout the

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**TABLE 1. Ventilatory response and hemidiaphragm function during postoperative recovery**

<table>
<thead>
<tr>
<th>Breathing Pattern</th>
<th>POD 1</th>
<th>POD 2</th>
<th>POD 3</th>
<th>POD 4</th>
<th>POD 5</th>
<th>POD 6</th>
<th>POD 7</th>
<th>POD 8</th>
<th>POD 9</th>
<th>POD 10</th>
<th>POD 11</th>
<th>POD 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT (mL/min)</td>
<td>3.8±1.82</td>
<td>3.6±2.61</td>
<td>4.1±1.86</td>
<td>3.7±2.82</td>
<td>3.8±0.80</td>
<td>3.6±0.70</td>
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<tr>
<td>I breathed/min</td>
<td>29.2±7.89</td>
<td>15.5±2.33</td>
<td>16.57±2.42</td>
<td>14.55±3.20</td>
<td>15.98±3.34</td>
<td>15.31±2.58</td>
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<tr>
<td>Vr (L)</td>
<td>0.17±0.06</td>
<td>0.20±0.09</td>
<td>0.25±0.11</td>
<td>0.27±0.07</td>
<td>0.25±0.07</td>
<td>0.2±0.06</td>
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<tr>
<td>Tl (mL)</td>
<td>0.56±0.23</td>
<td>1.22±0.17</td>
<td>1.20±0.15</td>
<td>1.34±0.15</td>
<td>1.31±0.12</td>
<td>1.29±0.22</td>
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<tr>
<td>VT/VT, h/s</td>
<td>0.13±0.03</td>
<td>0.20±0.04</td>
<td>0.21±0.06</td>
<td>0.29±0.03</td>
<td>0.19±0.06</td>
<td>0.20±0.02</td>
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</tr>
<tr>
<td>Length (Lmax), mm</td>
<td>15.29±2.99</td>
<td>15.19±5.32</td>
<td>14.99±3.46</td>
<td>14.89±2.87</td>
<td>14.56±2.16</td>
<td>13.72±2.05</td>
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<tr>
<td>COS</td>
<td>16.08±5.75</td>
<td>17.39±5.76</td>
<td>17.19±5.49</td>
<td>17.3±5.04</td>
<td>17.90±5.55</td>
<td>18.52±5.47</td>
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<tr>
<td>CRU</td>
<td>1.58±1.67</td>
<td>2.67±2.38</td>
<td>3.99±1.98</td>
<td>4.59±1.75</td>
<td>4.77±2.18</td>
<td>5.72±2.47</td>
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<tr>
<td>Short, %Lmax</td>
<td>1.45±0.56</td>
<td>2.67±1.61</td>
<td>5.75±1.89</td>
<td>7.88±2.59</td>
<td>12.28±2.22</td>
<td>8.56±2.03</td>
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<tr>
<td>Vel (Lmax)/s</td>
<td>2.2±2.38</td>
<td>2.05±1.51</td>
<td>3.45±1.96</td>
<td>3.71±1.62</td>
<td>3.96±1.10</td>
<td>4.65±1.68</td>
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<tr>
<td>COS</td>
<td>3.74±0.63</td>
<td>2.82±1.51</td>
<td>4.10±1.57</td>
<td>6.22±2.67</td>
<td>5.42±1.87</td>
<td>7.87±2.19</td>
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</tr>
<tr>
<td>CRU</td>
<td>3.22±1.28</td>
<td>2.84±0.50</td>
<td>2.96±0.87</td>
<td>2.57±1.05</td>
<td>2.33±1.21</td>
<td>1.62±0.60</td>
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<td></td>
</tr>
<tr>
<td>Length (L)</td>
<td>2.94±1.09</td>
<td>4.12±1.69</td>
<td>3.44±1.36</td>
<td>4.0±1.26</td>
<td>3.67±0.43</td>
<td>3.33±1.63</td>
<td></td>
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<tr>
<td>COS</td>
<td>15.27±2.96</td>
<td>15.18±5.32</td>
<td>15.00±3.46</td>
<td>14.52±3.63</td>
<td>14.51±2.14</td>
<td>13.75±2.94</td>
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</tr>
<tr>
<td>CRU</td>
<td>16.72±5.81</td>
<td>17.46±5.72</td>
<td>17.18±5.20</td>
<td>17.47±5.67</td>
<td>17.55±5.57</td>
<td>18.51±5.50</td>
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<tr>
<td>Short (s)</td>
<td>1.84±1.69</td>
<td>1.84±0.43</td>
<td>1.94±1.75</td>
<td>2.75±1.94</td>
<td>4.88±2.25</td>
<td>5.53±2.55</td>
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<tr>
<td>COS</td>
<td>1.65±0.80</td>
<td>1.64±1.68</td>
<td>6.73±2.22</td>
<td>9.09±3.56</td>
<td>9.14±3.89</td>
<td>10.14±4.73</td>
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Values are means ± SD. COS, left costal segment; CRU, left crural segment; Short, segmental shortening/breath; Vel, segmental mean velocity of shortening/breath; ENG, peak-integrated electromyographic activity/breath; Length(s), convention as for length, except preinspiration baseline length defined at first point of shortening in end expiration (XIemc); Short(s), convention as for Short, except %Xlmax.
remainder of the recovery period. The immediate postoperative days (A; POD 2) were characterized by increased respiratory rate and decreased tidal volume as summarized in Table 1. VI and VT/TI were not different throughout recovery; \( f \) decreased \((P < 0.001)\), VT increased \((P < 0.001)\), and TI lengthened \((P < 0.001)\) throughout the recovery. Specifically, the mean \( f \) on POD 2 was more rapid than on any other measured postoperative day \((P < 0.01)\). Similarly, VT was less \((P < 0.01)\) and TI shorter \((P < 0.01)\) on POD 2 compared to every other postoperative day measurement. The rapid recovery of breathing pattern is illustrated in Fig. 1, to allow comparison with indexes of segmental shortening described below. Group mean \( f \) and VT values from each postoperative measurement are normalized, as a percentage of the maximum value of the respective variable measured on any POD.

\[ \text{FIG 1. Group mean respiratory rate and tidal volume during postoperative recovery. Results are expressed as \% of the maximum value recorded on any study day during recovery. Solid circles show } f; \text{ solid squares show VT; bars, } \pm SD. \]

**Segmental length and shortening.** Characteristics of the costal and crural hemidiaphragm did not recover as quickly as, or in synchrony with, the indexes of breathing pattern. For consistency, the usual calculations of end-expiratory length and shortening based on airflow \( (L_{\text{FRC}} \text{ and } %L_{\text{FRC}}) \) are reported in these results. During recovery, the mean intertransducer distance at end expiration did not change significantly for either costal or crural segments of the left hemidiaphragm. Measurement on POD 2 revealed that as implanted, the costal and crural pairs were separated by 15.28 and 16.65 mm, respectively. As noted in Table 1, during recovery costal resting length decreased, and crural length increased, only slightly.
The effect of laparotomy and implantation on breath-by-breath segmental shortening was emphatic, and recovery from the postoperative perturbation was protracted. During recovery, there was a marked overall (P < 0.002) increase in costal shortening, as noted in Table 1. Mean costal shortening increased nearly threefold from an initial low of only 1.8% of resting length (%LFR) on POD 2. Comparison of sequential measurements reveal that costal shortening increased between POD 2 and POD 10, then apparently plateaued at that level of activity. Specifically, costal shortening on POD 2 is different than on either POD 10, 15, or 22 (P < 0.05), although shortening measurements within the range of POD 7-22 cannot be confidently differentiated. Crural performance presented an equivalent recovery, amounting to approximately a sixfold overall adjustment in shortening (P < 0.001), achieved by continuous improvements through POD 2-10, with an apparent stability of shortening within the range POD 10-22. Specific comparison confirmed that crural shortening on POD 2 was different than on POD 7, 10, 15, or 22 (P < 0.01), although shortening on any of POD 7-22 could not be confidently stated to be different. This increase-to-plateau recovery of segmental shortening is illustrated in Fig. 2, expressed as a percentage of maximal shortening from any recovery measurement. Despite differences in the absolute amount of shortening per segment, after normalization it is apparent that both segments present a very similar pattern of recovery. Comparison of Figs. 1 and 2 confirm the incongruity between the abrupt reversion in breathing pattern and the gradual recovery of segmental shortening, postoperatively.
The mean rate of shortening showed recovery adjustments that were analogous to the aforementioned changes in shortening. As noted in Table 1, costal mean velocity of shortening increased from 2.32 to 4.65 \( %LFRC/sec \) (\( P < 0.009 \)). Similarly, crural velocity increased from 1.74 to 7.67 \( %LFRC/sec \) (\( P < 0.001 \)). Once again, velocity of shortening of both segments appears to plateau at POD 7-10.

The maximum shortening capability of costal and crural segments was evaluated at the completion of the recovery measurement series, with transcutaneous supramaximal phrenic nerve stimulation administered during a brief anesthesia. Performance of both segments was not different; costal and crural shortened 49.1 \( \pm \) 6.5 and 49.3 \( \pm \) 3.5 \( %LFRC \), respectively.

The pattern of recovery adjustments in segmental length and shortening was not altered when the calculations were based on a resting length which was not chosen traditionally to coincide with inspiratory flow but instead on the maximal preshortening length found in end expiration. As seen in Table 1, this less "flow bound" determination of inspiratory onset increased resting length and the magnitude of costal shortening slightly, especially during later recovery days. However, segmental resting length was still not different throughout recovery, although costal and crural shortening increased significantly (\( P < 0.001 \)), apparently plateauing at POD 7-10.

Segmental EMG activity. Function of the bipolar electrodes allowed recordings of electromyographic activity in costal and crural segments in four animals. These values are listed in Table 1. Through recovery, moving average EMG of the costal segment seemed to decrease slightly while crural activity remained unchanged; overall there was not a significant change in EMG activity of either segment. This result contrasted sharply with tidal volume and shortening of the corresponding segment.

Tidal volume and segmental shortening were closely related; between VT and costal shortening \( r = 0.58 \) (\( P < 0.002 \)), for VT and crural shortening \( r = 0.40 \) (\( P < 0.016 \)), and costal to crural shortening was correlated at \( r = 0.49 \) (\( P < 0.002 \)). However, for the course of recovery, segmental shortening and peak moving average EMG were not significantly related; between costal EMG and shortening \( r = 0.22 \) (\( P < 0.33 \)), and for crural EMG and shortening \( r = 0.30 \) (\( P < 0.193 \)).
Patterns of segmental motion during recovery. Inspiratory length change was characterized by three recognizable patterns corresponding to different stages of recovery. Sequential recordings revealed consistent progression among these patterns during recovery. Length and EMG activity of a costal segment in synchrony with inspiratory airflow, reproduced from a single animal, are illustrated in Fig. 3. The sets of traces A, B, and C correspond to 2, 6, and 10 POD, respectively. It is apparent in the first set of tracings from POD 2 that recordings of flow and EMG during inspiration are clearly synchronous with lengthening of the diaphragmatic segment. By POD 6, it can be seen that costal length moves through a biphasic pattern during inspiration; an initial lengthening followed by late inspiratory shortening. The third set of tracings demonstrate that recovery has progressed to a simple segmental shortening, synchronous with inspiratory airflow and EMG activity.

The progression from inspiratory lengthening to biphasic lengthening then shortening to simple shortening of increasing magnitude was observed in most costal and crural segments. Any deviations were clearly defined. A few individual segments did not show the complete progression, but commenced instead with biphasic motion or even a barely detectable amount of simple shortening. In any one animal, costal and crural segments usually recovered through these patterns at differing rates. However for all segments and animals, it was universally true that
once monophasic shortening appeared there was never a regression to biphasic shortening; similarly there was no reversion to lengthening after biphasic shortening was seen. Lengthening never "flipped" directly to shortening without a biphasic transition, and motion of every segment ultimately culminated in simple shortening.

These tracings also provide a qualitative reinforcement of the curious relationship between segmental shortening and moving average EMG. Trace A, POD 2, shows a peak inspiratory EMG of ~4.5 V, while the immediately adjacent costal segment is lengthening. The EMG activity in trace C, POD 10, during simple shortening was slightly less than the EMG noted during lengthening.

The balloon-catheter systems did not reliably withstand the rigors of implantation and recovery, with less than half the catheters enduring through recovery. Pressure recordings from the remainder were analyzed conservatively for evidence of positive or negative deflections in phase with inspiration. Five pleural catheters were functional at the first preoperative measurement; all showed phasic negative inspiratory pressure. Four abdominal balloon catheters were functional at the first postoperative measurement; two recorded a positive deflection, while two recorded a negative inspiratory deflection, including the animal shown in trace A. Three of these abdominal catheters persisted; all were positive in phase with inspiration by POD 4, including the animal shown in these traces. Thus regional pressure recorded in the left, midthoracic pleural space was negative and abdominal left upper quadrant was positive, even while segmental shortening at the location of the sonomicrometer transducers was in transition from lengthening to biphasic motion.
Histology. At necropsy, gross examination revealed the diaphragm to be mobile and undistorted. As they traversed the abdominal cavity, the lead-in wires attracted some omental adherence but adhesion to any portion of the diaphragm was limited. There was no evidence of traction or torsion affecting the abdominal surface of the diaphragm. On the rostral surface the location of the underlying sonomicrometer implants usually could not be identified by visual inspection. Gross and microscopic examination did not find evidence of fibrotic degeneration surrounding the pairs of sonomicrometry transducers or extending into the intervening muscle. As illustrated in Fig. 4, at the conclusion of recovery each sonomicrometry transducer had come to be encased within a discrete fibrotic capsule. The photomacrophgraph is an approximately X8 magnification of a section of the diaphragm showing normal muscle abutting against the fibrotic capsule, which had surrounded the outer transducer of a pair, with a fibrotic encased pair of

FIG 4 Photomacrophgraph of canine diaphragm after removal of sonomicrometry transducer. Tissue specimen collected at 26 days postimplantation. Highlighted arrow marks silhouette of transducer lead-in wire, providing absolute scale since external wire diameter was 0.35 mm. C, cavity occupied by sonomicrometer transducer ("crystal"). Solid arrows outline edge of thin (1-1.5 mm) fibrotic capsule encasing transducer.
lead in wires lying just beyond. The section is representative of the amount of capsular fibrosis and was selected to provide absolute scale. Because the diameter of the lead-in wires was 0.35 mm, the thickness of the fibrotic capsule was approximately 0.8-1.1 mm. Thus the usual pair of transducers was separated by two intervening capsules accounting for 2.5 mm and an additional 13-14 mm of normal muscle. The bipolar stainless steel EMG wires did induce some fibrotic reaction, but this did not impinge on the adjacent transducers or intervening muscle.

![Graph](image)

**FIG 5** Tidal volume and segmental shortening for a single animal during recovery and laparotomy. Solid triangle shows Tidal volume, litres; solid circle shows costal shortening, %LFR; solid square shows crural shortening. %LFR. Small symbols indicate recovered function, before laparotomy on POD 22 that is marked by **thick vertical stripe**. Large symbols at POD 2 and 24 indicate function on the 2nd POD after the initial operation and laparotomy, respectively.

**Case Study.** In two animals we also examined the effect on segmental length and shortening of a second laparotomy, with conditions mimicking the original laparotomy and implantation except that peridiaphragmatic contact was avoided. Both animals exhibited the relative lack of effect of the second laparotomy that is illustrated for a single animal in Fig. 5. Tidal volume is seen to increase from 0.158 liters on the 2nd POD after the original laparotomy to 0.268 liters at the conclusion of recovery measurements on POD 21. A second laparotomy was performed on POD 22, and on the subsequent 2nd POD (original POD 24), VT decreased to 0.20 liters. Thus VT was 59% and 75% of the recovered value 2 days after the two laparotomies. Concurrently, costal shortening was 28% of the recovered value on POD 2, yet still 95% of the recovered value 2 days after the second laparotomy. Similarly, crural shortening was noted to be 21% and 86% of the recovered value 2 days after the respective laparotomies.
DISCUSSION

Data Summary. These results indicated that reliable chronic implantation of sonomicrometry transducers in the diaphragm could be achieved. Once in place they allowed direct measurement of diaphragm motion for an awake, spontaneously breathing canine reclining in a posture typical for that species. Costal and crural shortening during resting inspiration were noted to be ≈5 and 8% of end-expiratory length, respectively. Structure and performance of the segments were not degraded by the chronic transducers. Maximal segmental shortening during percutaneous phrenic stimulation was 49 %LFRC, and fibrotic degeneration of the muscle was restricted to a discrete capsule encasing each transducer. Sequential postoperative measurements revealed that this stable performance was reached as the culmination of a progressive recovery of segmental shortening. Segmental recovery was distinct in time from postoperative changes in breathing pattern and proceeded deliberately through a recognizable sequence of inspiratory lengthening, biphasic lengthening with shortening, and increasing magnitudes of monophasic shortening. The mechanism of the postoperative degradation of segmental function could not be ascribed to conditions of anesthesia or simple laparotomy.

These results rest on several reasonable assumptions. It is implied that a significant alteration of performance accompanies laparotomy and diaphragmatic manipulation. It is also suggested that direct length measurements provide legitimate evaluation of postoperative diaphragm performance; an insight that is distinct from indices of breathing pattern, pressures, or EMG. We must be confident that the progressive results were not spurious, and that a plausible mechanism can be advanced for postoperative dysfunction and progressive recovery. Finally, the performance and structure of the stable, sonomicrometer-implanted diaphragm segment must reasonably approximate our best contemporary estimate of "normal" function. Each of these prerequisites will be considered in detail.

Postoperative diaphragm dysfunction. Several recent investigations (6, 10, 21, 24) have assembled convincing, albeit indirect, evidence that diaphragm function is impaired after upper abdominal surgery. Ford et al. (10) showed that after such a surgical procedure, patients exhibited decreases in tidal volume, tidal swings in transdiaphragmatic pressure, the ratio of gastric to esophageal pressures, and the ratio of changes in abdominal to rib cage diameters. These postoperative indicators of decreased diaphragm activity all reverted to near-normal values within 24-48 h after surgery. In a second study by the same group (21), the abnormalities in pressure and magnetometer evidence of a shift from abdominal to rib cage breathing
were confirmed by controlled, pre- and postoperative measurements in anesthetized canines undergoing cholecystectomy. Other investigators have reported similar observations (6, 24). Cumulatively this work gives credence to the long standing hypothesis (18) that postoperative complications including basilar atelectasis are related to decreased excursion and reflexic dysfunction of the diaphragm.

Our findings extend this concept of disturbed diaphragmatic activity after upper abdominal surgery. It is noteworthy that our animals did not have cholecystectomy or significant trauma to any visceral organ, only laparotomy with manipulation of upper abdominal contents and a trivial insult to the diaphragm abdominal surface. Nonetheless, we made the reasonable assumption that the diaphragm dysfunction we saw after implantation originated via the same mechanism as with cholecystectomy. As in the preceding studies, traditional indexes quickly returned toward normal; here breathing pattern appeared to revert, and abdominal and pleural pressure waves were qualitatively normal by POD 4. However, previous investigations did not address the duration of postoperative diaphragm abnormalities. We know that clinical complications of upper abdominal surgery do not vanish after POD 4, and some existing literature suggests that certain measurements of lung volume, transdiaphragmatic pressure, and breathing pattern may not reach preoperative values by POD 7-10 (4, 24). Our direct measurements of costal and crural shortening demonstrate that segmental function had not stabilized before 7-10 POD, and even after 10 POD there was a slight additional, but insignificant improvement in shortening. In fact we can be more specific. We also noted that in most animals the pattern of costal and crural shortening did not recover to simple shortening synchronously. Therefore we can safely imply that during many postoperative days there was differential function of costal and crural segments. This observation supports the developing thesis (1, 5, 27) that the diaphragm may operate as two segments that are functionally as well as anatomically distinct.

Evidence of dysfunction. Despite the attraction of our direct segmental measurements, we must critically assess the possibility that there was not a diaphragmatic abnormality during the "recovery" from upper abdominal surgery. Could not the apparent constancy of EMG activity during recovery indicate that segmental activity was unchanged, and that the patterns of segmental lengthening and negative abdominal pressure merely reflected a temporary ventilatory predominance of intercostal and accessory muscles? If there was genuine segmental diaphragm dysfunction postoperatively, why was EMG activity not found to be diminished after surgery?
Although the previously cited investigations [10,21] do suggest that there is a temporary, surgically related shift from abdominal to rib cage breathing, this adjustment should have resolved by POD 4 even though segmental shortening was still abnormal as seen on POD 6 in Fig. 3. The preceding studies provide ample pressure measurements to suggest that diaphragm function is intrinsically disturbed after upper abdominal surgery. Moving average quantification of EMG activity usually provides reliable correlation with muscle force and neural output (15), but we believe that in this study there is a technical discordance between the precision of sonomicrometry length measurement and EMG between postoperative days. Although the trauma to the diaphragm was trivial, we know from other experiments (7) that the implantation of transducers and fine-wire EMG electrodes precipitated significant local edema. This interstitial fluid would be expected to decrease the tissue "impedance" between the electrode poles, changing the potential difference sensed in the volume conductor, and artifactually increasing the recorded electrical signal (2,3). As the edema was resolved and ultimately replaced by a thin wrapping of fibrosis around transducer and electrode, the environmental conductance would be degraded. Although the EMG recording might adequately reflect a changing electrical activation on a single day, we are very skeptical about the EMG precision and stability for evaluation of quiet breathing between postoperative days. In contrast we know (16) that the same transition from modest interstitial edema to a fibrotic capsule would have an insignificant effect on the velocity of ultrasound, and thus would not affect calculation of intertransducer length between postoperative days. Therefore the relative constancy of the sequential recovery EMG recordings probably can be interpreted only as evidence that at least some amount of segmental, inspiratory, electrical activity was present throughout recovery.

Mechanism of postoperative dysfunction. These results provide some insight into the probable mechanism of the postoperative inhibition. Previous evidence suggests that the diaphragm dysfunction is related to the upper abdominal location of the procedure, and unrelated to anesthesia (21), postoperative analgesic (24), or postoperative pain (4). The persistent abnormalities of segmental shortening that we describe for up to 10 POD further discredit effects of anesthesia or pain as etiologic factors. The presence of postoperative segmental disability in the absence of cholecystectomy or other significant trauma to abdominal viscera or diaphragm also discount diaphragmatic injury and loss of physical contractility as etiologic factors. A recent human investigation employing percutaneous phrenic stimulation suggested that contractility as reflected in maximum transdiaphragmatic pressure
generation was not different before and after upper abdominal surgery (6). Our observation in two animals that thorough simulation of the surgical procedure, except for peridiaphragmatic manipulation, failed to reproduce the segmental dysfunction supports a reflex inhibition. Afferent traffic arising from the abdominal splanchnics could selectively inhibit phrenic output in favor of increased thoracic breathing (19, 22), or less likely, a low level phrenic afferent discharge might inhibit phrenic output (11).

Recent investigations (12) in the anesthetized canine have indicated that even in quiet tidal breathing the EMG of abdominal muscles, especially the transversus abdominus, is active in expiration. Presumably these muscles would also have been adversely affected by the laparotomy, and would have recovered concurrently.

Because abdominal muscle shortening was not measured we cannot know the relative expiratory contribution of the abdominals during the diaphragm recovery. Normal segmental function. Between 10-22 days after implantation, these animals seemed to present stable segmental function. The animals did not exhibit the persistent deviation from the usual physiology of respiration that forced the abandonment of a previous animal preparation (9) by an earlier group of investigators in this laboratory. Specifically, the persistent relative tachypnea and negative abdominal pressure swing during resting inspiration, that plagued the aforementioned animals (9) which were implanted by thoracotomy, were never exhibited by the animals in this experiment.

In this study, between 10 and 22 days after implantation, these measurements may represent a contemporary estimate of "normal" function. Although this is a plausible hypothesis, it is impossible to validate (or completely discredit). We presume that the measurement of "recovered" costal and crural shortening from each pair of crystals is representative of the complete segment; a reasonable assumption because shortening in an earlier acute study was noted to be uniform across a segment (16). In that same report (16) detailing acutely anesthetized canines, the values of resting, tidal, costal, and crural shortening were 5.7 and 10.6 %LFRG, respectively, at a tidal volume comparable to this study. The preceding values were calculated over inspirations defined by the onset of airflow, occurring at low rate/min, with stable length during end expiration. Thus the values are comparable to, and noticeably less than, our costal and crural calculations of 4.59-5.32 and 7.89-8.56 %LFRG, respectively. This difference is not unexpected because a relative increase in diaphragm-abdominal contribution to tidal volume might be expected for supine anesthetized canines (20). Finally, the maximal segmental shortening with percutaneous phrenic stimulation recorded
after 22 days in these canines matched or slightly exceeded the corresponding values for acutely implanted animals (16). This implies that these awake chronically implanted animals were fully recovered and stable without evidence of loss of contractile ability of costal and crural segments.

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REFERENCES


Intrabreath segmental distinctions.

Costal and crural diaphragm in early inspiration: free breathing and occlusion. Manuscript

Thematic overview.

This study examined isometric contraction of the diaphragm against an inspiratory occlusion, with particular emphasis on the early moments of inspiration which had been long been of special interest because of P_{0.1}. This study demonstrated clearly, by direct measurement, that diaphragm segmental contraction was not isometric during inspiratory effort against an occluded airway.

Of additional importance to this overall body of work, was the evidence of differential function of costal and crural segments during obstructed ventilation, and during specific early portions of the breathing cycle. This static examination of a tiny portion of the breath was our first successful effort to supplant simple whole breath, peak tidal measurements of segmental performance, to begin to examine segmental function during intrabreath development.
Costal and crural diaphragm in early inspiration: free breathing and occlusion

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EASTON, PAUL A., JEAN-WILLIAM FITTING, AND ALEJANDRO E. GRASSINO. Costal and crural diaphragm in early inspiration: free breathing and occlusion. J. Appl. Physiol. 63(4): 1622-1628, 1987.--Changes in length of costal and crural segments of the canine diaphragm were measured by sonomicrometry within the first 100-300 ms of inspiration during CO\textsubscript{2} rebreathing in anesthetized animals. Both segments showed small but significant decreases in end-expiratory length during progressive hypercapnia. Although both costal and crural segments showed electromyographic activity within the first 100 ms of inspiration, in early inspiration crural shortening predominated with minimal costal shortening. Neither segment contracted isometrically early in inspiration in the presence of airway occlusion. The amount of crural shortening during airway occlusion exceeded costal shortening; both segments showed increased shortening with prolonged occlusion and increasing CO\textsubscript{2}. Costal and crural shortening at 100 ms was not different for unoccluded and occluded states. These observations suggest that neural control patterns evoke discrete and unequal contributions from the diaphragmatic segments at the beginning of an inspiration; the crural segment may be predominately recruited in early inspiration. Despite traditional assumptions about occlusion pressure measurement (P\textsubscript{a1}), diaphragm segments do not contract isometrically during early inspiratory effort against an occluded airway.

length, shortening; hypercapnia, canine, sonomicrometry, airway occlusion.
EVIDENCE of different innervation and mechanical effects have earned for costal and crural diaphragm segments some recognition as separate respiratory muscles (1,2,18). Recent experiments utilizing sonomicrometry have allowed direct measurement of the relative shortening of the two segments. Peak tidal shortening has been noted to be greater for crural than for costal during both resting (16) and CO₂ stimulated breathing (4,19). However, no experiment to date has evaluated the relative performance of the segments during the early moments of inspiratory activity. Since transmission of central output via the phrenic and subsequent biochemical activation of the muscle probably is complete within the first 20 ms of inspiration (15), direct measurements of costal and crural shortening during the initial 100-300 ms may allow some insight beyond the mechanical events. If we presume that the measured shortening reflects the electrical activation of the corresponding segments, then we may gain some insight into the differential pattern of neural activation affecting the two segments at the genesis of an inspiration.

In addition, direct examination of segmental shortening in early inspiration should provide new information about some previous work in which pressure generated during the first 100-150 ms of an inspiration against an occluded airway (P₁₀₀₅₅) has been interpreted as an index of the overall summed output from the respiratory controller to the muscles participating in the inspiration (6,24). For occlusion pressure to provide reliable insight about global "respiratory drive", several assumptions are thought to be operative. A usual precondition is that contraction of the respiratory muscles in early inspiration should be isometric; otherwise the transformation of neural output to measured pressure will be distorted according to the length-tension and force-velocity relations of the muscles. For occlusion pressure (P₁₀₀₅₅) to be comparable during any series of measurements, the length-tension relationship also requires that end-expiratory muscle length remain constant from breath to breath. There is already some information which suggests that neither of these assumptions is inviolate. In some postures, expiratory reserve volume may decrease during progressive hypercapnia (5). Recent direct, sonomicrometer measurements of canine diaphragm length have demonstrated significant peak shortening during completely occluded inspiration (4,16). The extent of any violation from these assumptions should be apparent for the diaphragm after a direct measure of segmental length and shortening in early inspiration.

Therefore we employed the technique of sonomicrometry to evaluate directly the length and contractility of costal and crural segments of the canine diaphragm.
Costal and crural diaphragm: Results

Early inspiration-
during the initial 100-300 ms of both normal and occluded inspirations during CO₂ stimulated ventilation.

METHODS

Experimental procedure and techniques. Eight mongrel dogs (mean weight 21.1 kg; range 15-30 kg) were anesthetized and studied in the supine position. Anesthesia was induced with sodium thiopental (15 mg/kg), and continued with a mixture of achloralose (50 mg/kg) and urethane (250 mg/kg). Small additional boluses of achloralose-urethane were given during the duration of the experiment to maintain a light level of anesthesia with brisk corneal reflexes. During the measurements, the animals breathed spontaneously through a cuffed endotracheal tube. Arterial blood pressure was monitored through a femoral arterial catheter; pressure remained within 10% of base-line values throughout the experiments in all animals.

The left hemidiaphragm was exposed through a midline abdominal incision. Piezoelectric transducers mounted on small (1.5 cm diameter) bases were attached to the surface of the diaphragm with cyanoacrylate glue (16). One pair of transducers was fixed onto a flat portion of each of the costal and crural segments of the left hemidiaphragm; opposing transducers in each pair were 15 ± 3 mm apart. On each segment, a bipolar hook electrode was attached adjacent to the pair of transducers. After implantation, the abdominal incision was tightly sutured. When measurements were complete, all transducers were recovered and inspected.

Dynamic measurement of the changing distance between the transducers of each pair was provided by the sonomicrometer (model 120, Triton Technology, San Diego, CA). Measurement of diaphragm length by sonomicrometry has been described in detail (16). Briefly, when electrically excited, the emitter piezoelectric transducer resonates, radiating ultrasound waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a measurable voltage. A quartz crystal clock oscillator measures the transit time of the waves, and since the conduction velocity in muscle is known, the sonomicrometer provides the inter-transducer distance. Electromyogram (EMG) signals from the hook electrodes were amplified (MkIII, TECA; White Plains, NY) and band-pass filtered (16 Hz-1.6 kHZ) The output signals were then rectified and processed by resistance-capacitance leaky integrators with a time constant of 100 ms, to provide moving averages of the EMG's of costal and crural diaphragm.

The endotracheal tube was attached through a unidirectional valve to a closed breathing circuit, with resistance <1.0 cmH₂O/L/s. The valve was pierced
by a pressure tap for measurement of mouth pressure. The inspiratory limb was connected to a pneumotachygraph (no. 2, Fleisch) for measurement of flow and volume (respiratory integrator, Hewlett-Packard, Waltham, MA). A pneumatic device for intermittent occlusion was adjacent to the inspiratory side of the valve; it was occluded for approximately the first 0.5 sec of inspiration at 20-to 30-s intervals. On the expiratory limb, CO₂ was sampled and analyzed continuously (model LB-2 infrared CO₂ analyzer, Beckman, Schiller Park, IL). Ventilatory and occlusion pressure responses to progressive hypercapnia were elicited by a modification of the Read technique (17), rebreathing 6% CO₂ in O₂ from a 4 liter bag.

Analysis of ventilation, pressure, and diaphragm length. Ventilatory and pressure responses to hypercapnia, ΔV/ΔPACO₂ and ΔP/ΔPA CO₂, were linear and slopes were calculated by linear regression using the method of least squares. The slope of the mouth pressure response to hypercapnia during normal, unoccluded breathing was calculated at 100 ms after the beginning of inspiration (ΔPNO.1/ΔPA CO₂). The slope of the occlusion pressure response to hypercapnia was calculated at 0.1, 0.2, and 0.3 seconds after the beginning of inspiration against occlusion (ΔPO.1/ΔPA CO₂, ΔPO.2/ΔPA CO₂, ΔPO.3/ΔPA CO₂).

EMG activity was quantified into arbitrary units based on the peak height of the integrated signal. The peak height of the unoccluded breath, before to each occluded breath was measured and considered as 100%; EMG during the occluded breath was expressed relative to this amount. EMG activity was specifically examined at 0.1 s after the beginning of occluded inspiration.

From the sonomicrometry recordings, the muscle length at end expiration was titled "length at FRC" and expressed as LFRC, and muscle shortening was expressed as a percent change from the resting length or %LFRC. Muscle shortening during any given breath, either unoccluded or occluded, was calculated using the LFRC immediately preceding the breath.

Three aspects of muscle length and shortening were specifically examined. 1) Baseline LFRC was noted at the beginning of hypercapnia, and the change in costal and crural segmental end-expiratory length (LFRC) was determined throughout rebreathing and the change was expressed as percent of the baseline LFRC. 2) The records of costal and crural length during hypercapnia allowed measurement of shortening (%LFRC) at 0.1 and 0.3 s of multiple, normal, unoccluded inspirations. During the hypercapnia, inspiratory airflow was obstructed during the expiratory phase of many of the measured normal inspirations, so shortening could also be measured at 0.1 and 0.3 s of the following
Costal and crural diaphragm: Results
Early inspiration.

occluded inspiration. The amounts of shortening at equivalent times for pairs of free or occluded inspirations was then compared. 3) Shortening at 0.1, 0.2, and 0.3 s of occluded inspiration was measured and compared for both costal and crural segments.

These aspects of muscle length and shortening and EMG activity are presented here, at four levels of PCO₂ PACO₂: 52.8 ± 2.3, 60.7 ± 3.4, 69.3 ± 2.8 and 77.4 ± 3.4 Torr PACO₂. For each animal these values represent the measurements taken at the beginning, ending, and two equally spaced intermediate points during CO₂ rebreathing.

Statistical Analysis. At individual levels of PACO₂, costal and crural shortening at 0.1 sec was compared using Student's paired t test. Similarly costal and crural EMG at 0.1 sec was compared by Student's paired t test. The slopes of normal and occluded pressure responses, ΔPN0.1/ΔPACO₂, ΔP0.1/ΔPACO₂, were also compared by paired t test. At 0.1 and 0.3 sec, occluded vs. unoccluded shortening was compared for each segment at all levels of PACO₂ using a three way analysis of variance (ANOVA) with repeated measures on 2 factors (8). The slopes of occlusion pressure responses to hypercapnia, ΔP0.1/ΔPACO₂, ΔP0.2/ΔPACO₂ and ΔP0.3/ΔPACO₂ were compared using a two way ANOVA with repeated measures on one factor, comparing pairs of slopes by Tukey's test (8,21). All values examined at four levels of PACO₂, including changes in EMG activity at 0.1 s and costal and crural LFRC and shortening at 0.1, 0.2, and 0.3 s, were also examined using a two way ANOVA with repeated measures on one factor.

RESULTS

CO₂ response. With this agent and dose of anesthetic, group mean ± SD ΔVI/ΔPACO₂ and intercept remained at 0.40 ± 0.06 (SD) and -15.03 ± 3.63 l/min/Torr, respectively. The slope of the pressure response at 100 ms for normal, unoccluded breathing (ΔPN0.1/ΔPACO₂), was significantly less than the slope of the occluded response at equivalent time (ΔP0.1/ΔPACO₂); 0.03 ± 0.02 compared to 0.13 ± 0.03, (P<0.01). The mean slopes of the occlusion pressure responses at 0.1, 0.2, and 0.3 s were 0.13, 0.28, and 0.40 cmH₂O/Torr respectively; values that showed a significant increase from 0.1 to 0.2 s and from 0.2 to 0.3 s (P<0.01).
Costal and crural diaphragm: Results
Early inspiration.

FIG 1  Diaphragm length at end expiration ($L_{FRC}$) during hypercapnia. Mean change in segmental $L_{FRC}$ at 4 levels of $PACO_2$, as a percent of the baseline $L_{FRC}$ at $PACO_2$ 52.8 Torr. Open squares, costal length change; fill circles, crural change; bars show ±SE.

For the group the hypercapnic stimulus commenced at 52.8 ± 2.3 and terminated at 77.4 ± 3.4 Torr $PACO_2$. Variables describing the mechanical and electrical function of the diaphragm are presented at these two extremes as well as two intermediate values of 60.7 ± 3.4 and 69.3 ± 2.8 Torr $PACO_2$.

Diaphragm length during hypercapnia. Compared with the direct sonomicrometry measurement of end-expiratory resting length of the diaphragm ($L_{FRC}$) at the onset of hypercapnia, there was a consistent decrease in $L_{FRC}$ through the progressive increase in $PACO_2$. This change is shown for four levels of $PACO_2$ in Fig. 1. The changes were consistent and similar for both costal and crural segments with a maximum decrease of 3% of initial $L_{FRC}$. This amount was a significant decrease for both segments ($P<0.01$).
Segmental shortening in early inspiration. Figure 2 demonstrates that for normal free breathing, crural shortening was predominately by 300 ms at each of the four levels of PACO₂. Although less than crural, costal shortening was moderate, reaching 6% at 77.4 Torr PACO₂. Although not shown in the figures, the mean peak tidal shortening of normal unobstructed costal and crural segments at PACO₂ 77.4 Torr was 22 and 27% LFRC, respectively. So during the highest degree of chemical stimulation by 300 ms the crural had achieved ±41% of peak tidal shortening, whereas costal had completed 27% of its final peak shortening.
Further insight about the beginning of each inspiration is gained from Fig. 3. There was very little shortening of the costal segment during the first 100 ms of inspiration, reaching only 0.5% even at 77.4 Torr PACO$_2$. In contrast, the crural segment demonstrated much more early activity at each of the four levels of PACO$_2$. So at 100 ms the crural had completed ±10% of peak tidal shortening, whereas the costal had shortened barely 2.5% of its peak tidal amount for the highest level of CO$_2$ stimulation. Similar relative proportions of total shortening were accomplished at 100 and 300 ms at the lower levels of CO$_2$.

Thus, during CO$_2$-stimulated breathing, peak tidal shortening of crural is greater than costal and crural shortening seems to predominate within the earliest moments of the inspiration. Costal activity is quite different. Although costal shortens little at first and ultimately reaches a lesser peak tidal shortening, there is a relatively greater increase in costal shortening between the 100-300 ms period during early inspiration.
EMG activity. EMG activity of costal and crural segments was consistently present during the first 0.1 s at all levels of CO₂. These changes are reported at the four levels of PACO₂ in Fig. 4; segmental EMG activity during the occluded state is illustrated. As seen in the graphic, mean costal EMG appeared to be less than crural for the two lower levels of PACO₂ but there was not a significant difference between the segmental EMG values at any of the four levels of PACO₂. Since peak EMG activity increased with increasing hypercapnia, both costal and crural segmental EMG activity at 0.1 sec increased significantly (P<0.01) as hypercapnia increased.
Costal and crural diaphragm: Results

Early inspiration.

Diaphragm contractility during occlusion. The diaphragm was not isometric during occlusion. Direct sonomicrometry measurements of the amount of shortening of costal and crural segments at 0.1 sec after occlusion are included in Fig. 5 and 6. Although present, the amount of shortening of costal diaphragm was negligible; maximum shortening after 0.1 sec at 77.4 Torr PACO₂, was only 0.2 ± 0.8% of the LFRC at the onset of the occlusion. In contrast active shortening of the crural segment did occur by 0.1 sec of occlusion, and it increased significantly (P<0.01) from 0.6 ± 0.5 to 2.6 ± 2.4% of LFRC at the extremes of hypercapnia. At each of the four hypercapnic levels, shortening during occlusion at 0.1 s was significantly greater (p<0.02) for the crural than for the costal segment.

Segmental shortening was evaluated as occlusion persisted from 0.1 to 0.2 and 0.3 s. As seen in Fig. 5, although costal shortening at 0.2 s increased significantly (p<0.05) during hypercapnia, the maximum change, which was seen at 77.4 Torr PACO₂, was still only 1.7 ± 3.1% LFRC. Even 0.3 sec after occlusion, the shortening only exceeded 1% at 69.3 Torr PACO₂, and reached a maximum of 4.1% at 77.4 Torr PACO₂. Thus, even after 300 ms at the highest level of CO₂ stimulation, the amount of costal shortening during occlusion was in fact a very small proportion of its peak tidal shortening.
Early inspiration:

As seen in Fig. 6, more shortening activity of the crural segment was noted at each of the three 100-ms intervals after occlusion. Crural shortening increased significantly during hypercapnia at each of 0.1 (P<0.01), 0.2 (P<0.025), and 0.3 (P<0.01) s. The maximum deviation from isometricity for the segment was 8.1 ± 7.7% LFR at 77.4 Torr PACO₂, occurring 0.3 s after occlusion.

Early segmental shortening: occluded versus unoccluded. After an evaluation of the usual early segmental length changes and the progressive departure from isometricity when contracting against an occlusion, it was informative to directly compare unoccluded and occluded pairs of inspirations. The results are included in Figs. 2 and 3. As seen in Fig. 3, the crural shortening at 0.1 sec at the four levels of PACO₂ was actually clustered about the line of identity. When considered at all four levels of PACO₂, the shortening during early inspiration of the crural segment was not different between occluded and unoccluded conditions. Although all values were less than for the crural, shortening activity of the costal at 100 ms was also similar for both the occluded and unoccluded conditions. However by 300 ms as seen in Fig. 2, all values were seen to rest below the line of identity. Therefore, when considered at all four levels of PACO₂, occluded shortening was less than unoccluded shortening for both costal (P<0.01) and crural (P<0.02) segments.
DISCUSSION

Data summary. For this animal preparation, with a $\Delta V/\Delta P_{ACO_2}$ of 0.40 l/min per $P_{ACO_2}$, costal and crural segments showed EMG activity by 100 ms after occlusion, activity which was consistent and increased with increasing hypercapnia. Direct measurement of end-expiratory position made via sonomicrometry revealed a small but significant shortening of both costal and crural fiber length during progressive hypercapnia. Crural shortening was predominant and the costal segment nearly inactive at 100 ms. Although at 300 ms crural shortening was still much greater than costal, there had been a larger increase relative to baseline in shortening of the costal segment in the intervening 200 ms. Neither costal nor crural segments were exactly isometric within the first 100-300 ms of inspiration against an occlusion. For both segments the amount of shortening increased with increasing time after the onset of occlusion, and at equivalent times and levels of hypercapnia the departure from isometricity of the crural segment was much greater than the minimal shortening of the costal. The amount of shortening demonstrated by each individual segment at 100 ms was not different between the free breathing and occluded states.

Assumptions regarding shortening. Interpretation of these results rests on several assumptions. We presume that a suitable CO$_2$ responsiveness persisted during the anesthetic, that the relative function of costal and crural segments during hypercapnia was not unduly affected by the anesthetic agent, and that the decreasing segmental lengths recorded during inspiration reflected active contraction of the respective segment and not just passive length change. A moderate ventilatory response to CO$_2$ of 0.40 l/min/Torr was retained, which is less than the awake but comparable to the anesthetized values of $\Delta V/\Delta P_{ACO_2}$ for a similarly sized mammal from another report (7). Although we are not aware of any reason why this anesthetic agent should cause a differential, detrimental effect on the neural activation of either segment of the diaphragm, preservation of a normal costal-crural relationship must remain an assumption until similar observations can be made in an unanesthetized preparation.

The presence and linear increase of EMG activity with increasing CO$_2$ during the first 100 ms provides an important background for the inspiratory length changes found by sonomicrometry. There is no a priori reason why either segment or even the entire diaphragm must be active at the very outset of an inspiration; early recruitment of non-diaphragmatic muscles could initiate inspiration and contribute to a passive change in costal or crural length (and participate in any measured pressure change such as P0.1). The early segmental EMG activity we
showed does not exclude activity of other muscles but does suggest that at least part of the costal and crural length changes resulted from neural activation and active contraction.

Any interpretation of these results must consider a discordance in the precision of the sonomicrometry length measurements and the EMG. As described in the methods, the EMG activity was quantified as a moving average with a 100 ms time constant. Given this procedure and the the imprecision which could be introduced by even tiny amounts of noise during earliest inspiration, the EMG values could not be extrapolated to accommodate the resolution of the segmental shortening information. As shown in Fig. 4 and described in the preceding paragraph, segmental EMG activity was present in early inspiration with generous variability, an could be roughly quantitated. However, we could not reliably compare the relative EMG values of the two segments within the 100- to 200-to 300-ms intervals, in any fashion analogous to the analysis of differential shortening of the two segments.

Relative segmental shortening in early inspiration. These records showing different shortening behavior of the two segments might be viewed as the mechanical result of simultaneous and equal neural activation of two areas of a single muscle. The greater crural shortening would reflect the contraction of that portion of the diaphragm against less mechanical impedance than was being faced by the costal section. More probably the segments should be viewed as separate muscles operating in a mechanically parallel arrangement (13). Despite separate innervations, fortuitously the neural activation of the two muscles would remain identical; again the different segmental shortening would relate solely to the unique mechanical orientation of each segment. Without evidence of differential segmental EMG activity to accompany this differential segmental shortening, we cannot formally refute these hypotheses. However, there is a more attractive explanation.

Other work has shown that during CO₂ rebreathing the peak EMG of costal and crural segments is not equivalent (22,23); the maximal increase from baseline of crural EMG is greater. This corresponds with sonomicrometry measurements of segmental peak tidal shortening during CO₂ rebreathing in this and other studies (4,19); again, the maximal increase from baseline of crural shortening is greater than costal. Together this evidence suggests that for peak tidal shortening, the greater crural response is a reflection of a proportionally larger increase in peak tidal neural activation of that segment during CO₂ stimulated breathing. We might anticipate therefore that this emphasis on crural activation would also extend to
other intervals in inspiration including the first 300 ms. As stated earlier, the EMG data in this study do not allow us to directly examine that assumption. However, van Luteren et al (21) have observed that during hypercapnic-stimulated breathing there was a definite inequality in the timing of EMG activity measured for the two segments. Crural EMG activity consistently preceded activity of the costal (21). Thus it seems reasonable to suggest that there is a preferential neural activation of the crural segment in early, as well as at peak inspiration.

On this background, the differential pattern of segmental shortening we noted in early inspiration may be interpreted as reflecting the neural control pattern which suberves the actions of the two segments. Crural shortening predominates while costal shortening is minuscule at 100 ms, suggesting that for the diaphragm the genesis of an inspiration is primarily a crural event. Although this does not give information about involvement of other inspiratory muscles, apparently the central controller differentially activates neurons subserving the C5 phrenic nerve roots and thus the crural segment (2) before those of the costal. The observations taken 200 ms later suggest that the presumed neural and electrical dissociation continues; further development of shortening within the next 200 ms is different for each segment. Between 100 and 300 ms costal activity "catches up" somewhat on crural; a greater proportion of peak tidal shortening of costal is achieved through this 200 ms period. Taken together these differences in costal-crural shortening at peak and at 100 and 300 ms suggest unique central neural outputs expressed through differences in total amount, initiation, and development of segmental shortening.

Functional residual capacity during hypercapnia. During rebreathing of CO₂ it is usually assumed that sequential measurements, such as occlusion pressure recordings, are generated at an unchanging functional residual capacity (FRC). However, while rebreathing CO₂, seated humans often decrease their expiratory reserve volume, thus changing FRC and increasing both end-expiratory length of the diaphragm and its pressure generation against occlusion (13). Although traditionally, supine humans and animals have not been thought to measurably decrease FRC with rebreathing, this experiment allowed a unique opportunity for direct evaluation of the constancy of segmental length at FRC. For the supine anesthetized canine the results are clear; there was a small but significant decrease in end-expiratory length of both costal and crural segments, which could translate into a slight increase in FRC (assuming a constant residual volume). Although the end-expiratory length decrease was small, this alteration is well within the precision of resolution of the measuring system. This result is in agreement with a
recent investigation, that showed a progressive decrease in FRC during CO₂ rebreathing in supine humans (12). In theory this decrease in LFRC could invalidate the linear coupling that is assumed between summed neural output and occlusion pressure. However, the potential for real underestimation of occlusion pressure seems very slight for a length change of this magnitude given the length-tension characteristics of the mammalian diaphragm (9, 14).

Nonisometric contraction during airway occlusion. Occlusion pressure measurements, notably at 100 ms (P0.1), have persisted as an apparently informative but controversial measure of total neural output. Generally a linear relation between occlusion pressure and increasing CO₂ is observed. During inspiratory elastic loading, even as the minute ventilation response to an increasing chemical stimulus begins to attenuate, occlusion pressure response increases suggesting logically that overall neural output still must be mounting (20). Occlusion pressure changes parallel measurements of integrated diaphragmatic EMG (11) or phrenic electroneurography (3) in most instances with exceptions such as flow loading (10) and the sitting posture (5) (where muscles other than diaphragm presumably contribute disproportionately to increasing inspiratory effort). For circumstances where there is relative constancy of the shape of the pressure wave, occlusion pressure (P0.1) seems to approximate an artificial summation of the various neural stimuli which orchestrate the multiple muscles contributing to an inspiration. But what are the implications of the nonisometric contraction we have observed during the airway occlusion measurement?

That the inspiratory muscles contract isometrically against an obstructed airway was an original assumption of occlusion pressure measurements. Any shortening during occlusion would be expected by length-tension and force-velocity characteristics to decrease occlusion pressure correspondingly. This data demonstrates that during 100 ms of occlusion there was no significant costal shortening, but crural shortening was detectable and at the highest PACO₂, approached 3% LFRC or 9% of unobstructed tidal shortening for that level of stimulus. Although pressure loss expected as a result of this shortening would be very small, clearly the segments do not contract isometrically. Some degree of chest wall distortion was implied at 300 ms; although the amount of crural exceeded costal, the occluded shortening of both segments was less than at a comparable time without occlusion. Recent reports (4, 16) suggest that if airway occlusion is continued throughout inspiration that both segments continue to shorten, and the final amount of crural shortening cannot be differentiated from the peak shortening of an unoccluded breath. In fact in this experiment during the
initial 100 ms of occlusion pressure measurement crural shortening was indistinguishable from the crural activity at 100 ms of free breathing, although by 300 ms this had changed.

Since the airway occlusion does not ensure isometric contraction, what is the role of the obstruction in the generation of the occlusion pressure measurement? Does the obstruction just give a convenient means of measuring the pressure?

This data suggested that the costal segment made the same contribution to the measured pressure in either occluded or unoccluded situations. Suppose our thesis correlating shortening and EMG activity was mistaken; in the unoccluded state, the costal might have received generous neural activation at 100 ms and made a significant contribution to pressure by a near-isometric contraction while facing some large load. But the introduction of an airway obstruction will not alter neural activation of the costal at 100 ms, so after occlusion with this unchanged neural activation the costal shortens the same small amount and generates the same pressure. Since neural activation of crural at 100 ms would also be unchanged by occlusion, the fact that the crural did not contract isometrically after occlusion suggests that it also responded as if facing a similar load and generated the same pressure after occlusion as when unoccluded. But pressure was greater after occlusion, so what was the origin of the additional pressure? There are at least two other possible contributing sources for inspiratory occlusion pressure. The accessory or intercostal inspiratory muscles might also be active in early inspiration; if they were strongly stimulated or faced an impedance relatively lower than the diaphragm, they could contribute to occlusion pressure. Alternatively pressure in earliest inspiration could be produced by relaxation of expiratory muscles and release of chest wall elastic recoil. From this data we cannot determine if either of these possibilities is applicable. This evidence that the diaphragm does not participate as expected through isometric contraction demonstrates the complexity of the generation and interpretation of occlusion pressure.

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REFERENCES


Segmental effects of mechanical stress

Diaphragm length adjustments with body position changes in the awake dog. Manuscript ③

Effect of anesthesia on canine diaphragm length. Manuscript ④

Velocity of shortening of inspiratory muscles and inspiratory flow. Manuscript ⑤

Thematic overview.

Together these three studies explore related mechanical attributes of diaphragm segmental function.

In our examination of length adjustment with position change, we noted that a rough equivalence of segmental tidal shortening was maintained despite changing posture. This outcome suggested that intrinsic diaphragm response to maintain tidal excursion of the respective segments was linked to the control of phasic expiratory activity of the abdominal musculature.

The induction of anesthesia was associated with a distinctive alteration in the resting length of the crural segment compared to costal segment. This result suggested that the two segments differed in their inherent tonic activation, and that this divergence was exposed by anesthetic.

The relation between segmental shortening and velocity of shortening, and mean inspiratory flow, was confirmed to be constant, linear, and reproducibly altered by inspiratory load. It was significant that this basic functional characteristic was similarly applicable to both segments, but was not confirmed for intercostal muscles under study.
Diaphragm length adjustments with body position changes in the awake dog.

J.W. FITTING, P.A. EASTON, R. ARNOUX, A. GUERRATY, AND A. GRASSINO. Hôpital Notre-Dame, Université de Montréal, and Meakins-Christie Laboratories, McGill University, Montreal, Quebec H3A 2B4, Canada; and Division de Pneumologie, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

FITTING, J.W., P.A. EASTON, R. ARNOUX, A. GUERRATY, AND A. GRASSINO. Diaphragm length adjustments with body position changes in the awake dog. J. Appl. Physiol. 66(2): 870-875, 1989. - Sonomicrometry was used to measure end-expiratory length and tidal shortening of the costal and crural diaphragm in awake chronically instrumented dogs in the right lateral decubitus, standing, and sitting postures. End-expiratory length did not change significantly in standing but fell by 11.5% for the costal and by 14.4% for the crural segment in sitting, when compared with decubitus position. Tidal shortening of both segments did not change significantly in the three postures. From decubitus to sitting, diaphragmatic electromyogram (EMG) activity increased only in some dogs, not significantly for the group. The inspiratory swing of abdominal pressure was always positive in decubitus and negative in standing and sitting. In the latter two postures, abdominal pressure increased gradually during expiration and fell in inspiration, suggesting a phasic expiratory contraction of abdominal muscles. We conclude that diaphragmatic tidal shortening is maintained in the different postures assumed by the awake dog during resting breathing. It seems that the main compensatory mechanism for changes in diaphragmatic operational length is a phasic expiratory contraction of the abdominal muscles rather than an increase in diaphragmatic EMG activity.

respiratory muscles; abdominal muscles; optimal muscle length; body posture; diaphragmatic electromyogram; sonomicrometry
A CHANGE IN BODY POSTURE from recumbent to upright is associated with the removal of the weight of abdominal contents from the diaphragm. Consequently, the diaphragm resting length must be shorter in the upright position. Until recently, this postural change in diaphragm length could not be quantified and was only assumed from the observation of compensatory changes in activation of inspiratory and/or abdominal muscles. These parameters indicate that the diaphragm operational length is suboptimal in the upright posture. Indeed, two different strategies can potentially compensate for a reduced efficacy of the diaphragm in the upright posture. One strategy consists in increasing the neural drive to inspiratory muscles. The other strategy consists in an activation of abdominal muscles, either tonic or phasic during expiration, which maximizes the diaphragm end-expiratory length (2). When looked for, both strategies were observed. Thus an increased inspiratory drive was found in upright awake humans (12) and anesthetized animals (22, 26). An activation of the abdominal muscles was also found in upright awake humans (9, 18, 25) and anesthetized animals (11).

Recently, sonomicrometry allowed the first measurement of the actual length changes of the diaphragm in anesthetized dogs in various postures (22). In that study, in which activity of abdominal muscles was not measured, the diaphragm resting length decreased appreciably in the upright posture and reached a suboptimal level in terms of its length-tension relationship. Despite this disadvantage, the tidal shortening of the diaphragm was in part maintained through an increase in neural drive. Another study in anesthetized dogs showed that the expiratory contraction of abdominal muscles partially prevented the fall of diaphragm end-expiratory length in the upright posture (15).

In anesthetized animals, the reflex responsible for activation of abdominal muscles in the upright posture was postulated to originate from airways stretch receptors and to be mediated by the vagus (8). In the unanesthetized state, however, the vagal reflexes are less active, and it has been suggested that the activation of abdominal muscles may depend on other mechanisms, such as conscious reactions (8). Therefore the results obtained in anesthetized animals may not reflect the events during wakefulness. To assess the role of consciousness in these postural adjustments, we measured diaphragm length with sonomicrometry in awake dogs.
METHODS

Eight mongrel dogs weighing 20-30 kg were instrumented during halothane anesthesia. Through a midline laparotomy, two pairs of piezoelectric crystals were implanted between the muscle fibers of the left hemidiaphragm, on a flat portion of each of the costal and crural segments. In the costal segment, the crystals were positioned in the area of apposition. Each pair was implanted along the same fiber bundle, 15-25 mm apart. As described in a separate paper (14), the wires showed only limited adhesion to the diaphragm at necropsy after 3-4 wk. Fibrosis of ~1 mm was found around each crystal but did not extend to the muscle between the two crystals. Fine-wire electromyogram (EMG) electrodes were implanted in adjacent areas of each segment, close to the central tendon in the costal segment. A 5-cm-long latex balloon attached to a polyethylene catheter was placed in the midabdomen, close to the anterior wall. The abdomen was closed, and the wires and catheter was externalized through a subcutaneous tunnel. During the same procedure, a chronic tracheostomy was performed. The dogs were then allowed to recover from the anesthesia and the operation. After an initial period of rapid shallow breathing and diaphragmatic inhibition, the tidal shortening of the diaphragm increased progressively and reached a plateau at the 10th postoperative day. The recovery course of diaphragmatic function after implantation and the validation of the model have been described in another paper (14). The present study was performed on average on the 12th postoperative day. The dogs were studied in three body postures; right lateral decubitus (RLD), standing on four legs (STA), and sitting (SIT), as indicated by silhouettes in Fig. 1. In each run, time was allowed for the dogs to attain a stable position and breathing pattern before initiating the measurements.

The piezoelectric crystals were connected to a sonomicrometer (Triton Technology, San Diego, CA) via fine isolated wires. The sonomicrometer measures accurately and continuously the distance between the crystals of each pair on the basis of the transit time of ultrasonic waves propagating from one crystal to the other. The application of sonomicrometry to the measurement of diaphragm length has been described previously (21).

The EMG was recorded and amplified (TECA TE4, White Plains, NY), band-pass filtered between 100 and 600 Hz, rectified and integrated by a resistance-capacitance circuit with a 100-ms time constant. Inspiratory flow was measured with a pneumotachograph (Fleisch no. 3) connected to the endotracheal cannula.
Tidal volume was measured as the integration of flow over time (respiratory integrator, Hewlett-Packard, Waltham, MA). Abdominal pressure (Pab) was measured with the abdominal balloon, which was filled with 1 ml of air and connected to a pressure transducer (Validyne MP-45). All measurements were recorded on an eight-channel paper recorder.

![Diaphragm end-expiratory length measured in 3 postures and normalized as 100% for right lateral decubitus value. RLD, right lateral decubitus; STA, standing; SIT, sitting. Open bars, costal segment; hatched bars, crural segment. Values are means ± SE; n=8. *P<0.01.](image)

The muscle length at end-expiratory volume was termed LFRC. In each position, the value of LFRC was normalized using the RLD value as 100%. EMG activity was quantified using the peak height of the integrated signal and was normalized using the RLD value as 100%. On average, 25-30 breaths were analyzed in each posture. Reported values are means ± SD unless otherwise stated. Values of breathing frequency, tidal volume, end-expiratory length, tidal shortening, and EMG activity were analyzed in the three postures with a two-way analysis of variance (ANOVA) with repeated measurements on one factor, followed by comparisons among pairs of means using Tukey's test.

**RESULTS**

Breathing pattern. Breathing frequency was not significantly different in the three postures. Tidal volume was higher in STA (P <0.01) and in SIT (P <0.01) than in RLD (Table 1).
TABLE 1. Breathing pattern, diaphragm end-expiratory length, and tidal shortening

<table>
<thead>
<tr>
<th></th>
<th>Decubitus</th>
<th>Standing</th>
<th>Sitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT, liters</td>
<td>0.269±0.042</td>
<td>0.339±0.059</td>
<td>0.342±0.043</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>15.6±4.1</td>
<td>15.0±2.7</td>
<td>13.2±2.5</td>
</tr>
<tr>
<td>COS LFRC,%</td>
<td>100.0±0.0</td>
<td>95.4±3.5</td>
<td>88.5±6.7</td>
</tr>
<tr>
<td>CRU LFRC,%</td>
<td>100.0±0.0</td>
<td>98.2±5.0</td>
<td>85.6±10.1</td>
</tr>
<tr>
<td>COS TS,%LFRC</td>
<td>4.8±2.2</td>
<td>5.8±4.0</td>
<td>7.3±2.9</td>
</tr>
<tr>
<td>CRU TS,%LFRC</td>
<td>7.5±3.5</td>
<td>7.9±4.0</td>
<td>8.4±3.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8. f, respiratory frequency; VT, tidal volume; COS, costal; CRU, crural; LFRC, end-expiratory length; TS, tidal shortening.

Diaphragm end-expiratory length. In SIT compared with RLD, LFRC decreased by 11.5% in the costal segment (P <0.01) and by 14.4% in the crural segment (P <0.01). In STA compared with RLD, costal and crural LFRC decreased slightly, but not significantly. In SIT compared with STA, LFRC decreased significantly both in the costal (P <0.05) and in the crural (P <0.01) segment (Table 1, Fig. 1).

Fig. 2. Inspiratory swing of abdominal pressure measured in the 3 postures.

Diaphragm tidal shortening. Costal tidal shortening was 4.8%LFRC in RLD, 5.8%LFRC in SIT. Crural tidal shortening was 7.5%LFRC in RLD, 7.9%LFRC in
STA, and 8.4% LFRC in SIT. For either segment, none of the differences in tidal shortening was significant among the three postures (Table 1).

Diaphragm EMG. Electrical activity could be measured in six dogs in the costal segment and in five dogs in the crural segment, but the signal had been lost during the postimplantation period in the remaining dogs. With body position changes from RLD, EMG activity changed in different directions in the different dogs. On the average, costal diaphragm EMG activity decreased by 9.6 ± 14.1% in STA and increased by 39.8 ± 64.2% in SIT, neither change being significant. Crural EMG activity decreased by 25.5 ± 25.2% in STA and increased by 29.8 ± 43.5% in SIT, again neither change being significant.

Abdominal pressure. Pab could be measured in five dogs, but the signal had been lost during the postimplantation period in the other three dogs. The inspiratory swing in Pab (ΔPab) was +1.1 ± 1.3 cmH2O in RLD, -1.2 ± 0.7 cmH2O in STA, and -3.4 ± 2.3 cmH2O in SIT. In each animal, inspiratory ΔPab went from positive in RLD to negative in STA, and to more negative in SIT. Figure 2 shows the individual
inspiratory ∆Pab in the three postures. In all cases in STA and SIT, Pab increased gradually during expiration and fell during inspiration, as indicated in Fig. 3 for one dog. This positive expiratory swing in Pab was variable among dogs. Figure 4 represents the relationship between the LFRC and the expiratory swing in Pab. It can be seen that the dogs behaved differently, and the fall in LFRC from lateral decubitus to sitting was inversely related to the positive Pab generated during expiration.

![Diagram](https://via.placeholder.com/150)

Fig. 4. Relationship between diaphragm end-expiratory length in sitting position (expressed in % of value in RLD) and abdominal pressure swing during expiration.

**DISCUSSION**

LFRC. Although never proved experimentally, it has been accepted that the diaphragm resting length must be shorter in the upright than in the recumbent posture. This assumption was confirmed recently by using sonomicrometry to measure the diaphragm length of anesthetized dogs in different postures (15, 22). In the present study, similar measurements were performed in awake dogs, which assumed natural postures likely to affect diaphragm length. We found that diaphragm end-expiratory length did not change significantly from right lateral decubitus to standing, but fell significantly in sitting, by 11.5% for the costal and by 14.4% for the crural segment. These length changes are less than those measured under anesthesia. In anesthetized dogs with corneal reflexes suppressed, Newman
et al. (22) observed that LFRC fell by 20% for the costal and by 23% for the crural segment from supine to upright. In anesthetized dogs with corneal reflexes maintained, Farkas et al. (15) observed that a similar postural change was associated with a fall of 10.2% in costal LFRC of 21% in crural LFRC. In the latter study, the fall in diaphragm length was partially prevented by a phasic contraction of abdominal muscles during expiration. Indeed, when this reflex activation was abolished by vagotomy, a further decrease in length was observed (15).

We have indirect evidence that abdominal muscles were contracting phasically when our awake dogs assumed the standing and the sitting postures. In view of the multiple abdominal muscles and of their uneven degree of activation (17), we did not record their EMG activity. Instead, we used Pab as an index of the global mechanical output of the abdominal muscles and of the diaphragm. The pattern of Pab differed consistently according to the posture. In lateral decubitus position, the inspiratory swing in Pab was always positive, reflecting diaphragmatic contraction. In contrast, it was always negative in standing and sitting. This negative swing was not due to a paradoxical movement of the diaphragm since tidal shortening of both segments did not vary with postures. Inspection of the tracings indicates that in standing and sitting Pab increased gradually during expiration, and fell relatively abruptly in inspiration (Fig. 3). This pattern of pressure development can only be explained by a phasic contraction of expiratory muscles, followed by relaxation at the onset of inspiration. It is likely that the abdominal muscles play the major role in this process, since they alone can oppose the gravitational force exerted by abdominal viscera in the sitting and in the standing dog. This interpretation is supported by the recent observation of De Troyer et al. (10) that the transversus abdominis is consistently and strongly activated during expiration in sitting and standing awake dogs. It is possible that the rib cage expiratory muscles assist the abdominal muscles in building up Pab during expiration. As indicated by Fig. 4, the postural adjustment of diaphragm end-expiratory length was conditioned by the amplitude of the positive expiratory swing in Pab. Thus some dogs defended their diaphragm LFRC in the sitting position by contracting abdominal muscles in expiration, whereas others let their LFRC fall to a certain degree. This relationship represents direct experimental evidence of the "operational length restoring response" described by Mead (20). According to Fig. 4, the crural segment might be more affected than the costal by the contraction of abdominal muscles. This was predicted by Road et al. (24) on the basis of different
passive length force curves, the crural segment being lengthened more than the costal segment for a given increment in Pab.

The two segments of the diaphragm also have different active length-force relationships [24]. From two previous studies in anesthetized dogs [22, 24], it can be estimated that in right lateral decubitus (nondependent diaphragm) costal LFRC is 101% of optimal length (Lo) and crural LFRC 90% Lo. However, we have shown in another study that anesthesia selectively increases crural LFRC by 7-8% (16). These data and the results of the present study suggest that the crural segment is more severely affected by the sitting position in the awake dog.

Tidal shortening. If the neural drive directed to and the load opposed to the diaphragm do not change when its operational length becomes suboptimal, then tidal shortening is bound to decrease. This was illustrated indirectly by the study of Danon et al. [7], who showed that tidal volume fell by half when C1 quadriplegics with constant phrenic pacing were tilted from supine to 55°. In upright anesthetized dogs, tidal shortening of both segments of the diaphragm decreased when compared with recumbent values [22]. However, the fall in tidal shortening was moderate and probably less than could be expected from the length-force relationship, since neural drive to both segments increased markedly. Indeed, for a given transdiaphragmatic pressure, diaphragmatic EMG increased by 220%, which is similar to the values found by Druz and Sharp in humans [12]. This increase in inspiratory neural drive corresponds to the "operational length compensating response" described by Mead [20].

In the present study in awake dogs, tidal shortening of the diaphragm was better maintained in face of a suboptimal length, since it did not change significantly and even showed a trend to increase between lateral decubitus and sitting. An increase in EMG activity of the diaphragm was obviously not the main compensatory mechanism, since it occurred only in some dogs and was not significant for the group. Conversely, in each case Pab increased phasically during expiration as a result of abdominal muscle contraction. In so doing, the abdominal muscles not only restore partially LFRC but also decrease the load faced by the diaphragm. At the onset of inspiration, Pab decreases abruptly and thereby assists diaphragmatic contraction. With the help of this mechanism, the diaphragm could even shorten without neural activation during inspiration. This operational length restoring response was present in all cases but was of uneven amplitude. It was
when the restoring response was the least important that the largest compensating response, i.e., increase in diaphragmatic EMG activity, was observed. This is illustrated in Fig. 5, which shows two extreme patterns of response. In these two dogs, the events occurring from standing to sitting are presented, since tidal volume changed <4% between these two positions. One dogs was a "nondefender", in that he did not recruit much the abdominal muscles and let his diaphragm LFRC fall. He showed an unusually large increase in daphragm EMG. The other was a "defender" and recruited more his abdominal muscles. As a consequence, his diaphragm LFRC was maintained, and no increase in diaphragm EMG was observed in the sitting position.

Compensatory mechanisms. Diaphragm length compensatory mechanisms have been studied in different conditions. In awake humans during resting breathing, a body postural change from recumbent to upright is associated with both an increase in neural drive to inspiratory muscles (12, 13) and an activation, tonic or phasic, of abdominal muscles (9, 12, 25). During CO2 rebreathing runs, Grassino et al. (18) observed that the end-expiratory anteroposterior diameter of the abdomen was stable in supine subjects but decreased to a variable extent in sitting subjects as a result of the expiratory contraction of abdominal muscles. In these subjects, they found as well that the first 0.1 s of inspiration, after the release of abdominal tone, was characterized by a negative Pab swing and the absence of
diaphragmatic EMG activity. Their findings in sitting humans during CO₂ rebreathing are compatible with ours in sitting animals during quiet breathing. However, the associated changes in diaphragm length were qualitatively evaluated in the former and measured in the present study.

Postural changes have been simulated by pressure breathing, which can as well shorten diaphragm operational length. In awake humans breathing against a positive mouth pressure, Green et al. (19) reported that either end-expiratory lung volume increased and was then associated with an increase in diaphragmatic EMG, or end-expiratory volume did not change as a result of contraction of expiratory muscles, in which case no change in diaphragmatic EMG was observed. Banzett et al. (1) applied a negative pressure to awake quadriplegic patients in a cuirass and observed a 0.5-liter increase in FRC and an increase in diaphragmatic EMG. Positive-pressure breathing gave variable results in anesthetized animals. It was shown to increase diaphragmatic EMG in rabbits (5) but to inhibit the diaphragm and to activate phasically expiratory muscles in cats (3, 4). Awake monkeys submitted to positive pressure breathing showed most often an expiratory strategy with activation of abdominal muscles and no change in end-expiratory abdominal diameter or diaphragmatic EMG (6).

Finally, postural changes have also been simulated by immersion. In awake sitting humans, decreasing the water level from shoulders to hips elicits similar thoracoabdominal changes as a postural change from recumbent to upright. This maneuver is associated with an increase in neural drive, mainly to inspiratory muscles but also to expiratory muscles (23).

Thus both the inspiratory and the expiratory strategy have been observed as compensatory mechanisms, depending on the species and state of wakefulness. Until this study, no species had been studied both in the anesthetized and in the awake state. In dogs with moderately deep anesthesia, the upright posture elicits an important increase in diaphragmatic EMG, with no evidence of restoring response from the abdominal muscles (22). In lightly anesthetized dogs, the upright posture elicits a clear activation of abdominal muscles during expiration (15). Finally, in awake sitting dogs, the most consistent compensatory mechanism is a phasic expiratory contraction of abdominal muscles, with no significant increase in diaphragmatic EMG activity. Therefore it appears likely that the state of
wakefulness plays a role in the choice of a compensatory mechanism for a disadvantageous operational length of the diaphragm.

In summary, we have measured diaphragm length in awake dogs in three natural postures and have shown that: 1) end-expiratory length is maintained in standing but is decreased in sitting; 2) tidal shortening is maintained in all postures; 3) the main operational length compensatory mechanism is a phasic expiratory contraction of abdominal muscles; and 4) the degree of length compensation varies among animals.

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EFFECT OF ANESTHESIA ON CANINE DIAPHRAGM LENGTH

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General anesthesia has been shown to induce a cephalad shift of the end-expiratory position of the diaphragm in recumbent human subjects. The authors used the technique of sonomicrometry in chronically instrumented dogs to measure the length changes occurring in the costal and crural diaphragm during anesthesia. Seven dogs were studied in lateral decubitus, first awake and then during pentobarbital anesthesia. The end-expiratory length (LFRC) of the crural segment increased gradually and reached a plateau after 30 min of anesthesia. Costal LFRC did not change. The results were similar when the hemidiaphragm under study was placed in a gravity dependent or in a nondependent position. In the awake state, variable levels of post-inspiratory or tonic diaphragmatic EMG activity were observed, which disappeared during anesthesia. The authors conclude that anesthesia induces a 7-8% increase in end-expiratory length of the crural but not of the costal diaphragm. This selective adjustment is not due to a pressure gradient effect, but is compatible with a loss of tone in the crural diaphragm. (Key words: Lung; Diaphragm length; FRC. Measurement techniques: EMG; sonomicrometry. Respiratory muscle tone.)

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A REDUCTION IN functional residual capacity (FRC) has been repeatedly observed in human subjects during general anesthesia.\textsuperscript{1-5} Although the exact mechanism underlying this change in lung volume is still unknown, it has been proposed that the initial effect of anesthesia is to decrease the outward recoil forces of the chest wall.\textsuperscript{5} Such an alteration of the pressure-volume characteristics of the chest wall could be explained by a loss of muscle tone, as suggested by the fact that FRC does not decrease further when paralysis is added to anesthesia.\textsuperscript{2,5} In fact, both a reduction in internal rib cage dimensions\textsuperscript{1} and a cephalad shift of the diaphragm\textsuperscript{3,8} have been observed with the induction of anesthesia.

Using fluoroscopy, Froese and Bryan have demonstrated that general anesthesia induces a cephalad shift of the end-expiratory position of the diaphragm.\textsuperscript{6} This displacement was predominant in the dependent parts of the muscle. However, this technique provides only a lateral view of the diaphragm silhouette and does not quantitate the change in muscle length. Furthermore, it does not dissociate the contribution of the two segments of the diaphragm, costal and crural, which are distinct muscles and may therefore react differently to anesthesia.\textsuperscript{7}

The length of respiratory muscles can be measured accurately with the method of sonomicrometry.\textsuperscript{8} Using this technique in chronically instrumented dogs, we measured the length of costal and crural diaphragm in the awake state and during anesthesia.

Methods

MODEL

Seven mongrel dogs weighing 20-30 kg were instrumented during halothane anesthesia. Through a midline laparotomy, two pairs of piezoelectric crystals were implanted between the muscle fibres of the left hemidiaphragm, one pair in the costal segment and one pair in the crural. Each pair was implanted along the same fibre bundle, 15-25 mm apart. Fine wire electromyogram (EMG) electrodes were implanted in adjacent areas of each segment. A 5 cm long latex balloon attached to a polyethylene catheter was placed in the mid-abdomen, close to the anterior wall. The abdomen was closed and the wires and catheter were externalized through a subcutaneous tunnel. During the same procedure, a chronic tracheostomy was performed. The dogs were then allowed to recover from the anesthesia and the operation. After an initial period of rapid shallow breathing and diaphragmatic
inhibition, the contractility of the diaphragm increased progressively and reached a plateau at the 10th postoperative day. The recovery course of diaphragmatic function after implantation and the validation of the model have been described previously.9 The present study was performed, on average, on the 12th postoperative day.

TECHNIQUES

The piezoelectric crystals were connected to a sonomicrometer (Triton Technology, San Diego, CA) via fine isolated wires. The sonomicrometer measures accurately and continuously the distance between the crystals of each pair on the basis of the transit time of ultrasonic waves propagating from one crystal to the other. The application of sonomicrometry to the measurement of diaphragm length has been described previously.8

The EMG was recorded and amplified (TECA TE4, White Plains, NY), band-pass filtered between 100 and 600 Hz, rectified and integrated by an RC circuit with a 100 ms time constant. Inspiratory flow was measured with a pneumotachograph (Fleisch no. 3) connected to the endotracheal canula. Tidal volume was measured as the integration of flow over time (Respiratory Integrator, Hewlett-Packard, Waltham, MA). During the measurements, the abdominal balloon was filled with 1.0 ml of air. All measurements were recorded on an eight-channel paper recorder. In some dogs, EMG and flow signals were recorded on a tape recorder.

Fig. 1: Schematic transverse section of the animal. Left panel: the dog is in right lateral decubitus, with the left hemidiaphragm in a nondependent position. Right panel: the dog is in left lateral decubitus, with the left hemidiaphragm in a dependent position. The dots indicate the location of the crystals in each segment.
Awake recordings were performed with the dogs in the right lateral decubitus (RLD) posture. Before starting the recording, time was allowed for each dog to reach a breathing pattern which was regular and similar to that recorded on the previous days. After the awake recording had been completed, the dogs were anesthetized with an intravenous injection of pentobarbital sodium (20 mg·kg⁻¹). Care was taken to keep the anesthetized dogs in exactly the same position as they had been in when awake. The measurements were recorded continuously during the first 30 minutes of anesthesia.

In three dogs, the recordings in the awake state were made first with the dogs in the left lateral decubitus (LLD) position, and then in the RLD position. The anesthesia was then induced in the same RLD position. After 30 minutes of anesthesia, the measurements were made with the dogs again in the LLD position, within 5 min after the position change.

In three dogs, the anesthesia protocol was performed twice. On the first occasion, the dogs were studied in the RLD position, first awake and then anesthetized. On the second occasion, they were studied in the LLD posture, first awake and then anesthetized.

The crystals being implanted in the left hemidiaphragm, the non-dependent diaphragm was studied in the RLD position, and the dependent diaphragm in the LLD posture (fig. 1).

ANALYSIS OF RESULTS
The distance between the crystals at end expiration was termed LFRC. This intertransducer distance reflects the change in length of the whole fibre, since the magnitude of contraction has been shown to be uniform along a fibre. In each position, the value of LFRC was normalized using the awake state as 100%. Measurements were made on 20 breaths in the awake state and on 10 breaths every 5 min during anesthesia. Reported values are means ± SD. In the RLD position (n=7), the difference in LFRC of each segment of the diaphragm between the awake state and after 30 min of anesthesia was analyzed with the Wilcoxon Signed-Rank test for two groups arranged as paried observations.
Results

**RLD POSTURE (n=7)**

Breathing frequency was 15.0 ± 3.1 breaths per minute in the awake state, and 10.1 ± 3.9 breaths per min after 30 min of anesthesia. Tidal volume was 0.255 ± 0.060 l in the awake state, and 0.316 ± 0.105 l after 30 min of anesthesia.

Costal diaphragm LFRC did not change significantly with anesthesia, increasing by 0.8 ± 1.8% after 30 min. Crural diaphragm LFRC increased gradually during anesthesia, approaching a plateau after 30 min (fig. 2). At this time, crural LFRC had increased by 7.6 ± 3.4%, which represents a significant change from the awake state (P<0.01).

![Diagram of DIAPHRAGM RESTING LENGTH (LFRC)](image)

**EFFECT OF POSITION ON DIAPHRAGMATIC FIBER LENGTH**

*In the same anesthesia (n=3):* In the awake state with dogs in the LLD position, costal LFRC was 3.7 ± 1.0% longer and crural LFRC 1.7 ± 0.3% longer than in the RLD position.

With the awake LFRC normalized as 100% in each position, comparisons were made between the LFRC in the awake state and after 30 min of anesthesia. Costal LFRC decreased by 0.3 ± 0.9% in RLD, and by 2.1 ± 1.6% in LLD. Crural LFRC increased by 6.8 ± 3.8% in RLD, and by 6.7 ± 4.3% in LLD (fig. 3). Thus,
Costal and crural diaphragm: Results
Anesthesia

crural LFRC increased and costal LFRC did not in the two positions.

![Graph showing end-expiratory length (LFRC) of costal and crural diaphragm in awake state and after 30 min of anesthesia.](attachment:graph.png)

Fig. 3: End-expiratory length (LFRC) of costal and crural diaphragm in the awake state and after 30 min of anesthesia. Comparison between the nondependent side (right lateral decubitus) and the dependent side (left lateral decubitus) during the same anesthesia. Values are means ± SE.

In separate anesthesias (n=3). Comparisons were made between the LFRC in the awake state and during anesthesia with the dogs in both LLD and RLD positions. After 30 min, costal LFRC had decreased by 0.3 ± 0.9% in RLD, and by 0.6 ± 1.2% in LLD. Crural LFRC increased gradually in both positions to reach a plateau after 30 min. At that time, the increase was 6.8 ± 3.8% in RLD, and 9.9 ± 7.7% in LLD (Fig. 4). Thus again, crural LFRC increased and costal LFRC did not in the two positions.

![Graph showing changes in LFRC over time in awake and anesthetized conditions.](attachment:time_graph.png)

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Fig. 4: End-expiratory length (LFRC) of costal and crural diaphragm in the awake state and during anesthesia. Comparison between the nondependent side (right lateral decubitus) and the dependent side (left lateral decubitus) during separate anesthesias. Values are means ± SE.

ELECTROMYOGRAPHY

The EMG could be measured in the costal and crural segments in three dogs, but only in one of the segments in four dogs. Therefore, a quantitative comparison of the costal and crural EMG patterns was not undertaken. In the awake state, it was found in every animal that the electrical activity of the diaphragm persisted during part of expiration, as measured from flow. As can be seen in figure 5, the EMG activity increased during inspiration and then decreased very gradually over most of the expiratory time. In some breaths, this post-inspiratory activity persisted even until the onset of the next inspiration. In contrast, in every animal, the EMG activity ceased abruptly at the end of inspiration during anesthesia. This change usually occurred within the first breaths following induction of anesthesia.
ABDOMINAL PRESSURE

Abdominal pressure could be measured successfully in four dogs. From the awake state to anesthesia in the same position, the end-expiratory abdominal pressure decreased minimally in two dogs and did not change in the other two dogs. The mean change was $-0.3 \pm 0.4$ cm H$_2$O.

Fig. 5: EMG recording of the crural diaphragm in one dog. From top to bottom: integrated EMG, raw EMG, inspiratory flow. A: Awake; B: Anesthetized.

Discussion

A cephalad displacement of the end-expiratory position of the diaphragm has been previously documented during anesthesia in human subjects, both by fluoroscopy$^6$ and by computerized tomography$^3$. The present study provides, for the first time, a direct measurement of the length changes occurring in the
The results show a clear difference between the two segments of the diaphragm during anesthesia, the crural segment increasing its end-expiratory length up to +7.6% after 30 min, whereas the costal length did not change. To interpret our data, we must take into account the observation of Froese and Bryan⁶ that the cephalad shift of the diaphragm occurring with anesthesia or with paralysis predominates in the dependent parts of the muscle. The authors explained this effect by the difference in gradients of hydrostatic pressure existing across the diaphragm in recumbent positions. In the awake state, pressure increases by 0.2 cm H₂O per cm of height on the thoracic side, and by 1.0 cm H₂O per cm of height on the abdominal side. Therefore, in horizontal positions, higher transdiaphragmatic pressures (Pdi) must exist across the dependent parts than across the non-dependent parts of the diaphragm. This vertical gradient of Pdi could explain that the cephalad displacement predominates in the dependent parts when all activity is suppressed in the diaphragm.

In our study, the dogs were lying in the RLD position, i.e., in a situation where the left costal segment was in the least dependent position and the crural segment in a more dependent position (fig. 1, left schema). Therefore, the possibility existed that the selective lengthening of the crural segment observed during anesthesia was due to a vertical pressure gradient across the diaphragm. To distinguish between a postural effect and a segmental effect, we studied three dogs in both lateral postures. In the LLD posture, the left costal segment is in the most dependent position and the crural is in a relatively less dependent position (fig. 1, right schema). If the adjustments in the segmental end-expiratory lengths were due to a pressure gradient effect, the pattern should be inverted in LLD, i.e. costal LFRC should increase more than crural LFRC during anesthesia. This was obviously not the case, the crural segment always lengthening, and the costal not lengthening, regardless of the position. This result was found whether the two positions were studied during the same anesthesia or each posture was studied during a separate anesthesia. We conclude therefore that the difference in length change between costal and crural diaphragm during anesthesia is not due to a postural effect, but to distinct segmental properties. These results do not contradict those of Froese and Bryan.⁶ Indeed, fluoroscopy revealed a predominant shift of the diaphragm silhouette in the dependent areas but does not allow interpretation of the contribution of each segment to this movement.

The cephalad shift of the diaphragm occurring with anesthesia has been attributed to a loss of tone in this muscle.⁶ The presence of tone in the diaphragm is
debated because it is impossible to distinguish it with certainty from a noisy signal. Muller et al. addressed this problem by studying step changes in baseline activity between the awake state and REM sleep or anesthesia. They observed consistently more baseline activity in the awake state than in the other two conditions, which suggested the presence of tone rather than noise. Our findings are similar to those of Muller et al., in that we observed post-inspiratory activity and, sometimes, tonic activity in the awake state, which disappeared during anesthesia. Moreover, our EMG signals were recorded via implanted, rather than surface, electrodes, and were thereby free of interference from other muscles. If the selective lengthening of the crural segment was due to this mechanism, the loss of tone should be more important in this segment than in the costal segment. However, some reasons support the hypothesis that tonic activity may be more important in the crural than in the costal segment. When muscle spindles have been described in the diaphragm, they were found in the crural segment, either exclusively or predominantly. The spindles play an important role in regulating tonic activity by their facilitating effect on alpha-motoneurons, and their activity varies in proportion with the level of cortical activity.

It can be argued that our results might be due to the light level of anesthesia, rather than to anesthesia per se. A phasic expiratory activation of abdominal muscles has been documented in a majority of subjects during light anesthesia, decreasing with deepening anesthesia. Therefore, it is theoretically possible that the increase in end-expiratory diaphragm length might be due to an increase in abdominal pressure secondary to abdominal muscle contraction, and not to a loss of tone in the diaphragm itself. However, this mechanism was ruled out in humans by Froese and Bryan, since they measured a similar cephalad displacement of the diaphragm during anesthesia and during muscle paralysis. In the absence of EMG recording of abdominal muscles, we cannot comment on their activation, but we think, nevertheless, that this mechanism did not account for the length changes that we observed. Firstly, there is no apparent reason for an abdominal muscle contraction to affect the length of only the crural part of the diaphragm, especially since the costal part represents about five-sixths of the diaphragm area. Secondly, according to the in vivo passive pressure-length characteristics of the diaphragm measured by sono-micrometry, the baseline abdominal pressure should increase by approximately 7 cmH₂O to produce the crural lengthening that we observed. On the contrary, the baseline abdominal pressure fell slightly or did not change in the four dogs in which it could be measured.

It should be mentioned that, in contrast to humans, dogs do not manifest, as
a rule, a fall in FRC with anesthesia. During halothane anesthesia, FRC has been shown to decrease in supine,\textsuperscript{18} but not in prone, dogs \textsuperscript{19}. During thiopental anesthesia, FRC did not change significantly in dogs in prone, supine, and lateral positions.\textsuperscript{20} FRC was not measured in this study, but we attempted to indirectly evaluate the volume change induced by the diaphragmatic lengthening. Taking the actual length of each segment and the area of the diaphragm dome measured in dogs of similar weight,\textsuperscript{8} assuming a piston-like displacement of the diaphragm and considering the relative areas of each segment, and finally assuming an FRC of 1 litre,\textsuperscript{20} we calculated that the length changes that we measured would produce a 5-6\% fall in FRC. The interspecies difference in FRC decrement during anesthesia, which is still not explained, raises the possibility that the increase in diaphragm LFRC may be greater in humans than in dogs. However, the decrease in FRC observed in humans is not only due to diaphragmatic displacement, but also to changes in rib cage dimensions and intrathoracic fluid volume.\textsuperscript{21}

Finally, the time course of crural lengthening is noteworthy. Most of the length change occurred within the first minutes of anesthesia, but an additional elongation appeared slowly over the next 20 min. In man, FRC decreases within minutes after induction of anesthesia, and remains unaltered over the next half-hour. It is unknown if the slow elongation that we measured in dogs occurs in humans. If it does, the lack of further change in FRC could be due to the small size of this additional lengthening, or to compensatory volume changes from other thoracic structures.

In summary, we demonstrated that in dogs anesthesia induces a 7-8\% increase in end-expiratory length of the crural diaphragm with no change in the costal diaphragm. This difference seems to be due to segmental properties and not to a pressure gradient effect. In partial data, we observed prolonged post-inspiratory EMG activity and, in some cases, tonic activity of the diaphragm in the awake state, which disappeared during anesthesia. It remains to be established if the selective lengthening of the crural diaphragm is secondary to a preferential loss of tone in this segment.

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Velocity of shortening of inspiratory muscles and inspiratory flow

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FITTING, JEAN-WILLIAM, PAUL A. EASTON, AND ALEJANDRO E. GRASSINO. Velocity of shortening of inspiratory muscles and inspiratory flow. J. Appl. Physiol. 60(2):670-677, 1986. Respiratory muscle length was measured with sonomicrometry to determine the relation between inspiratory flow and velocity of shortening of the external intercostal and diaphragm. Electromyographic (EMG) activity and tidal shortening of the costal and crural segments of the diaphragm and of the external intercostal were recorded during hyperoxic CO$_2$ rebreathing in 12 anesthetized dogs. We observed a linear increase of EMG activity and peak tidal shortening of costal and crural diaphragm with alveolar CO$_2$. For the external intercostal, no consistent pattern was found either in EMG activity or in tidal shortening. Mean inspiratory flow was linearly related to mean velocity of shortening of costal and crural diaphragm, with no difference between the two segments. Considerable shortening occurred in costal and crural diaphragm during inspiratory efforts against occlusion. We conclude that the relation between mean inspiratory flow and mean velocity of shortening of costal and crural diaphragm is linear and can be altered by an inspiratory load. There does not appear to be a relationship between inspiratory flow and velocity of shortening of external intercostals.

diaphragm; diaphragm length; external intercostal muscle; flow-velocity; hypercapnia; airway occlusion; sonomicrometry; canine.
INTUITION SUGGESTS that the inspiratory muscles must shorten at a rate proportional to inspiratory flow (1,13). However, this assumption has hitherto not been confirmed by experimental evidence. The relationship between flow and velocity is likely to depend on several factors, including the length of the different respiratory muscles at various lung volumes, the recruitment of these muscles for achieving various flow rates, and the relative agonist or fixator function of each muscle. Indeed, in a previous investigation in which diaphragmatic length was measured with sonomicrometry (16), it was found that there was not a unique relation between instantaneous flow and instantaneous velocity within a single breath. However, this observation did not consider the validity of the assumption for mean values of inspiratory flow and velocity of shortening. Therefore, we studied the relationship between mean inspiratory flow ($V_{T}/T_{i}$) and mean velocity of shortening of three inspiratory muscles, the costal and crural segments of the diaphragm and the external intercostal, over a wide range of ventilation.

METHODS

Twelve mongrel dogs, weighing 16 - 30 kg, were studied in the supine posture during anesthesia. After induction with thiopental sodium (15 mg/kg), constant anesthesia was initiated with a $\alpha$-chloralose (50 mg/kg) and urethane (250 mg/kg) mixture. During the experiment, small additional boluses of chloralose and urethane were given to maintain a constant, light level of anesthesia with brisk corneal reflexes. A femoral arterial catheter was inserted to monitor blood pressure, which remained within 10% of baseline values in all animals. Auffed endotracheal tube was inserted transorally during induction. The animals breathed spontaneously during all measurements.

The left diaphragm was exposed through a midline abdominal incision. Two pairs of piezoelectric crystals (Triton Technology, San Diego, CA) were attached to the diaphragm with cyanoacrylate glue, as previously described (16). The crystals were placed on relatively flat portions of costal and crural segments; the distance between the two opposing crystals of each pair was 15 - 20 mm. Two bipolar hook electrodes were inserted, one each in
crural and costal segments. Crystals and electrodes were always placed on
the left diaphragm. After implantation, the abdominal incision was closed.

The external intercostal muscle was exposed on the midaxillary line
through a lateral chest wall incision at the level of the sixth intercostal space,
and one pair of piezoelectric crystals was glued along the axis of the fibres.
The crystals were 10 - 15 mm apart and embedded in Vaseline to ensure a
constant medium for the propagating ultrasonic waves. A bipolar hook
electrode was then inserted in the same muscle, and the incision was closed.
All crystals were inspected and recovered at autopsy. The distance between
the crystals and hence the muscle length changes was determined by the
sonomicrometer (Triton Technology) on the basis of the transit time of
ultrasonic waves propagating from the first or transmitter crystal, to the
second or receiver crystal (16). Signals from the electromyogram (EMG) hook
electrodes were amplified with a TECA MkIII amplifier (White Plains, NY)
and band-pass filtered (16 Hz-1.6 kHz for the diaphragm, 16 Hz-3.2 kHz for
the intercostal). The signals were then rectified and integrated with an RC
leaky integrator with a 0.1-s time constant.

The endotracheal tube was attached via a unidirectional valve (Hans
Rudolph model 2500) to a low-resistance breathing circuit, with the
inspiratory limb connected to a pneumotachygraph (Fleisch no. 2), and both
inspiratory and expiratory limbs were attached to a small 4- to 5-litres
rebreathing bag. Hypercapnia was elicited by a modification of the Read
technique (18), with the animal rebreathing 6% CO2 in O2 from a 4- to 5-l
bag. In 11 dogs a pneumatic device for intermittent occlusion was attached
to the inspiratory side of the valve which was occluded at ≈30-s intervals.
CO2 was sampled continuously from the expiratory limb, and fractional
concentration of expired CO2 (FECO2) measured by infrared CO2 analyser
( Beckman model LB-2). Tidal volume was obtained by integration of flow
(Respiratory integrator, Hewlett Packard, Waltham, MA).

The muscle length at end-expiratory volume was termed LFRC and
muscle shortening expressed in %LFRC. The velocity of shortening was
expressed as %LFRC per second (%LFRC/s). The mean velocity of shortening
was calculated as the maximal shortening divided by the time elapsed
between the point of initial departure from LFRC and the point of maximal
shortening. EMG activity was quantified using the peak height of the
integrated signal. The peak EMG of the first unoccluded breath of each run
was considered as 100% and the peak integrated EMG of all subsequent
breaths was expressed relative to this base line. An average of 20 breaths was analyzed for each rebreathing run. Linear regressions were calculated by the method of least squares. The linearity of the relationships was estimated by visual examination of the individual plots and calculation of \( r \) for each slope; the average and smallest individual values of \( r \) are reported. Values of EMG activity, shortening, and velocity for costal and crural diaphragm were compared using Student's paired \( t \) test.

RESULTS

VENTILATION. During \( \text{CO}_2 \) rebreathing, mean \( \pm \) SD end-tidal \( \text{PCO}_2 \) (\( \text{PETCO}_2 \)) increased from 44.4 \( \pm \) 5.0 to 85.2 \( \pm \) 12.0 Torr and ventilation (\( V_I \)) \( \pm \) SD rose from 4.8 \( \pm \) 2.3 to 20.8 \( \pm \) 7.4 l/min. The ventilatory response to \( \text{CO}_2 \) (\( \Delta V_I/\Delta P_A\text{CO}_2 \)) was linear (\( r=0.96 \)), with a mean value \( \pm \) SD of 0.40 \( \pm \) 0.19 l/min/mmHg. Average values of tidal volume and respiratory frequency are plotted in Fig. 1. In this figure, it can be seen that in these animals the initial ventilatory response was achieved mainly by an increase in tidal volume, with a subsequent common increase in both tidal volume and frequency.

FIG. 1. Relationship between tidal volume and breathing frequency during progressive hypercapnia at 4 levels of alveolar \( \text{CO}_2 \) partial pressure: 45, 55, 65, and 75 Torr. Values are means \( \pm \) SE; \( n = 12 \).
**Diaphragm.** The role of the diaphragm in ventilatory output in response to CO₂ stimulation is dissociated into four diagrams in Fig. 2. The points marked in Fig. 2, as in Figs 3 and 4, correspond to four levels of pCO₂: 45, 55, 65, and 75 Torr. This four-quadrant figure will be considered in sequence, moving counterclockwise from the upper right quadrant.

**FIG. 2.** Diaphragmatic determinants of ventilatory output in response to CO₂ stimulation. Values are mean ± SE; n = 12. EMG, electromyogram; CRU, crural; COS, costal; PA_C0₂, alveolar CO₂ partial pressure; VT, tidal volume; L_FRC, muscle length at end-expiratory volume.

The changes induced by CO₂ in peak EMG of the costal and the crural diaphragm are shown in the right upper quadrant. In every animal, EMG activity increased linearly with CO₂ stimulation. Furthermore, the increment was significantly greater in crural than in costal diaphragm (Table 1).
FIG. 3. Relationship between tidal shortening and 1) mean velocity of shortening (upper panel) and 2) peak velocity of shortening (lower panel) of costal (COS) and crural (CRU) diaphragm. Values are means ± SE; n = 12. LFRC, muscle length at end-expiratory volume.

The upper left quadrant shows that tidal shortening increased linearly with EMG activity, with no difference between the costal and the crural segment (Table 1). The mean value ± SD for tidal shortening was 5.1 ± 2.9 %LFRC for costal and 8.4 ± 6.3 %LFRC for crural at the beginning of the rebreathing, increasing to 21.7 ± 11.5 %LFRC and 29.9 ± 11.6 %LFRC at the end. During the runs, LFRC decreased by 2.7 ± 2.1% (Mean ± SD) for costal and by 5.2 ± 4.3% for crural. However, the difference of initial LFRC among two successive runs was not significant (P > 0.05), paired t test): -1.5 ± 2.2% (mean ± SD) for costal and -1.2 ± 3.8% for crural. As might be expected from the previous EMG-CO2 and tidal shortening-EMG relations, tidal
shortening increased linearly with $P_{A\text{CO}_2}$ and the slope was significantly steeper for crural than for costal segments (Table 1). It should be noted that the lower coefficients of correlation pertaining to the costal segment were lower only because this segment did not increase shortening during CO$_2$ rebreathing in a single dog, in which this segment did not increase shortening during CO$_2$ rebreathing. For the relationships affected by this deviant result the second smallest individual value of $r$ is reported in Table 1.

The lower left quadrant of Fig. 2 shows the correlation of tidal shortening and tidal volume. Shortening of both costal and crural increased linearly with tidal volume, with no significant difference between the segments (Table 1).

Finally, the lower right quadrant illustrates the usual result of a ventilatory response to a chemical stimulus, showing the correlation between tidal volume and $P_{A\text{CO}_2}$.

The relations between tidal shortening of the diaphragm and peak and mean velocity of shortening are shown in Fig. 3. In general, both peak and mean velocities increased linearly with shortening.

![Diagram](image)

**FIG. 4.** Relationship between mean inspiratory flow ($V_{I}T_{i}$) and mean velocity of shortening of costal (COS) and crural (CRU) diaphragm. Values are mean± SE; $n = 12$. $L_{FRC}$, muscle length at end-expiratory volume.

The relationship between mean velocity of shortening of costal and crural diaphragm and mean inspiratory flow ($V_{I}T_{i}$) is presented in Fig. 4. There was a linear relation between these variables in each dog, without differences in slopes between costal and crural segments (Table 1). However, there was a wide range of slopes so that although mean velocity of shortening of costal and crural diaphragm always increased linearly with mean inspiratory flow, the increment varied from dog to dog.
The breaths which occurred during total airway occlusion were analysed and compared to the preceding free breaths; Table 2 presents the ratios of EMG, shortening and velocity of shortening of occluded versus free inspirations. It is apparent that during occlusions considerable shortening occurred in the costal, with even greater shortening in the crural, segments. In fact, crural shortening was not significantly different between occluded and unoccluded inspiratory efforts. Accordingly, both peak and mean velocities of shortening were appreciated during occlusions. However, the velocity did not exceed 15% of the maximal velocity of shortening of the crural segment, as obtained in a previous study (16). Figure 5 shows the values of peak shortening during occluded breaths plotted against the shortening observed in free breaths, at the initiation and at the completion of the rebreathing runs. Similar plots for peak velocity and mean velocity are shown in Figs. 6 and 7, respectively. As presented in Table 2, the peak EMG activity was higher during occluded breaths; this was primarily due to a prolongation of inspiratory time and not to an increase in the rate of rise of integrated EMG.
External intercostal. Mean ± SD for tidal shortening was 1.6 ± 4.0 \%L_{FRC} at the onset and 3.4 ± 6.9 \%L_{FRC} at the completion of the rebreathing runs. However, for external intercostals, no consistent pattern was found for EMG or shortening. There was marked variability between animals. Two dogs showed increasing EMG activity and increasing shortening; one showed increasing EMG but no length change; one showed a stable EMG and a slight increase in shortening; three showed a decreasing EMG, with a slight increase in shortening in one and a stable shortening in the other two; three had no inspiratory EMG activity at all, two with a stable shortening and one with a stable lengthening of the muscle. In addition to such wide variation, responses within individual animals were seldom linear.

FIG.5. Relationship between shortening of the diaphragm during occluded and unoccluded inspirations. Open and closed symbols indicate values at the onset and termination of CO₂ rebreathing.
Values are mean ± SE; n = 11. COS, costal; CRU, crural; L_{FRC}, muscle length at end-expiratory volume.

FIG. 5. Relationship between peak velocity of shortening of the diaphragm during occluded and unoccluded inspirations. Open and closed symbols indicate values at the onset and termination of CO_{2} rebreathing. Values are mean ± SE; n = 11. CRU, crural; COS, costal; L_{FRC}, muscle length at end-expiratory volume.

FIG. 6. Relationship between peak velocity of shortening of the diaphragm during occluded and unoccluded inspirations. Open and closed symbols indicate values at the onset and termination of CO_{2} rebreathing. Values are mean ± SE; n = 11. CRU, crural; COS, costal; L_{FRC}, muscle length at end-expiratory volume.
DISCUSSION

Ventilatory stimulus. Since our aim was to study the relation between inspiratory flow and velocity of shortening of diaphragm and external intercostal muscles, we needed a wide and consistent range of ventilations. We chose chloralose-urethane because this agent provided a light but constant level of anesthesia. Consequently, the ventilatory response to CO$_2$ was brisk (0.40 l/min/Torr, or 0.019 l/min/kg/Torr). This compares favourably to other investigations which have demonstrated 0.32 l/min/Torr, or 0.005 l/min/kg/Torr, in thiopental sodium-anesthetized goats (14) and 0.61 l/min/Torr, or 0.012 l/min/kg/Torr, in halothane anesthetized humans (21).

DIAPHRAGM. To meaningfully assess the correlation between inspiratory flow and velocity of shortening of the diaphragm, it is necessary to ensure that this muscle shortens actively in the conditions chosen for the study. In this experiment EMG activity of costal and crural diaphragm increased linearly with $P_{ACO_2}$ in every dog. Moreover, the slope of the EMG response to CO$_2$ was significantly greater for crural than for costal diaphragm. This finding is similar to the observation by Van Lunteren et al. (22), although they noted a much greater response of crural diaphragm than in this study, 15.5 %/Torr vs. 8.0 %/Torr, respectively. These differences may be explained by experimental design. In the previous study a period of resting breathing was taken as a control, whereas we chose the first breaths of each rebreathing run. In the previous study a period of resting breathing was taken as a control, whereas we chose the first breaths of each rebreathing run. Furthermore, as evidenced by reported standard errors, their results showed more scatter for crural than for costal.

We found a linear relation between the shortening and the EMG responses to CO$_2$, with no difference between the costal and the crural segment. This suggests that both segments play a constant and similarly agonist role in the range of ventilation studied. Accordingly, the response of
the crural segment in terms of tidal shortening was found to be significantly greater than that of the costal.

In the past, generally it has not been thought possible to speculate about the relation of inspiratory flow and velocity of shortening of inspiratory muscles because of the complex arrangements of the respiratory muscles (23). With sonomicrometry we are now able to elucidate this relationship and to show that mean velocity of shortening of costal and crural diaphragm is linearly related to mean inspiratory flow. Although we measured length changes at only one location of each segment, this relation probably holds for each segment in its entirety, since tidal shortening measured by sonomicrometry has been found uniform in different locations of the diaphragm (16).

FIG. 8. Relationship between inspiratory muscle shortening and tidal volume in 4 dogs. Dots indicate average of costal and crural diaphragm; crosses indicate the external intercostal. L_FRC, muscle length at end-expiratory volume; DI, diaphragm; IC, intercostal.
Although linear in each individual dog, the relation between flow and velocity varied widely among the dogs. One possible explanation is that some interanimal difference in chest wall configuration may exist, so that the apportionment of tidal shortening between the diaphragm and the other inspiratory muscles may vary. Another possibility is that there was a variable recruitment of rib cage muscles according to the level of anesthesia; if the diaphragm was the only active inspiratory muscle remaining, it had to shorten more not only to take over the inspiratory function of the rib cage muscles but also to compensate for the distortion of the rib cage which was no longer stabilized by the intercostals. We can test this hypothesis only for the external intercostal, which we studied. In Fig. 8, the tidal shortening of the diaphragm (average of costal and crural) and of the external intercostal is related to tidal volume for four dogs. If the diaphragm was required to shorten more to compensate for a failing intercostal, its slope should be inversely proportional to that of the intercostal. This is apparently not correct, since in the two dogs with a steep diaphragmatic slope (nos. 5 and 7) the intercostal shortened increasingly in one but not in the other. On the other hand, in the two dogs with a lesser diaphragmatic slope (nos. 1 and #10), the intercostal also shortened increasingly in one but not in the other. Thus, these external intercostal data cannot confirm the hypothesis, but suggest that shortening of this muscle does not play a consistent role in inspiration. It must be considered however that the variability of the flow-velocity relation could be due to a similar interaction between the diaphragm and the other rib cage muscles, such as the parasternal, scalenus and sternocleidomastoid muscles.

To what extent can inspiratory flow be considered to reflect velocity of shortening of inspiratory muscles? We provided evidence that in anesthetized dogs the mean velocity of shortening of costal and crural diaphragm increases linearly with mean inspiratory flow but that the interanimal variability of this relation does not permit quantitative predictions. We do not know if this observation is applicable in humans, but suggest that the interindividual variability of the slope velocity/flow should be less than in our study. In conscious humans the chest wall configuration is probably much more stable than in canines and anesthetic variation is absent.

Using sonomicrometry in anesthetized dogs during resting breathing, Newman et al. observed that peak crural shortening was similar during
completely occluded and unoccluded breaths (16). The costal segment also demonstrated considerable shortening during occlusions, although it was less than during non occluded breaths. Our data confirm these observations over a wide range of ventilation. Clearly, a significant distortion of the chest wall must occur during occlusions to permit this amount of diaphragmatic shortening. Two possible explanations must be considered. Perhaps most of the inspiratory neural output is directed to the diaphragm, so that intercostal and accessory muscles are not activated sufficiently to stabilize the rib cage. Alternatively, the neural output may be more evenly distributed among the inspiratory muscles but the unfavourable mechanical arrangement of the rib cage muscles prevents them from counterbalancing the action of the diaphragm. At least in theory, the smallest possible shortening of inspiratory muscles during an occlusion, i.e. the shortening necessary to accommodate for the extension of muscular elastic components and for the expansion of gas, can be achieved only by a particular distribution of the neural output such that no muscle predominates, and it is not known whether such a condition exists or not. Furthermore, the supine posture may not be optimal for studying rib cage function. In summary, these observations show that the usual assumption of an isometric or quasi-isometric contraction of the diaphragm occurring during an inspiratory effort against an occlusion is wrong, at least in supine anesthetized dogs. We speculate that the same phenomenon may occur in humans, although probably to a lesser degree since rib cage compliance and distortion are probably less.

The observation that the diaphragm shortened at a non negligible velocity during occlusions, i.e. in conditions of zero flow, indicates that the relation that we found between velocity of shortening and inspiratory flow is likely to be variable. Indeed, between the two extreme conditions of this study, i.e. unimpeded inspirations and inspiratory efforts against an infinite resistance, the relation between flow and velocity would be expected to be modified by any added inspiratory resistance. Thus when an inspiratory load is added, the velocity of shortening should decrease relatively less than inspiratory flow. It is not known whether the relation between flow and velocity of shortening remains linear in the face of various added resistances.

Our observation may contribute to some earlier attempts to correlate flow and velocity (23). Several authors used the pressure-flow relation to study the global force-velocity relation of the inspiratory muscles. Using
maximal voluntary inspirations without and with added resistances, Agostoni and Fenn (1) and Hyatt and Flath (13) found a linear relation between pressure and flow. Using phrenic stimulations in humans and cats, Pengelly et al. (17) also noted a linear relation. The discrepancy between the linear pressure-flow relation and the hyperbolic force-velocity relation described first by Hill (12) in isolated muscles was attributed to the complexity of the respiratory system (1,13). On the other hand, Goldman et al. found a curvilinear relation between transdiaphragmatic pressure (Pdi) and inspiratory flow while controlling chest wall configuration changes and diaphragmatic EMG (10). The first three studies and the last one present a methodological difference in the way the various flow rates were obtained. Three groups (1,13,17) maximally stimulated the inspiratory muscles and obtained different flow rates by adding resistances. According to our results, this could have caused the relation between flow and velocity to depart from the unloaded conditions, in a amount which was different and unknown for each resistance. At the point of zero flow the diaphragm was probably still shortening with significant velocity. Thus it may not be surprising that the pressure-flow relation obtained in these conditions did not reflect the curvilinearity of the force-velocity relation. However, in the study of Goldman et al. the different flow rates were performed by the subjects without added resistance (11). Therefore, according to our thesis, the velocity of shortening of the diaphragm was changing linearly with flow. The only points of the Pdi-flow diagram (Fig. 6 of reference 10) where the flow-velocity relation must be altered are the points corresponding to the static manoeuvres. At these points where flow equaled zero, the diaphragm probably still had a certain velocity of shortening. Thus if velocity was expressed on the abscissa in place of flow, only the points corresponding to the static manoeuvres would have to be displaced to the right, and this would tend to increase the curvilinearity of the relation.

**External intercostal muscle performance.** The striking observation regarding external intercostals was the distinct inhomogeneity of both electrical and mechanical events. Obviously, such a finding raises the possibility of a technical problem. However, the EMG hook electrodes were always carefully placed, in the same location, and superficially enough to avoid the underlying internal intercostal muscle. Their position was confirmed at the completion of each experiment. Similarly, the piezoelectric crystals were always placed in the same location and carefully aligned along
the axis of the muscle fibres. They were still firmly attached at the end of
each experiment. There was no technical problem that could be invoked to
explain these highly variable results. Indeed, the presence of this
heterogeneity in both electric activity and length changes tends to support a
real physiologic variability. In these conditions, the external intercostal
muscles presented the whole gamut of behaviours, ranging from passive
length change to isometric contraction, to increasing active shortening.

Other reports suggest that all intercostal muscles are not activated
homogeneously. The parasternal intercostals, which shorten during
inspiration (2), always present a phasic electrical activity during inspiration
in anesthetized dogs (5), awake cats (9), and awake humans (6,8,10,20). Except
during isovolume manoeuvres, the neural activation of the
parasternal muscles appears closely coupled to that of the diaphragm (8,10).
On the contrary, the parasternal and the lateral intercostals are activated
separately as shown during loaded breathing (19) and inspirations performed
mainly by the rib cage (11).

Indeed, it is reported that the external intercostals are less consistently
active than the parasternals. In humans, the external intercostals have been
found to be phasically active during resting breathing with normal tidal
volumes (19) or only during deeper inspirations (20). In anesthetized dogs,
they have shown predominantly tonic and not phasic activity (15). In cats,
the same muscles are generally inactive in anesthetized or decerebrate
animals, but they show primarily a postural activity in wakefulness and
sleep (9). These distinct patterns of activation have led to the conclusion that
the parasternal intercostals are primary phasic respiratory muscles, whereas
the external intercostals have an important postural role and are only
secondary phasic respiratory muscles. This is emphasized by the higher
spindle density in external than in parasternal intercostals (9).

A phasic inspiratory activity was present throughout the rebreathing
runs, but only in some of our dogs. It is possible that such a variation in
electric activity was related to the level of anesthesia. It has been shown that
the inspiratory intercostals are electrically inactive during even light
halothane anesthesia (21). If the same effect occurs with chloralose-
urethane, we may have maintained our dogs in a transitional zone by
choosing a light level of anesthesia, so that small changes in the level of
anesthesia could have resulted in variable degrees of inhibition of the
external intercostals.
The lateral intercostal muscles, external and internal, have been shown to elevate the ribs into which they insert, when contracting at end-expiratory lung volume in anesthetized dogs (7). By expanding the rib cage, they can be considered as inspiratory muscles and thus expected to shorten when activated in inspiration. However, the lack of consistent neuromechanical coupling that we observed is not surprising when the complexity of the rib cage and the different groups of muscles involved in its movements are considered. It is conceivable that the length of an interosseous intercostal muscle, bound to an upper and to a lower rib, could be affected by any other muscle acting directly or indirectly on these ribs. Thus in the absence of electric activity, it could be passively shortened or lengthened. When activated it could be shortening (stiometric contraction), lengthening (pliometric contraction) or not changing its length (isometric contraction). Thus, it has been observed recently that external and internal intercostal muscles shorten in the upper spaces and lengthen in the lower spaces during inspiration in anesthetized dogs (3). This finding may explain the lack of consistency that we observed in a midlevel interspace. In conclusion, although we do not know the etiology of the variable behavior of the external intercostal in this anesthetized preparation, its velocity of shortening is obviously not reflected by inspiratory flow.

In summary, during ventilation stimulated by progressive hypercapnia, we found that: 1) EMG activity and tidal shortening of costal and crural diaphragm increased linearly with PCO₂, with the crural activity exceeding costal; 2) inspiratory effort against occlusion was associated with considerable shortening of both segments of the diaphragm; 3) The relationship between mean velocity of shortening of the diaphragm and mean inspiratory flow was linear, individual, and altered by an inspiratory load; and 4) There was no correlation between velocity of shortening of an external intercostal muscle and inspiratory flow.

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Effects of chemical stimulation on segmental function.

Costal and crural diaphragm function during CO₂ rebreathing in awake canines. Manuscript 6

Thematic overview.

The relative function of the segments was examined during conditions of CO₂ rebreathing. The differential activity of costal and crural which was exhibited during resting breathing was consistently altered and accentuated during CO₂ stimulated ventilation.

Careful examination of intrabreath segmental performance, with normalization of time and amplitude of activity to allow precise comparison despite variations in breathing pattern, was essential in the characterization of segmental function. Crural segmental activity was more prominent than costal in both early inspiration and PIIA at rest and with stimulation. With increasing CO₂ stimulated breathing, crural segmental predominance in early inspiration was accentuated and its distinctive, predominate changes in PIIA confirmed.

The distinctive response and flexibility with chemical stimulation of the crural segment, helped elucidate its role as a muscle recruited for "adjustment" of diaphragm mechanics and "interaction" with cooperating muscles, in contrast to the costal segment with its apparent mission as a pure respiratory pump.
Costal and crural diaphragm function during CO2 rebreathing in awake canines.


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ABSTRACT

If costal and crural diaphragm segments can perform as separate muscles, then CO₂ stimulated ventilation may elicit differential segmental function. We report measurements of diaphragm segmental length, shortening, and EMG activity from 10 canines that were chronically implanted with sonomicrometry transducers and EMG electrodes then studied a mean 16 days post implantation, while awake and breathing spontaneously during CO₂ rebreathing. During increasing hypercapnia, segmental shortening and EMG activity per whole tidal breath progressively increased, but these segmental responses could not be differentiated at any level of CO₂. With increasing CO₂ there was a significant increase in resting end expiratory length of both diaphragm segments. Examination of costal and crural diaphragm segments during the complete, intra-breath, inspiratory-expiratory cycle revealed distinctive segmental function. During resting ventilation, relative crural shortening preceded and exceeded costal shortening in earliest inspiration; costal and especially crural segmental shortening persisted beyond the peak of inspiratory flow into early expiration. During resting ventilation, crural EMG activity was greater than costal in earliest inspiration and showed more persistent end inspiratory/early expiratory (PIIA) activity. During CO₂ stimulated breathing, at ETCO₂ 59.5 Torr, neither costal nor crural segments shortened appreciably during the inspiratory flow of earliest inspiration. And at ETCO₂ 59.5 Torr, crural shortening considerably exceeded costal shortening during early inspiration and outlasted costal shortening during expiration; for both segments, most of the peak tidal shortening persisted after inspiratory airflow terminated. At increased ETCO₂, crural EMG activity preceded costal activity in earliest inspiration and was dominant into expiration, while costal EMG activity terminated abruptly with the end of inspiratory flow. Thus costal EMG PIIA was not evident during hypercapnia, while crural EMG PIIA was significant. These results suggest that in the awake, spontaneously breathing canine that 1) costal and crural diaphragm segments exhibit differential function during room air and CO₂ stimulated breathing; 2) crural segmental activity predominates in early inspiration; 3) at the onset of inspiration, neither diaphragm segment fully accounts for inspiratory flow; 4) both segments exhibit PIIA during resting breathing, but crural PIIA dominates during hypercapnic stimulation.

INDEX TERMS: costal, crural, canine, shortening, sonomicrometry, EMG, awake, nonanesthetized, CO₂ stimulated ventilation.
INTRODUCTION

Little is known of the function of the costal and crural diaphragm segments, including length, shortening, and EMG activity, during CO$_2$ stimulated ventilation, in intact, awake and spontaneously breathing mammals (5,29). Previous investigations have employed sonomicrometry to provide direct measurements of diaphragm segmental motion during stimulated ventilation, but these measurements were made in a surgical, supine position, in close temporal relationship to laparotomy or thoracotomy, while the animals remained anesthetized (3,6,15,22,30). With rare exceptions (25,26), measurements of diaphragm EMG activity during hypercapnia have required the same acute, anesthetized conditions. Recently, we described a technique for the chronic implantation of sonomicrometry transducers and EMG wires into costal and crural diaphragm, with complete recovery, allowing subsequent measurement of normal diaphragm segmental function during awake, spontaneous breathing (7). Chronic implantation of sonomicrometry transducers not only provides direct and dynamic measurement of diaphragm length and shortening, but also reinforces the validity of any accompanying measurements from chronically implanted, diaphragm EMG electrodes. Even after apparently trivial implantation procedures, some inhibition of diaphragm segmental function may persist, and the presence of phasic inspiratory EMG activity does not guarantee an effectively shortening muscle (7,29). Therefore, presumption of normal EMG activity is strengthened by concurrent evidence that each segment is actually shortening as expected.

Evidence continues to accumulate in support of costal and crural diaphragm segments as separate muscles. Differing segmental innervation and mechanical actions on the rib cage (4,23) and a closer crural association with gastroesophageal events (1) have been noted. Measurements of segmental length and/or EMG activity in acutely anesthetized mammals have suggested that crural may overshadow costal activity early in inspiration, and that crural shortening and responsiveness to CO$_2$ stimulation may be relatively greater (3,6,15,22,31,32). But this evidence of differential segmental function has not been confirmed in the awake, spontaneously breathing mammal. How predominant is crural shortening, without anesthetic? What is the relative segmental EMG and shortening activity as each inspiration begins? Does differential function of diaphragm segments extend to end inspiration, or post inspiratory inspiratory activity (PIIA)?

To address these questions, we measured length, shortening, and EMG activity during CO$_2$ stimulated ventilation, in the left hemidiaphragm of awake and
spontaneously breathing canines, who had previously been chronically implanted and recovered to apparently normal function.

METHODS

Surgical Preparation. Ten tracheostomized mongrel dogs (mean weight 24 Kg; range 18-30 Kg), had sonomicrometer transducers and EMG wires implanted during laparotomy and were studied after diaphragm segmental shortening had fully recovered. This technique of chronic diaphragmatic implantation of sonomicrometry and EMG wires and the 7-10 day progressive recovery of segmental shortening have been described fully in a recent publication (7). Briefly, implantation was performed under general anesthesia with thiopental sodium and halothane. The left hemidiaphragm was exposed through a midline abdominal incision, and spherical (33) ultrasonic transducers were implanted between muscle fibres on a flat portion of each of the costal and crural segments of the hemidiaphragm. Costal transducers were implanted in the mid portion of the segment, approximately midway between central tendon and chest wall (27,10). Crural transducers were inserted posteriorly in the mid paravertebral aspect of the muscle. On each segment immediately adjacent to each pair of transducers, a fine wire stainless steel, bipolar electromyogram (EMG) electrode was attached. All wires were externalized via subcutaneous skin tunnel, and the animals were recovered.

Measurement Techniques. Measurements of resting and CO2 stimulated ventilation were made a mean of 16 days post-implantation (range 10-23 days post-implantation). Measurements were performed with the animals awake, relaxed, and breathing quietly while lying in the right lateral decubitus position. The animals were familiar with the location, routine, and personnel of the recording. During the measurements, the animals breathed spontaneously through a cuffed endotracheal tube which was attached through a unidirectional valve to a low resistance open breathing circuit (<1.0 cmH2O/L/s), connected to a pneumotachygraph (Fleisch #2) for measurement of airflow. On the expiratory limb, CO2 was sampled and analyzed continuously (Model LB-2 infrared CO2 analyzer, Beckman Instruments, Schiller Park, IL). Ventilatory and diaphragmatic responses to progressive hypercapnia were elicited by a modification of the Read technique (18), rebreathing 6% CO2-93% O2 from a 4-5 L bag.

Measurements were made for flow, costal and crural length and shortening on all ten animals. For technical reasons, costal and crural EMG activity was available in seven and five of the ten animals, respectively.
Dynamic measurement of the changing distance between the sonomicrometer transducers of each pair was provided by the sonomicrometer (Model 120, Triton Technology, San Diego, CA). Measurement of diaphragm length by sonomicrometry has been described in detail (7,15). Briefly, when electrically excited, the emitter piezoelectric transducer resonates, radiating ultrasound waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a measurable voltage. A quartz crystal clock oscillator measures the transit time of the waves, and because the conduction of velocity in muscle is known, the sonomicrometer provides the intertransducer distance. EMG signals from the wire electrodes were amplified (Mark III, TECA, White Plains, NY) and band pass filtered (16 Hz-1.6 KHz). The output signals were then rectified and processed by passage through resistance-capacitance, leaky "integrators" with a time constant of 100 ms, to provide moving averages of the electromyograms of costal and crural diaphragm.

Analysis of Ventilation. All signals were recorded on a strip chart recorder and simultaneously gathered by a single board analog-to-digital system (Model 2801-A, Data Translation, Marlboro, MA) directly to hard disk on a microcomputer (PC, IBM, Boca Raton, FL) for subsequent examination using a series of dedicated analysis programs written by one of the authors (PE). The flow signal was evaluated for respiratory timing and digitally integrated; minute ventilation (VI), respiratory frequency (FREQ), tidal volume (VT), inspiratory time (TI), mean inspiratory flow (VT/TI), and inspiratory fraction of respiration (TI/TOT) were calculated breath-by-breath.

Whole Breath Analysis. Using the sonomicrometry data from each diaphragm segment, the computer algorithm identified the muscle length for each breath which corresponded to the onset of inspiratory flow. In each breath, the computer compared this value to the data samples of muscle length within the final third of the preceding expiration, and identified the maximum resting end expiratory muscle length. This length at end expiration in mm was titled LEEX, this length defined the baseline, end expiratory length for muscle shortening per breath. This length has been titled LfRC in our previous reports (6,7). From this resting, end expiratory length, the shortening per each breath was expressed as a percentage change from resting length and titled %LEEX. Similarly, segmental mean velocity of shortening per breath, and segmental peak velocity of shortening per breath, were calculated as a percent change of end expiratory length per second and expressed as %LEEX/s. As a final step in the computer algorithm, for each breath the muscle length corresponding exactly to the onset of inspiratory flow was identified and this pre-inspiratory length, was recorded in mm and titled LP1F. Then maximum
segmental shortening for the breath was expressed again as a percentage of baseline pre-inspiratory length %LPoIF.

EMG activity was quantified arbitrarily per breath as the maximum difference in volts between end expiratory baseline and the peak height of the moving average signal.

Ventilatory, segmental shortening, and EMG responses to hypercapnia (ΔVI/ΔETCO₂, ΔCOS %ILEX/ΔETCO₂, ΔCRU %ILEX/ΔETCO₂, ΔCOS EMG/ΔETCO₂, ΔCRU EMG/ΔETCO₂), were calculated by linear regression using the method of least squares.

All aspects of muscle length and shortening and EMG activity were calculated at resting ventilation and four levels of alveolar PACO₂ (ETCO₂): 37.9 ± 2.9, 47.4 ± 3.9, 53.1 ± 3.8, 59.5 ± 5.0, and 66.4 ± 6.0 Torr ETCO₂. For each animal these stepwise increments represent measurements taken at room air before hypercapnia, and then during the beginning, ending, and at two equally spaced intermediate points during the CO₂ rebreathing. These measurements defined whole breath, or "tidal" breath activity of inspiratory flow and respiratory timing; diaphragm length, shortening, and velocity; and EMG activity.

**Intrabreath Analysis.** After calculating peak tidal, whole breath values, the computer algorithm calculated the intrabreath development or "shape" of inspiratory airflow, diaphragm length, shortening, and EMG activity for each breath. Briefly, for each whole breath, the peak inspiratory airflow, maximum segmental shortening and peak EMG activity were identified. Then throughout the duration of each breath (TTOT), the percent of peak segmental activity was determined after each successive 5% "slice" of the total time of the breath (%TTOT). That is at each 5% increment, inspiratory flow, segmental shortening and EMG activity were expressed as a percent of the maximum tidal value for the breath. This created a profile for each breath of segmental shortening and EMG standardized as a percentage of the peak whole breath value, against time standardized as a percent of TTOT. At each of the five levels of CO₂, the breath profiles were calculated for each canine and then averaged for the group.

Four events within each breath were examined specifically. 1) Segmental shortening and EMG activity at the onset of inspiratory flow (IO) was identified as the shortening and EMG activity after the first 5% of the TTOT. Segmental shortening and EMG activity were also examined within the breath profile at three additional points corresponding to: 2) the peak of inspiratory flow (IP), 3) the end of inspiratory flow (IF), and 4) early expiration (EE) which was defined as 15% of TTOT after the end of inspiratory flow (IF). The early expiratory time highlighted
by EE was expected to coincide with post inspiratory inspiratory activity PIIA, of both shortening and EMG.

Statistical Analysis. After computer analysis, mean values were exported to spreadsheet software for review, to graphics software to output Figures 1-6, and to the PC version of SAS (24) for statistical analysis. Mean values for parameters of breathing pattern, segmental length and shortening, and EMG activity were tested across the five levels of CO₂ by two-way analysis of variance with repeated measures on one factor (11,24). Multiple comparison testing of the mean values of the individual periods was performed using Tukey's test (28). The slopes of the linear relations of costal and crural segmental shortening and EMG activity were compared by paired t test. Mean values for costal and crural shortening at each of the five levels of CO₂ were compared by paired t test. Intrabreath results for costal and crural segmental shortening and EMG at the onset of inspiratory flow (IO), peak inspiratory flow (IP), end of inspiratory flow (IF), and early expiration (EE), were also compared by paired t test. Standardized breath profiles at each level of CO₂ were plotted as mean ± SEM for each diaphragm segment.

RESULTS

CO₂ Responses. For this group, awake and breathing spontaneously in the right lateral decubitus position after full recovery from implantation, the mean ΔVI/ΔETCO₂ was 0.78±0.29 L/min/Torr (Table 1). The CO₂ responsiveness expressed as diaphragm segmental shortening was 0.74±0.46 and 0.71±.38 per cent shortening from baseline expiratory length per Torr ETCO₂ for costal and crural, respectively. This relative segmental shortening responsiveness to CO₂, ΔCOS %LeX/ΔETCO₂, and ΔCRU %LeX/ΔETCO₂, was not different between segments. With increasing hypercapnia, the relative segmental EMG responsiveness was similarly linear and not different between costal and crural; ΔCOS EMG/ΔCO₂ and ΔCRU EMG/ΔCO₂ were 0.17±0.16 and 0.21±0.14, respectively.

For the group the hypercapnic stimulus commenced at room air (ETCO₂ of 37.9±2.9 Torr) and terminated at mean 66.4±6.0 Torr. Variables describing the peak tidal, whole breath, performance of the mechanical and electrical function of the diaphragm are summarized at these two extremes as well as three intermediate values of 47.4±3.9, 53.1±3.8 and 59.5±5 Torr PaCO₂ (Table 1).

Across the range of CO₂ stimulation, VI increased from baseline 4.11±0.96 to 22.00±7.94 L/MIN, with respiratory frequency increasing from 15.83±1.88 to 24.11±6.23, tidal volume increasing from 0.25±0.05 to 0.90±0.21, and TI increasing from 1.08±0.14 to 1.24±0.26 s; all changes P<0.0001.
Table 1 Ventilatory response and segmental function during progressive hypercapnia.

<table>
<thead>
<tr>
<th>BREATHING PATTERN:</th>
<th>ETCO₂ (Tor)</th>
<th>37.39(2.85)</th>
<th>47.41(3.85)</th>
<th>53.09(3.81)</th>
<th>59.46(5.00)</th>
<th>66.40(6.01)</th>
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<tr>
<td>VT</td>
<td>4.11(0.96)</td>
<td>5.83(1.25)</td>
<td>11.94(3.30)</td>
<td>17.09(5.74)</td>
<td>22.00(7.94)</td>
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<tr>
<td>FREQ</td>
<td>15.83(1.88)</td>
<td>15.77(1.78)</td>
<td>17.96(3.20)</td>
<td>20.29(4.47)</td>
<td>24.11(6.21)</td>
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</tr>
<tr>
<td>VT</td>
<td>0.25(0.05)</td>
<td>0.37(0.10)</td>
<td>0.68(0.16)</td>
<td>0.53(0.17)</td>
<td>0.90(0.21)</td>
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<tr>
<td>Ti/TOT</td>
<td>1.09(0.19)</td>
<td>1.21(0.15)</td>
<td>1.38(0.16)</td>
<td>1.35(0.24)</td>
<td>1.24(0.29)</td>
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</tr>
<tr>
<td>VT/TI</td>
<td>0.29(0.05)</td>
<td>0.32(0.05)</td>
<td>0.41(0.05)</td>
<td>0.44(0.05)</td>
<td>0.47(0.04)</td>
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</table>

LENGTH (BASELINE END EXPIRATORY - LEEF):

<table>
<thead>
<tr>
<th></th>
<th>COS</th>
<th>CRU</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHORTENING (%LEEF):</td>
<td>14.30(3.37)</td>
<td>17.98(4.43)</td>
</tr>
<tr>
<td>MEAN VELOCITY (%LEEF/s):</td>
<td>5.11(3.59)</td>
<td>6.54(3.43)</td>
</tr>
<tr>
<td>PEAK VELOCITY (%LEEF/s):</td>
<td>11.20(9.12)</td>
<td>12.43(4.89)</td>
</tr>
<tr>
<td>EMG DIFF:</td>
<td>1.76(1.00)</td>
<td>2.77(1.09)</td>
</tr>
</tbody>
</table>
| LENGTH (PRE-INSPIRATORY FLOW - LPEF):
| SHORTENING (%LPEF): | 14.26(9.40) | 17.74(4.32) |
| CO₂ RESPONSE CURVES: Slope | Intercept |
| ΔV/ΔCO₂ | 0.78(0.29) | -2.94(12.53) |
| ΔCO₂ SHORTENING/ΔCO₂ | 0.74(0.46) | -2.94(22.11) |
| ΔCRU SHORTENING/ΔCO₂ | 0.71(0.38) | -2.15(17.25) |
| ΔCO₂ EMG/ΔCO₂ | 0.17(0.16) | -5.95(6.71) |
| ΔCRU EMG/ΔCO₂ | 0.21(0.14) | -6.56(6.67) |

**Breathing pattern:** VT, minute ventilation (L/min); FREQ, respiratory frequency (breaths/min); VT, tidal volume (L); TI, inspiratory time (sec); Ti/TOT, inspiratory fraction of respiration; VT/TI, mean inspiratory flow (L/sec). LENGTH: End expiratory segmental length (mm), baseline length defined as first point of shortening in end expiration (LLEE); COS, left costal segment; CRU, left crural segment. SHORTENING: Segmental shortening per breath, expressed as percent of baseline end expiratory length, (%LLEE). MEAN VELOCITY: Segmental mean velocity of shortening per breath, expressed as percent of baseline end expiratory length per second (%LLEE/s). PEAK VELOCITY: Segmental peak velocity of shortening per breath, (%LLEE/s). EMG DIFF: Peak integrated EMG per breath (volts); COS N=7, CRU N=5. LENGTH: Pre-inspiratory segmental length (mm), baseline length defined as first airflow (LPEF). SHORTENING: Segmental shortening per breath, expressed as percent of baseline pre-inspiratory length, (%LPEF). Values are mean (SD); N=10. RESPONSE CURVES: ΔV/ΔCO₂ change in minute ventilation per CO₂ (L/min/mMCO₂); ΔSH/ΔCO₂ change in segmental shortening per CO₂ (mm/mMCO₂). ΔEMG/ΔCO₂ change in segmental EMG per CO₂ (volts/mMCO₂).

Diaphragm length during hypercapnia. The mean end expiratory length in millimeters, the baseline length from which the diaphragm segments began to...
shorten during the subsequent inspiration, was 14.30±3.37 and 17.98±4.43 mm at a mean room air CO₂ of 37.9 Torr for costal and crural segments, respectively. At the highest ETCO₂ of 66.40 Torr, the end expiratory length was 14.62±3.52 and 18.97±4.76 for costal and crural segments, respectively. Thus, the maximum change in resting end expiratory segmental length over approximately 30 Torr of increasing hypercapnia was 0.3 mm and 1.0 mm for costal and crural segments respectively. As a percent of initial room air resting segmental length, these increases represented approximately 2% and 5.5% of resting costal and crural length respectively. Although modest in magnitude, the length increases were extremely consistent within each animal with increasing ETCO₂, and highly significant (P<0.007 and 0.0001) for costal and crural respectively (Figure 1).

Figure 1. Left diaphragm segmental end expiratory length during CO₂ rebreathing. Closed circles: costal segment; closed squares: crural segment; symbols are group mean, bars SD.

When baseline resting length was chosen at the first point of airflow, costal pre-inspiratory length (LPIF) was 14.26±3.40 and 14.47±3.51, and crural length (LPIF) was 17.74±4.32 and 18.62±4.73, at room air and 66.4 Torr ETCO₂ respectively. This change was significant as well (P<0.005 and 0.001 for costal and crural segments respectively). Thus, baseline segmental length defined either as the
longest segmental length in end expiration or as the segmental length at the first recorded point of airflow, increased consistently by a small but detectable magnitude during hypercapnic stimulation from room air through 66.4 Torr ETCO₂.

\[ \text{Figure 2. left diaphragm segmental shortening during CO₂ rebreathing.} \]

Convention as in Figure 1.

**Segmental shortening.** As seen in Figure 2, costal and crural segmental shortening increased progressively with increasing ETCO₂. Resting, room air, segmental shortening as a percent of end expiratory length (%LEEX) was 5.11±3.59 and 6.54±3.43 for costal and crural segments, respectively. Costal shortening increased to a maximum of 21.35±7.55 % %LEEX, and crural shortening increased to 22.58±8.27 %LEEX, at 66.4 Torr ETCO₂. Both costal and crural shortening increased significantly (P<0.0001) over this range of hypercapnia, as illustrated in Figure 2. However, this peak tidal shortening of costal and crural segments could not be differentiated at room air or at any individual level of CO₂ stimulation.

Calculation of segmental shortening as a percent of baseline segmental length at the beginning of airflow provided an equivalent result, a significant and similar increase in tidal segmental shortening for both costal and crural diaphragm with hypercapnia (Table 1).
Calculations of both mean and peak segmental velocity of shortening per breath revealed significant increases during rebreathing, which could not be differentiated between costal and crural diaphragm segments. Segmental mean velocity of shortening per breath was 3.48±0.77 and 3.94±2.38 %LEV/s at room air for costal and crural segments, respectively. This increased to 17.99±8.9 and 16.41±8.93 %LEV/s, at the highest ETCO₂ for both costal and crural segments, respectively (both P<0.0001). Segmental increases in peak velocity of shortening per breath were also significant and similar between costal and crural segments (Table 1).

**EMG activity.** The group mean, peak tidal moving average EMG measured from costal and crural diaphragm segments increased significantly (P<0.0001) for both segments across the range of hypercapnia. In arbitrary voltage units, mean moving average costal EMG increased from 1.76±1.00 to 4.59±2.23 volts while crural EMG increased from 2.77±0.99 to 6.76±2.28 volts, as ETCO₂ increased from 37.9 to 66.4 Torr, respectively.

**Intrabreath segmental diaphragm function.** Shortening and EMG activity of costal and crural segments were examined dynamically within each tidal breath. In contrast to the global, peak analysis per whole breath cited in the previous paragraphs, this analysis concentrated on the profile of inspiratory flow, segmental shortening, and segmental EMG that developed dynamically during each breath. The standardized breath profiles for costal and crural shortening and EMG activity, during room air (ETCO₂ 37.9) and moderate hypercapnic stimulated (ETCO₂ 59.46 Torr) breathing are shown in Figures 3-6.
Figure 3a. Inspiratory airflow and segmental shortening per breath during hypercapnia. Standardized intrabreath values for all respirations during room air breathing, mean ETCO₂ 37.9 Torr. Values of flow and segmental shortening standardized as percent of maximum per individual breath; standardized values noted sequentially after each 5% of total breath time (TTOT). Mean breath profile results for all animals are shown. Mean airflow is marked by solid black curve and open circles. Mean costal shortening±SEM shown by the light dashed line and crosshatched pattern; mean crural shortening±SEM shown by the light dotted line and horizontal hatching. IO marks the inspiratory flow after the first 5% of TTOT; IP marks the peak of inspiratory airflow; IF indicates the end of inspiratory flow; EE marks intrabreath events in early expiration, 15% TTOT after the end of inspiratory flow.

Resting intrabreath segmental shortening. Figure 3a illustrates inspiratory flow, costal segmental shortening, and crural segmental shortening for all resting tidal breaths for the group. Values of flow and shortening within each breath were standardized as a percent of the maximum per breath, and time was standardized as a percent of breath duration (TTOT); all resting breaths were averaged per animal, and the group mean±SEM expressed graphically in Figure 3a, with emphasis on beginning (IO), peak (IP), and ending (IF) of inspiratory flow and the first third of expiration (EE). During this resting ventilation, costal and crural segmental shortening was noted generally to be synchronous during development of
inspiratory flow. However, neither costal nor crural segmental shortening tracked inspiratory airflow at earliest inspiration; after 5% of TTOT when inspiratory airflow was already near 45% of peak, crural and costal shortening had achieved only 25 and 12% of peak tidal shortening, respectively. At this time of very earliest inspiration (IO), crural shortening preceded costal segmental shortening (P<0.08). By the moment of peak inspiratory airflow (IP), the segmental shortening of both costal and crural had developed together and overlapped. The peak of inspiratory airflow did not correspond in time with peak segmental shortening. Peak costal shortening lagged peak flow by 5% TTOT, while maximum crural shortening lagged peak inspiratory flow by 15-20% TTOT. Costal and crural segmental shortening diverged markedly during the decay of inspiratory airflow, and when inspiratory airflow was effectively terminated by 35% TTOT (IF), segmental shortening was persistent. In fact, as measurable inspiratory airflow was ending most costal segmental and crural segmental shortening remained. As airflow ended, more crural segmental shortening remained than costal, (P<0.013). Costal and crural segmental activity was not seen to converge until later in expiration. Early in expiration, (EE), both costal and crural segments suggested significant post inspiratory inspiratory activity (PIIA), expressed as segmental shortening, with crural greater than costal (P<0.0009). This profile suggested that at room air, costal and crural segmental functions were not identical; crural segmental shortening preceded costal in earliest inspiration, maximum crural shortening lagged both peak airflow and peak costal shortening, and crural shortening remained significantly greater than costal shortening as inspiratory airflow ended. Moreover, neither costal nor crural segments appeared to account for the earliest development of inspiratory airflow, and shortening of both segments persisted beyond obvious inspiratory airflow suggesting PIIA.
Resting intrabreath segmental EMG. An EMG-flow breath profile during resting ventilation, analogous to the flow-shortening profile of Figure 3a, is illustrated in Figure 3b. It shows standardized, group mean, inspiratory airflow, and costal and crural EMG activity, during resting ventilation at a mean ETCO₂ of 37.9 Torr. Since costal EMG could be measured in 7 of 10 animals and crural EMG in 5 of 10 animals, slightly more scatter is exhibited in the range of values illustrated by mean±SEM. As expected, both costal and crural EMG activity apparently precedes the respective shortening activity; EMG activity occurs approximately in synchrony with inspiratory airflow. Although the difference was not significant, crural EMG activity appeared to precede costal EMG activity at the earliest time in inspiration (IO), as was noted previously for costal and crural shortening activity in earliest inspiration. Both costal and crural EMG activity peaked approximately in synchrony with peak inspiratory airflow, but neither costal nor crural EMG decayed in synchrony with declining inspiratory airflow. By the ending of inspiratory airflow (IF), significant activity of both costal and crural EMG was still present. Costal and crural EMG activity diverged during the first third of expiration, with crural activity significantly greater than costal (difference P<0.09, N=4). This late inspiratory and expiratory persistence of costal and crural
segmental EMG activity was consistent with the persistent segmental shortening previously seen in Figure 3a; that is, the PIIA EMG activity corresponded to the PIIA suggested previously by segmental shortening.

Figure 4a. Inspiratory airflow, costal and crural shortening per breath during hypercapnic stimulated ventilation, mean ETCO₂ 59.5 Torr. Other conventions as in Figure 3a.

Intrabreath segmental shortening during hypercapnia. Group mean inspiratory flow and costal and crural segmental shortening are illustrated during moderate hypercapnic stimulation in Figure 4a. Compared to Figures 3a-3b, mean inspiratory flow persists for approximately 50% of TTOT, so expiratory time is proportionally a lesser amount of TTOT. From Table 1, the typical tidal breath expressed by Figures 3a-3b and Figures 4a-4b correspond to tidal volumes of 0.25 L and 0.83 L at ETCO₂ values of 37.9 and 59.5 Torr, respectively.

At this moderate level of hypercapnic stimulation, costal and crural shortening activity was dichotomous. During earliest inspiratory airflow, at 5% TTOT (IO), inspiratory airflow had risen quickly to 40% of the maximum tidal value, while crural segmental shortening was noted at 15% and costal segmental shortening at 3% of mean peak tidal (difference P<0.008). As with room air, neither costal nor crural segmental shortening appeared to track earliest inspiratory airflow.
and the shortening activity of crural clearly antedated the accompanying costal segmental shortening. The relative lack of shortening of the costal segment was striking; airflow had reached 40% of maximum per tidal breath despite minimal segmental shortening of costal diaphragm. Both costal and crural segmental shortening was equivalent at the time of peak inspiratory airflow, but the amount of shortening noted in both segments was still nearly maximal even as airflow was ending (IF). Costal, and especially crural segmental shortening, persisted far into expiration (EE) with crural shortening predominating (P<0.0009). Both segments showed more post inspiratory inspiratory shortening activity than had been evident during resting ventilation.

![Graph showing inspiratory airflow, costal and crural EMG per breath during hypercapnic stimulated ventilation, mean ETCO₂ 59.5 Torr. Other conventions as in Figure 3b.](image)

Figure 4b. Inspiratory airflow, costal and crural EMG per breath during hypercapnic stimulated ventilation, mean ETCO₂ 59.5 Torr. Other conventions as in Figure 3b.

**Intrabreath segmental EMG during hypercapnia.** The corresponding profile of costal and crural EMG activity and inspiratory flow during moderate hypercapnia is shown in Figure 4b. Mean inspiratory airflow and costal and crural EMG activity are illustrated at the same moderate ETCO₂ of 59.5 Torr as Figure 4a. Crural EMG activity in earliest inspiration was synchronous with mean inspiratory airflow and
significantly greater than costal electrical activation in earliest inspiration (difference $P<0.05$, $N=4$). Both costal and crural EMG activities peaked immediately after peak inspiratory flow. Thereafter, costal EMG activity declined precipitously and in conjunction with the decay of inspiratory airflow, in contrast to the relative persistence of crural EMG activity. From the EMG record, there was no evidence of costal segment PIIA, yet crural segmental EMG PIIA remained. At the termination of inspiratory airflow (IF), significantly more crural EMG activity persisted compared to costal (difference $P<0.035$). Both costal and crural EMG activity showed some persistence late into expiration, although the relative scatter prevented any differentiation between the segments.

At moderate hypercapnia, post inspiratory inspiratory activity (PIIA) was clearly different for the two segments. For costal, there was shortening but not EMG PIIA, suggesting that the expiratory persistence of costal shortening was not active. For crural, extensive PIIA shortening was noted, and at least part of this segmental shortening was active and reflected significant underlying EMG PIIA.

When the shortening and EMG PIIA of moderate hypercapnia is contrasted with that of resting ventilation, i.e. Figures 3a-3b and 4a-4b, both EMG and shortening differences are noted. For shortening, the perceived post inspiratory inspiratory activity of both segments becomes more significant at hypercapnic ventilation. The shape and duration of costal and crural inspiratory shortening changes and persists far into expiration with hypercapnia. However, examination of EMG PIIA for both segments indicates that this apparent shortening only partly correlates with active primary segmental activity as indicated by EMG. There is markedly less EMG PIIA of costal segment with hypercapnia compared to resting ventilation. Thus the segmental shortening that persists far into expiration during hypercapnic stimulated ventilation is partly "active" for crural, but strictly "passive" for costal.
Figure 5a. Costal and crural segmental shortening per breath during room air ventilation, mean ETCO₂ 37.9 Torr. Each value of segmental shortening standardized as percent of maximum per individual breath; standardized values then noted sequentially for each 5% of the breath duration (TTOT). All resulting breath profiles averaged per animal; mean results for all animals are shown. Open circles mark each 5% increment of the total breath duration (TTOT) as in Figures 3-4, IO marks onset of inspiratory flow at 5% TTOT; IP marks the time of peak inspiratory flow, IF shows end inspiratory flow, and EE shows early expiration 15% TTOT after end of inspiratory flow.
Relative segmental shortening. Figures 5a-5b illustrate the relative, group mean, standardized shortening of the two segments during resting and CO₂ stimulated ventilation. Each breath is represented as a continuous shortening-lengthening cycle, without imposing any beginning/ending related to inspiratory airflow. This presents relative segmental shortening throughout each breath cycle. The shortening relationship for the two segments during the room air breathing cycle is expressed in Figure 5a. From the onset of shortening during each breath cycle, even before inspiratory flow appears (IO), through peak flow (IP), costal and crural shortening develop equivalently. However, after peak inspiratory airflow the character of segmental shortening diverges; first, costal shortening falls off abruptly, then crural shortening recedes through expiration. A similar presentation of segmental shortening during moderate hypercapnia is shown in Figure 5b. The non-linearity of costal versus crural shortening is apparent. During inspiration and expiration, segmental shortening develops and resolves asynchronously; crural shortening appears first in the inspiratory cycle, then costal shortening accelerates during inspiratory flow only to recede abruptly into expiration where crural

Figure 5b. Segmental shortening per breath during moderate hypercapnia, mean ETCO₂ 59.5 Torr. Conventions as in Figure 5a. Intrabreath events marked by IO, IP, IF, and EE correspond to times shown in Figure 3.
shortening gradually recedes. However, these segmental differences in shortening do not reveal the relative electrical activation of the segments that accompanies the changing length.

Figure 6a. Costal and crural segmental shortening and EMG activity per breath during room air ventilation, mean ETCO₂ 37.9 Torr. Each value of segmental shortening and EMG activity standardized as percent of maximum per individual breath; standardized values then noted sequentially for each 5% of the breath duration (TTOT). All breath profiles averaged per animal; mean results for all animals are shown. Mean costal shortening and EMG activity shown by the light dashed line; mean crural shortening and EMG activity by the dotted line. Open circles mark each 5% increment of the total breath duration (TTOT) as in Figure 3; onset of inspiratory flow at 5% TTOT, the time of peak inspiratory flow, end inspiratory flow, and early expiration 15% TTOT after end of inspiratory flow, are marked by IO, IP, IF, and EE, respectively.

Segmental electromechanical coupling. Figures 6a-6b illustrate the relative, group mean, standardized EMG and shortening of the two segments during resting and CO₂ stimulated ventilation. These Figures show a breath as a continuous electro-mechanical cycle, without imposing any beginning/ending related to inspiratory airflow. This is an attempt to elucidate the relative segmental shortening expressed per measured EMG activity, throughout each breath cycle.
The EMG-shortening relationship for the two segments during the room air breathing cycle is expressed in Figure 6a. From the earliest baseline EMG activity in both segments, beyond earliest inspiratory airflow and nearly to peak airflow (IP), relatively more shortening was developed per EMG unit activity of the crural diaphragm segment. And, beyond peak inspiratory flow (IP), relatively greater shortening activity was expressed per remaining unit of crural EMG activity than for its costal counterpart. Towards end inspiration/early expiration (IF-EE), when PIA was considered, relatively more crural shortening and shortening per unit EMG persisted.

Since both costal and crural measurements are continuous, have a common time base, and are represented as a continuous cycle on X-Y axis, these graphics have some analogy with a traditional Lissajous interpretation of an X-Y oscilloscopic plot of two signals to estimate the relative phase or asynchrony that exists between the two signals. By this analogy, the phase of costal EMG-shortening and crural EMG-shortening are seen to be slightly different; the electro-mechanical relationships of costal and crural cycles are slightly "out-of-phase", particularly in the time corresponding to the mid to latter portions of inspiratory flow.

A similar presentation of segmental EMG and shortening is shown in Figure 6b. From this graphic the electromechanical coupling for each diaphragm segment during moderate hypercapnia can be compared, and the relative relationship can be contrasted with the status during room air breathing shown in the preceding Figure 6a. Overall the EMG-shortening relation of both segments is markedly different than the relation expressed in Figure 6a; during hypercapnia, the costal and crural EMG-shortening relationships are more divergent than during room air breathing. In the part of the cycle corresponding to early and developing flow (10-IP), relatively more shortening was developed per unit EMG activity of the costal diaphragm segment than for the crural. This contrasts with the relationship seen during early flow during room air breathing in Figure 6a. Although both costal and crural EMG-shortening relationships in early inspiratory flow follow a roughly parallel, linear relationship, both segments appear significantly more "out-of-phase" during the latter inspiratory airflow and expiration (corresponding to IF-EE). As airflow declines into expiration (corresponding to IP-IF), much costal electrical activity abates for relatively little decrease in shortening, compared to the crural segment. Then at the end of the electro-mechanical cycle, costal shortening dives precipitously while costal EMG activity remains relatively constant, in contrast to the linear, more parallel decline of crural EMG-shortening. This latter costal-crural
divergence is consistent with the relatively enduring EMG PIIA of crural, while costal EMG in expiration vanishes precipitously.

Figure 6b. Segmental shortening and EMG activity per breath during moderate hypercapnia, mean ETCO$_2$ 59.5 Torr. Conventions as in Figure 6a. Intrabreath events marked by IO, IP, IF, and EE correspond to times shown in Figures 3-5.

DISCUSSION

Data Summary. In these awake, chronically implanted canines, direct measurements of shortening and EMG activity suggested similar costal and crural diaphragm segmental function for traditional, peak tidal breath values, but revealed costal-crural divergence within the development of each breath cycle. CO$_2$ response curves expressed as segmental shortening or EMG activity per Torr ETCO$_2$ showed similar shortening and EMG responsiveness with CO$_2$ for the two segments. Examining breathing pattern, and variables describing segmental length, shortening and EMG across progressive steps of hypercapnic stimulation from room air ETCO$_2$ 37.9 Torr through ETCO$_2$ of 66.4 Torr, revealed significant sequential increases in respiratory frequency, tidal volume and TI, as well as segmental shortening, velocity of shortening, and EMG activity. But these tidal breathing, segmental responses to hypercapnia could not be differentiated between the two segments at
any level of CO₂. With increasing CO₂ there was a modest, but consistent and significant increase in resting end expiratory length of both diaphragm segments.

Function of costal and crural diaphragm segments during the complete, intra-breath, inspiratory-expiratory cycle was different and distinctive. During each standardized resting breath, relative crural shortening preceded and exceeded costal shortening in earliest inspiration, while costal and especially crural segmental shortening persisted beyond the peak of inspiratory flow into early expiration. This segmental shortening during resting breathing occurred with EMG activity which was more synchronous with inspiratory flow than segmental shortening. During resting breathing, crural EMG activity was greater than costal in earliest inspiration and showed more persistent end inspiratory-expiratory activity. Thus, during resting breathing there was evidence of post inspiratory inspiratory activity (PIIA) expressed in both EMG and shortening of costal and crural segments.

Functional differences of costal and crural segments were accentuated during CO₂ stimulated breathing. At ETCO₂ 59.5 Torr, neither costal nor crural segments could be seen to shorten appreciably as inspiratory flow developed quickly in earliest inspiration. Crural shortening considerably exceeded costal shortening in early inspiration and significantly outlasted costal shortening during expiration. The proportion of shortening activity for both segments that persisted long after the termination of measurable inspiratory airflow was striking, suggesting significant PIIA. Concurrent EMG activity in moderate hypercapnia suggested that much of the observed segmental shortening difference was active. Crural EMG activity significantly preceded costal activity in earliest inspiration and was more dominant into expiration, while costal EMG activity decayed abruptly in synchrony with inspiratory flow. Thus costal EMG PIIA was not present, while crural EMG PIIA was evident.

Finally, examination of EMG function and the shortening that was expressed per unit of EMG for each segment provided insight about the relative electromechanical coupling of the muscles. During CO₂ stimulated and even resting, room air ventilation, the two diaphragm segments appeared to be neuromechanically "out-of-phase". During a CO₂ stimulated breath, but not a room air breath, much more shortening was developed per unit EMG activity of the costal diaphragm segment compared to the crural. And as inspiratory flow declined into expiration during CO₂ stimulation, crural shortening decayed in a steady progression in tandem with decreasing EMG activity (shortening and EMG PIIA), while costal shortening receded abruptly on a relatively constant, minimal EMG activity (shortening without EMG PIIA).
Chronic implantation and normal segmental function. These measurements rest upon the reasonable assumption that the fully recovered, fully active, awake and spontaneously breathing canine with chronically implanted sonomicrometer transducers and EMG wires represents normal diaphragm function. In a previous publication, we have described in detail the techniques of implantation and sequence of recovery that provided a return to normal breathing pattern, uniphasic segmental shortening of appropriate magnitude synchronous with EMG, expected values of maximal segmental shortening with supramaximal phrenic stimulation, and histologic evidence of healthy, nonfibrotic muscle between the sonomicrometer transducers (7). Identical techniques were employed for this canine study group; in accordance with our documentation of post laparotomy/implantation recovery sequence (7) no measurements were made before day 10, and measurements presented here were taken a mean of 16 post operative days after implantation.

Because of inevitable effects imposed by anesthetic and supine posture typical of acute studies, we might expect some difference between the segmental function noted in this group and segmental function described in earlier publications (6,15,22,31,32). Measurements of segmental function in this group might be comparable to spontaneously breathing canines implanted with bipolar EMG electrodes and measured two weeks post-implantation (31,32). However, we retain some wariness regarding the interpretation of chronically implanted, fine wire diaphragm EMG electrodes when no accompanying measurement of segmental length and shortening is available. As noted in a previous publication (7), some apparently healthy, chronically implanted canines retained a long-standing partial inhibition of diaphragm segmental shortening function, and as illustrated in that publication, the presence of phasic, inspiratory EMG activity was not a guarantee that the diaphragm segment was shortening effectively (or at all) during inspiration.

Segmental shortening as recorded from the costal sonomicrometer transducers in these canines may represent shortening throughout the costal segment, if costal segmental shortening is relatively homogeneous as suggested in an early sonomicrometry study of the diaphragm in anesthetized canines (15). However, recent evidence suggests that significant regional differences in shortening may occur within the costal segment (27). If such regional differences are operative in these canines, then these recordings of costal shortening are most representative of the middle region of the costal segment (27). It should be noted that for the canines in this series, the position of the transducers along the costal segment, relative to origin and central tendinous insertion, was consistent.
Presentation and interpretation of the data from these chronically implanted canines depends upon sufficient measurement resolution to justify both the usual whole breath, tidal breathing parameters, as well as the bin style, standardized intrabreath profiles to graphically illustrate within breath events. Since all signals were gathered and permanently stored direct-to-digital, with 12 bit resolution at a sampling rate of 50Hz, the computerized recording and storage of muscle length and moving average EMG occurred with ample resolution and precision for this analysis. Within the breath profiles, the group mean value cited for each of the variables inspiratory flow, and costal and crural segmental shortening represents the mean of 150 to 300 analyzed breaths; mean values for costal and crural EMG represent proportionally fewer numbers of analyzed breaths since costal and crural EMG were available in 7 and 5 of 10 canines, respectively.

In the computer generated, graphic representation of standardized, within breath events of Figures 3-6, interpretation may be assisted by some additional explanation of certain computer calculations. For example, group mean inspiratory flow during resting breathing shows a peak inspiratory flow value which is less than 100%. This is a logical outcome of standardizing all intrabreath values as a percent of the maximum breath value for each animal, and then averaging the results for the entire group at each 5% TTOT. Unless every animal in the group presented a maximum inspiratory flow at exactly 20-25% TTOT, the group mean inspiratory flow value for that interval would be expected to average to something less than 100%. This group averaging of individually standardized intrabreath variables accounts for the maximum group mean values of segmental shortening which are seen to be less than 100%, indicating that maximum shortening did not necessarily occur at exactly the same time in each animal. At first glance, it may seem unusual that each breath "begins" with group mean inspiratory flow already at moderate levels. This is the logical result of standardizing breath duration, slicing each breath into aliquots of 5% TTOT. Thus the first calculations are presented after 5% of TTOT has elapsed, so the first recorded measurement on the X axis for inspiratory flow and segmental function will reflect not the activity at the absolute onset of inspiration, but after 5% of breath time has elapsed. This mode of calculation is evident by the 5% value as the first Y axis point. Since the breath profile is a continuum, events preceding the first 5% are not lost, and can be seen on the Y axis beyond 100% TTOT, as the breath cycle "wraps around" into the next breath.

Functional residual capacity during hypercapnia. In these awake, spontaneously breathing canines rebreathing CO2, there was a small but consistent,
and highly significant increase in the resting lengths of both costal and crural segments. Since the Y axis scaling of Figure 1 was selected to allow presentation of both costal and crural resting lengths on the same graphic, it does not emphasize visually this change in resting length over the range of hypercapnia, and the large standard deviations represent inter animal variability. If the resting lengths were to be illustrated as a change from resting length (as in a previous publication (7)), the magnitude of the resting length change would be accentuated visually. The increase in costal and crural segmental length with hypercapnia was not a statistical artifact. The increases represents approximately 2% and 5.5% of initial resting lengths of costal and crural segments, respectively. The relative magnitude of this change can be appreciated when compared to the tidal shortening during resting ventilation which was 5.1 and 6.5% for costal and crural segments, respectively. Thus the increase in resting end expiratory segmental length was a significant result of the moderate magnitude and consistency of the increase in resting length within each of the 10 animals with progressive increases in ETCO₂.

This increase in resting length contrasted with the equally consistent decrease in resting length with hypercapnia that we recorded in a previous study (6), where segmental resting length was measured by sonomicrometry in the acutely anesthetized, supine canine. Together these results suggest that in this mediumsized mammal, diaphragm segmental resting length decreases and FRC slightly increases during supine CO₂ rebreathing under anesthesia, while segmental length increases and FRC presumably decreases during spontaneous, awake, CO₂ rebreathing in the right lateral decubitus position. We believe that the positional effect on resting diaphragm length of supine and lateral decubitus would be roughly similar. These increases in resting segmental length during wakeful CO₂ rebreathing are in agreement with recent observations that FRC does decrease during wakeful CO₂ rebreathing in supine humans (12).

Such a progressive increase in end expiratory segmental length during CO₂ rebreathing might be related to increasing expiratory abdominal muscle activity, and an associated effect on FRC (14). Since the resting length of costal and crural segments is probably less than optimal length (L₀) during room air breathing (21,8), then this increased resting length during hypercapnia would tend to optimize the length-force characteristics of the segments and improve the pressure generation efficiency of both diaphragm segments during CO₂ stimulated breathing.

**Differential function of costal and crural segments.** Evidence continues to accumulate in support of the concept that costal and crural segments function differentially as separate muscles. The two segments are innervated by different...
phrenic nerve roots, and when stimulated the two segments exert a different mechanical action on the rib cage (4,23). Certain actions reveal clear divergence of costal and crural function, e.g. crural diaphragm activation during gastroesophageal reflexes (1). Apart from a single recent study which suggested that measurements from costal and crural branches of the phrenic nerve were not different in paralyzed decerebrate cats (17), most other existing evidence favors differential innervation, activation, and function of the two segments. Indeed, recent application of EMG and glycogen depletion methods have suggested that the diaphragm may be served as four identifiable, well-delineated motor unit territories corresponding to four primary branches of each phrenic nerve; three costal areas and a single crural area (10). In theory, diaphragm function could be differentiated even more discretely than simply as costal and crural segments.

Previous investigations measuring costal and crural segmental EMG and/or length by sonomicrometry in acutely anesthetized cats and canines, suggests unique activity during respiration for each segment. Evidence to date suggests that: 1) the onset of crural EMG activity precedes costal EMG activity in early inspiration, 2) the associated onset of crural segmental shortening precedes costal shortening in early inspiration, 3) crural EMG and shortening is greater than corresponding costal segmental values, and 4) with CO₂ stimulation there is a proportionally greater increase in crural segmental activity compared to costal (3,15,22,6,31,32).

This awake, chronically implanted group allows the opportunity to expand and refine this view of differential costal-crural function. These awake canines provide a different impression of the degree of predominance of crural compared to costal activity during tidal breathing at room air and during increasing hypercapnia. Peak tidal activity of crural shortening and EMG activity is slightly, but not significantly, greater during resting or hypercapnic stimulated breathing. While this may reflect simply an increased variability in the conscious animal, it can also be attributed to lesser EMG activation and shortening in the nonanesthetized crural diaphragm segment. The information presented by these animals regarding the change in segmental activity during progressive hypercapnic stimulation is clear; there was little difference in ΔCOS EMG/ΔCO₂ or ΔCRU EMG/ΔCO₂, and no difference in ΔCOS LEX/ΔCO₂ and ΔCRU LEX/ΔCO₂. This suggested that, unlike the anesthetized preparation, there is not a progressive, predominant increase in crural activation compared to costal, with increasing ventilatory stimulation.

The generally equivalent costal-crural peak tidal activity shown here during hypercapnia differs from the significantly greater shortening of costal compared to crural segments in a group of chronically implanted, non-anesthetized sheep (29).
Compared to the sheep, these canines exhibited relatively more crural and less costal shortening during hypercapnia. Such differences might be ascribed to ovine compared to canine diaphragm, posture (standing versus RLD), or implantation in different costal regions (27) for the two studies.

Although these chronically implanted canines may advance a lesser role for the crural segment during peak tidal breathing, they emphasize the relative predominance of the crural segment in earliest inspiration. A significantly greater EMG and shortening activity of crural over costal segments is confirmed during resting ventilation, and found to be greatly accentuated during CO₂ stimulated breathing. And this group allows a more complete, temporal definition of flow, shortening and EMG as each breath onsets. One recent study of awake, standing canines with chronically implanted EMG wires suggested that costal and crural segmental EMG activity lagged the onset of inspiratory flow by 19% and 11% of TI, during room air and 6.5% FICO₂ stimulated ventilation, respectively (26). Our canines suggest an alternate sequence of events at the beginning of a breath: diaphragm shortening rather than EMG activity lags the onset of inspiratory flow, with segmental EMG activity preceding evidence of the respective segmental shortening, and diaphragm EMG occurring synchronously with inspiratory flow. A similar but accentuated sequence is observed beginning each CO₂ stimulated breath. The sequence of events we describe suggests that: 1) earliest inspiratory flow is assisted by recoil or nondiaphragm inspiratory muscles, 2) active diaphragm contributions to inspiration logically begins with diaphragm EMG activity which appears to coincide with the very onset of inspiratory flow, 3) the EMG activity results in effective segmental shortening appearing shortly after EMG activity, in early inspiratory airflow.

Post inspiratory inspiratory activity (PIIA). These awake, implanted canines provide new information about inspiratory activity in late inspiration and early expiration for the two diaphragm segments. Such post inspiratory inspiratory activity (PIIA) is interpreted conventionally as having a braking effect on expiratory airflow, to retard the emptying of the lung (19). According to the presence or absence of PIIA, expiration may be viewed as early (Phase I) or later (Phase II), with PIIA present only in Phase I (20). Besides the EMG, our measurements of shortening can imply PIIA, so we differentiate these activities as PIIA EMG and PIIA shortening. Previous investigations of hypercapnia and diaphragm PIIA have provided varying results. EMG activity measured in anesthetized canines suggested that hypercapnia decreased PIIA, but only in the portion of animals who had exhibited relatively prolonged PIIA at rest (16). In awake cats, results were mixed;
hypercapnia reduced or obliterated PIIA only in a modest proportion of animals (9). In awake, spontaneously breathing, standing canines with chronically implanted costal and crural EMG electrodes, EMG PIIA was noted only in four of six animals during room air ventilation, and during hypercapnia EMG PIIA significantly decreased in the diaphragm segments (in the animals where it had been noted) (26). These latter results with EMG wires chronically implanted in canines, share some similarity with the results in this study. However, we found more consistent post inspiratory inspiratory activity of both costal and crural diaphragm segments during room air and CO₂ stimulated ventilation. During hypercapnia there was a striking alteration in diaphragm segmental EMG PIIA. The consistent decrease in costal segmental PIIA EMG activity with relative persistence of crural PIIA EMG activity during hypercapnia was one prominent indication of differential segmental activation in these canines.

Although we recognize the theoretical potential for interaction between the tracheostomy of our canines and post inspiratory inspiratory activity, we believe that any such effect would be slight and would not change our conclusions. Because of the tracheostomy, we might expect that upper airway braking could be decreased (19). If this occurred, then other respiratory muscles would be expected to compensate, increasing PIIA to preserve expiratory braking. If the diaphragm participated, then some compensatory, increased diaphragm PIIA might follow, and our results could include this slightly accentuated PIIA. However, we do not believe that this potential effect would account for the fundamental differences in PIIA of costal and crural segments that we observed during hypercapnia.

Segmental electro-mechanical coupling. These measurements provide new information regarding intrabreath flow, shortening and EMG events. But, can we synthesize the measured, segmental EMG activity with the shortening observed for these two segments, during increasing CO₂ stimulation? Interpretation of the segmental EMG/shortening interaction is complex. We must consider inherent mechanical characteristics of each individual segment which could result in a different shortening given identical EMG activation, superimposed mechanical forces from other respiratory muscles active in expiration and inspiration, and a primary effect in significantly different neural/EMG activation per segment.

The neuromechanical behavior of the two segments can be considered by viewing costal and crural diaphragm as two pumps which are arranged pneumatically in series (13), so that the pressures developed by the two segments on the rib cage are additive while crural segment has its predominant effect upon the abdomen without direct effect on rib cage. Within this scheme, the crural
segment would face abdominal hydrostatic pressure, while the costal diaphragm would face the additional elastance of the rib cage. Thus even for equivalent electrical stimulation, slightly less segmental shortening of costal might be expected. This interpretation seems comfortable during resting breathing, as illustrated by Figure 6a. Since we presume that awake, resting ventilation is carried out primarily by the diaphragm, then the relative differences in phase for costal and crural segments might be explained by slightly different neural activation and different elastance loads, of the segments. The costal loop in Figure 6a would be consistent with the additional neural activity required to overcome rib cage elastance, and the brisker rise and fall of the crural loop might correspond to the lesser elastance load and greater inherent compliance (21) of the crural segment.

In addition to any difference in mechanical advantage per diaphragm segment, the compounding effect upon diaphragm segmental function of other respiratory muscles must be considered. Our interpretation of the costal and crural shortening during moderate hypercapnic stimulation illustrated in Figure 6b must account for the role of abdominal expiratory and rib cage inspiratory muscles. In the supine anesthetized canine, we know that hypercapnia is associated with EMG activity and shortening of the transversus abdominus expiratory muscle (2), and that in the awake standing canine, transversus abdominus EMG activity increases with hypercapnia (25). This abdominal expiratory muscle contraction during hypercapnia (16) would enhance expiratory airflow and affect electromechanical coupling of both costal and crural segments in expiration. Abdominal expiratory activity would also impact on segmental activity in earliest inspiration, since subsequent, passive, diaphragm segmental recoil, along with intercostal/rib cage inspiratory muscles, could contribute significantly to airflow in earliest inspiration which occurred with minimal active segmental shortening. The relatively greater costal shortening per costal EMG seen in Figure 6b during inspiration may partly reflect the additional, abdominal expiratory generated, diaphragm recoil. Costal behavior later in inspiration and early expiration would be consistent with the abrupt decrease in costal PIIA against the higher elastance of the rib cage. Late inspiratory and expiratory movement of the crural segment could correspond to the more enduring crural EMG PIIA and a greater abdominal expiratory muscle influence on the crural segment.

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REFERENCES


Effects of chemical stimulation on segmental function.

Activity of costal and crural diaphragm during progressive hypoxia or hypercapnia.

Thematic overview.

The relative function of costal and crural segments was examined during both progressive hypoxic and hypercapnic stimulated breathing. The differential activities of the two segments were adjusted in a consistent and significantly different fashion by each of the chemical stimulants.

The overall CO₂ and hypoxic responsiveness of the two segments was not measurably different; again this underlined the limitations of whole breath examination to discern activities of individual respiratory muscles. The relative PIIA expressed both in EMG and shortening of the two segments was unique per chemical stimulant, with predominant alterations centred on crural. This suggested a distinctive activity and interaction of each segment, recruited in coordination with other abdominal and rib cage muscles, for each chemical stimulant.
Activity of costal and crural diaphragm during progressive hypoxia or hypercapnia.


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ABSTRACT

Since costal and crural diaphragm segments have different functional characteristics, ventilatory stimulation with hypoxia or hypercapnia may elicit distinctive, differential segmental function. We report measurements of diaphragm segmental length, shortening, and EMG activity from 11 canines that were chronically implanted with sonomicrometry transducers and EMG electrodes then studied a mean of 18 days post implantation (range 9-32 days), while awake and breathing spontaneously during CO₂ rebreathing and progressive isocapnic hypoxia. Ventilatory responses to hypercapnia and progressive hypoxia were moderate at 1.13 (0.31) ΔVI/ΔPaCO₂ and -0.98 (0.51) ΔVI/ΔSaO₂, typically measured in these awake canines to maximum stimuli of 60-65 mmHg PaCO₂ and 65-70 %SaO₂, respectively. Segmental ventilatory response curves expressing costal and crural segmental responsiveness to the chemical stimuli were not different. For the group, peak tidal values for breathing pattern and segmental function were compared at matching mean tidal volumes during CO₂ and hypoxic stimulated ventilation, respectively, corresponding to mean CO₂ of 49.4 PaCO₂ and SaO₂ 77%, respectively. At this level of matched tidal volume, respiratory rate was significantly greater and inspiratory time (TI) significantly less during hypoxia compared to hypercapnia. At matching tidal volume, peak, whole breath calculations did not reveal any significant difference in resting length, tidal shortening, or tidal EMG of costal or crural segments at this level of CO₂ or hypoxic stimulation. Examination of intrabreath profiles of flow, shortening, and EMG activity at these matched tidal volumes showed that inspiratory flow during hypoxia was significantly greater through early inspiration, with a more protracted peak flow, compared to CO₂. In both hypercapnic and hypoxic stimulated breathing, crural shortening preceded costal shortening, with corresponding early activity of crural EMG activity. Compared to resting ventilation, both costal and crural segments showed increased post inspiratory inspiratory activity (PIIA) with chemically stimulated ventilation, but the PIIA activity of the crural segment was significantly greater. During hypoxia, crural shortening PIIA was greater than costal and greater than crural shortening PIIA at similar %Ttot during CO₂ stimulation. Similarly, costal EMG PIIA was greater during hypoxia than hypercapnia, but significantly less than crural EMG PIIA with either chemical stimulus. In the awake, spontaneously breathing canine these results suggest that: 1) costal and crural diaphragm segments exhibit differential function during moderate levels of chemical stimulation, 2) both segments exhibit increased PIIA during stimulated breathing compared to resting ventilation, 3) segmental PIIA is not identical with
hypoxia and hypercapnia, 4) crural PIIA is greater than costal during hypercapnia, and especially during hypoxia.

INDEX TERMS: shortening, sonomicrometry, EMG, nonanesthetized canine, diaphragm segments, CO$_2$, O$_2$, differential function.
INTRODUCTION

Compared to hypercapnia, there is a traditional expectation that hypoxic ventilatory stimulation is characterized by a relatively greater change in respiratory rate (13,24,6,19). Besides such frequency dependent effects, evidence continues to accumulate suggesting that there are fundamental differences in respiratory muscle recruitment between hypoxia and hypercapnia. Differential effects of hypoxia and hypercapnia on upper airway muscles, glottic aperture, and expiratory braking in humans (10,2,20) have been documented. Hypoxia and hypercapnia have a measurable effect upon post inspiratory inspiratory (PIIA) activity of certain inspiratory muscles, and the relative activity of some expiratory muscles (15,16,22,34,3).

There is increasing support for costal and crural diaphragm segments as separate muscles. Differing segmental innervation and mechanical actions on the rib cage (5,28), as well as a closer crural association with gastroesophageal events (1), have been noted. In theory, the two segments could be affected differently by chemical stimuli. But to date, there has been little direct information about the relative effects of hypoxia and hypercapnia on the costal and crural diaphragm segments, especially in awake, intact mammals. In anesthetized cats, hypoxia and hypercapnia are known to have differential effects on diaphragm PIIA (22), while in awake canines, hypercapnia has shown variable effects on diaphragm segmental activity (30,7).

What is the relative effect of hypoxic and hypercapnic stimulated ventilation on costal and crural segmental function? Are the segments activated differentially by these chemical stimulants in the awake intact mammal? To address these questions, we measured length, shortening and EMG activity of the left hemidiaphragm during CO₂ and hypoxic stimulated breathing in awake canines, which previously had been chronically implanted with sonomicrometry transducers and bipolar EMG electrodes and recovered to normal function.

METHODS

Surgical Preparation. Each tracheostomized mongrel dog had sonomicrometry transducers and EMG wires implanted in costal and crural diaphragm. Animals were studied after diaphragm segmental shortening had recovered. The technique of chronic sonomicrometry and EMG implantation, and the 7-10 day progressive recovery of diaphragm segmental shortening, has been described fully elsewhere (9). Briefly, implantation was performed under general
anesthesia with thiopentol sodium induction and halothane. The left diaphragm was exposed through a mid-abdominal incision and ultrasonic transducers were implanted between muscle fibres on a flat portion of each of the costal and crural segments of the hemidiaphragm. Costal transducers were implanted in the mid-portion of the costal segment approximately midway between central tendon and chest wall in the region corresponding roughly to the SC2 branch of the phrenic nerve (14,32). Crural transducers were inserted posteriorly in the mid-perivertebral aspect of the muscle. On each segment immediately adjacent to each pair of transducers, a fine wire stainless steel bipolar electromyogram electrode was attached. All implants were secured by fine, synthetic, non-fibrogenic sutures (Prolene, Ethicon Ltd.). All wires were externalized by a subcutaneous skin tunnel and the animals were recovered.

**Measurement techniques.** Measurement of resting, CO₂, and hypoxic stimulated ventilation and of respiratory muscle function were performed with the animals awake and breathing quietly while lying in the right lateral decubitus position. The animals were familiar with the location, routine and personnel of the recordings. All measurements were made with the animals breathing through the tracheostomy which was attached through a unidirectional valve to a low resistance open breathing circuit (<1 cm H₂O/L/S), connected to a pneumotachograph (Fleish #2) for measurement of airflow. On the expiratory limb, CO₂ was sampled and analyzed continuously (Infared model CO₂ meter, Emetek, Thermox Instruments Division, Pittsburgh, Pennsylvania). Continuous measurements were made of inspiratory airflow, and costal and crural, length, shortening and EMG.

Ventilatory and diaphragmatic responses to progressive hypercapnia were elicited by a modification of the Reid technique (23), rebreathing 6% CO₂-93% O₂ from a 4-5 liter bag. Ventilatory and diaphragm segmental responses to hypoxia were elicited by a modification of the steady state technique of Weil et al (38,25). Progressive isocapnic hypoxia was elicited by continuous addition of nitrogen and CO₂ to a room air gas mixture to progressively decrease FIO₂, with SAO₂ monitored continuously by pulse oximeter (Pulse Oximeter, Biox 3700, Ohmeda, Bolder, Colorado, USA).

Dynamic measurement of the changing distance between the sonomicrometer transducers of each pair was provided by the sonomicrometer (Model 120, Triton Technology, San Diego, CA). Measurement of diaphragm length by sonomicrometry has been described in detail (21). Briefly, when electrically excited, the emitter piezoelectric transducer resonates, radiating ultrasonic waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a
measurable voltage. A quartz crystal clock also measures the transit time of the waves and because the conduction of velocity in muscle is known, the sonomicrometer provides the inter-transducer distance. EMG signals from the wire electrodes were amplified (Mark III, TECA, White Plains, NY) and band pass filtered (16 Hz-1.6 KHz). The output signals were then rectified and processed by passage through resistance-capacitance, leaky "integrators" with a time constant of 100 ms, to provide moving averages of the electromyograms of costal and crural diaphragm.

Analysis of Ventilation. All signals were recorded on a strip chart recorder. Simultaneously, signals were monitored on-screen and sampled at 50 Hz by a single board analog-to-digital system (Model 2801-A, Data Translation, Marlboro, MA) directly to hard disk on a microcomputer (PC, IBM, Boca Raton, FL) for subsequent examination using a series of dedicated acquisition and analysis programs written by one of the authors (PE). The flow signal was evaluated for respiratory timing and digitally integrated; minute ventilation (V\text{\textsubscript{e}}), respiratory frequency (F\text{\textsubscript{REQ}}), tidal volume (V\text{\textsubscript{T}}), inspiratory time (T\text{\textsubscript{I}}), mean inspiratory flow (V\text{\textsubscript{T}}/T\text{\textsubscript{I}}), and inspiratory fraction of respiration (T\text{\textsubscript{I}}/T\text{\textsubscript{tot}}) were calculated breath-by-breath.

Whole breath analysis. Using the sonomicrometry data from implanted muscle, the computer algorithm identified the muscle length for each breath which corresponded to the onset of inspiratory flow. In each breath, the computer compared this value to the data samples of muscle length in the final third of the preceding expiration and identified the maximum resting end expiratory muscle length. This baseline, resting length at end expiration in mm was titled L\text{BL}, i.e. baseline length prior to the onset of muscle shortening, in inspiration. This length has been titled L\text{FRC} in some of our previous reports (8,9), but L\text{BL} is utilized here and preferred since L\text{BL} can be extended to any inspiratory or expiratory muscle. From this baseline, end expiratory length, the shortening for each breath was expressed as a percentage change from resting length entitled %L\text{BL}. EMG activity was quantified arbitrarily per breath as baseline value in volts (EMG\text{BL}), and the maximum difference in volts (V) between baseline EMG and the peak height of the moving average EMG signal expressed as EMG\text{DIFF}.

These measurements defined whole breath or "tidal" breath activity of inspiratory flow and respiratory timing, as well as costal and crural diaphragm length, shortening, and EMG activity. Breath by breath values of breathing pattern, length, shortening, and peak difference of moving average EMG, where compared for resting ventilation preceding CO\textsubscript{2} and hypoxic stimulated ventilation. In order to compare the relative effect of hypercapnia and hypoxia on the diaphragm segments, for each animal, sections of stimulated ventilation were selected from the
hypercapnic and hypoxic response curves during moderate levels of each chemical stimulus to provide matching tidal volumes. For the group, all values of muscle length, shortening, and EMG were then compared between these tidal volume matched sections of hypercapnic and hypoxic stimulated ventilation.

**Intrabreath Analysis.** After calculating peak tidal, whole breath values, the computer algorithm calculated the intrabreath development or "shape" of inspiratory airflow, diaphragm shortening, and EMG activity for each breath. Briefly, for each whole breath, the peak inspiratory airflow, maximum segmental shortening and peak EMG activity were identified. Then throughout the duration of each breath (Ttot), the percent of peak segmental activity was determined after each successive 5% "slice" of the total time of the breath (%Ttot). That is at each 5% increment, inspiratory flow, segmental shortening and EMG activity were expressed as a percent of the maximum tidal value for the breath. This created a profile for each breath of segmental shortening and EMG standardized as a percentage of the peak whole breath value, against time standardized as a percent of Ttot. The breath profiles were calculated for resting, CO₂ stimulated, and hypoxic stimulated ventilation for each canine and then averaged for the group.

**Statistical Analysis.** After calculation, mean values were exported for review to spreadsheet software (Microsoft Excel, Microsoft, Redmond, WA), to graphic software to output figures 1-4 and to the PC version of SAS (29), for statistical analysis. Ventilatory (Vt), VT, segmental shortening, and EMG responses to hypercapnia ($\Delta V_{t}/\Delta P_{aCO_2}$, $\Delta V_{T}/\Delta P_{aCO_2}$, $\Delta COS \%L_{BL}/\Delta P_{aCO_2}$, $\Delta CRU \%L_{BL}/\Delta P_{aCO_2}$), were calculated by linear regression using the method of least squares (33,29); equivalent responses to progressive hypoxia were calculated as well. Mean values for parameters of breathing pattern, segmental length and shortening, and EMG activity were tested between the two conditions of resting ventilation, and between CO₂ and hypoxic stimulated ventilation, by paired t test (33,29). Intrabreath results were examined graphically by plotting the group mean standardized breath profiles for baseline and the two conditions of chemical stimulation, with inter-animal variability expressed as SEM.

**RESULTS**

Measurements were made during resting, hypoxic, and CO₂ stimulated ventilation in 11 chronically instrumented canines, mean weight 24 kg. The studies were conducted an average of 18 days after chronic implantation of sonomicrometry transducers and EMG electrodes, range 9-32 days post-implantation. Durability of
implanted transducers and electrodes was not universal; we report measurements for costal and crural shortening in 11 animals and for costal and crural EMG in 7 animals, respectively.

Ventilation and breathing pattern were virtually identical during resting breathing preceding either CO₂ or hypoxic stimulated ventilation. Mean VT and FREQ were 0.28 (standard deviation 0.04) and 0.30 (0.05) L, and 19.0 (4.1) and 18.7 (3.6) b/min for resting pre-CO₂ and resting pre-hypoxia, respectively. Mean VI was 5.78 (1.8) and 5.94 (2.1) L/min, for resting pre-CO₂ and pre-hypoxia ventilation, respectively. Since resting baseline ventilation was equivalent for both conditions, we present the values of length, shortening, and EMG activity for costal and crural as a single mean value for all resting measurements.

Ventilatory and segmental responses. Ventilatory responses to hypercapnia and to progressive hypoxia were 1.13 (0.31) ΔL/ΔPaCO₂ and -0.98 (0.51) ΔL/ΔSaO₂, typically measured in these awake canines to maximum stimuli of 60-65 mmHg PaCO₂ and 65-70 %SaO₂, respectively. To express the ventilatory chemoresponse more directly for each diaphragm segment, costal and crural segmental shortening during CO₂ and hypoxia, %LLBJ/ΔPaCO₂ and Δ%LLBJ/ΔSaO₂, were calculated. Typical segmental ventilatory response curves from a single animal are shown in Figures 1a,1b, expressed along with ΔVT/ΔPaCO₂ and ΔVT/ΔSaO₂. As seen in Figure 1a, costal and crural segmental CO₂ responsiveness was 0.34 and 0.37 %LLBJ/ΔPaCO₂, respectively. Similarly in figure 1b, costal and crural segmental hypoxic responsiveness was -0.36 and -0.37 %LLBJ/ΔSaO₂, respectively. Thus, costal and crural segmental chemoresponsiveness was not different at least in these awake animals over the range of stimuli studied.
Figure 1a. Costal and crural segmental response during progressive hypercapnia. Ventilatory response during CO₂ rebreathing, expressed by VT, costal and crural shortening. X axis shows ETCO₂ in mmHg. Y axis (left) shows amplitude of VT (L); values for VT marked by dotted line and solid triangles. Y axis (right) shows amplitude of segmental shortening in %LBL. Values for costal shortening marked by solid line and open circles, for crural shortening by solid line and closed squares.

Figure 1b. Costal and crural segmental response to progressive hypoxia. Ventilatory response to progressive isocapnic hypoxia, expressed by VT, costal and crural shortening. X axis shows % SaO₂. Other symbols and conventions as in figure 1a.
Tidal values. For the group, peak tidal values for breathing pattern and segmental function were compared at a matching mean tidal volume of 0.69 (0.14) and 0.60 (0.08) L, during CO₂ and hypoxic stimulated ventilation, respectively. This corresponded to a mean CO₂ stimulus of 49.4 PaCO₂ and SaO₂ 77%, respectively.

At this level of matched tidal volume, peak tidal values showed some differences in parameters of breathing pattern. Mean VI was 14.45 (3.75) and 16.43 (2.95) L/min for CO₂ and hypoxia respectively (diff NS). Any difference in VI was accounted for by greater FREQ during hypoxia, 29.6 (4.8), compared to 20.4 (3.1) L/min for CO₂ (p<0.01). Mean TI was greater during CO₂ than during hypoxia; at matching VT, TI during CO₂ was 1.23 (0.20) sec compared to 0.92 (0.13) sec during hypoxia, (p<0.01). However, with the relative increase in FREQ during hypoxia, corresponding TI/Ttot was not different between CO₂ and hypoxia, at 0.42 (0.05) and 0.45 (0.04) respectively.

Whole breath values for segmental function revealed a mean costal LBL of 16.08 (4.96) mm at rest, compared to 16.18 (5.02) and 15.87 (4.83) mm during CO₂ and hypoxia, respectively (hypoxic and hypercapnic diff NS). Similarly, mean crural LBL was 17.54 (5.79) mm at rest, 17.63 (5.98) and 17.46 (5.97) mm during CO₂ and hypoxia respectively (diff NS). Thus in these animals, there was no measurable change in resting length of costal or crural segments with either CO₂ or hypoxic stimulation. A similar equivalence was noted for tidal shortening of the segments for the two chemical stimulants. Mean costal shortening was 3.88 (3.73) %LBL at rest, increasing significantly to 8.23 (6.20) and 8.38 (7.22) %LBL during CO₂ and hypoxia, respectively. Similarly, mean crural shortening of 3.90 (2.65) %LBL increased to 8.11 (5.72) and 8.49 (4.94) %LBL during CO₂ and hypoxia, respectively (diff NS). So at this matched VT, mean shortening of both costal and crural segments was not different between CO₂ and hypoxia. This rough segmental equivalence was expressed in tidal segmental EMG values as well. Mean costal EMGDIFF increased significantly from 2.76 (0.78) volts (V) at rest, to 3.88 (1.84) and 3.93 (1.75) V during CO₂ and hypoxia, respectively (diff NS). Crural EMGDIFF was 3.50 (1.41) V at rest, increasing significantly to 4.92 (1.67) and 4.35 (1.59) V during CO₂ and hypoxia (diff NS).

Intrabreath segmental activity. Examination of the normalized intrabreath profiles of inspiratory airflow, segmental shortening, and segmental EMG, at the same matched values of tidal volume during hypercapnic and hypoxic stimulated
ventilation, revealed the relative activity of each muscle dynamically throughout each breath. These profiles of shape and segmental activity are shown in Figures 2-4. Unlike the approximate equivalence of segments suggested by peak tidal values, distinctive activities of the individual segments were apparent on examination of intrabreath activity. Beginning with room air, intrabreath shortening and EMG are shown in Figures 2a and 2b. Scrutiny of these resting ventilation figures revealed that, as seen in Figure 2a, costal shortening peaked earlier than crural, and both segments showed some post inspiratory inspiratory activity (PIIA) but crural segmental PIIA predominated. From Figure 2b, costal segmental EMG can be seen to more closely approximate inspiratory flow than did crural EMG; costal EMG had its onset with flow and showed minimal PIIA. Meanwhile crural EMG can be seen to be active prior to inspiratory flow, with more PIIA.

Figure 2a. Costal and crural segmental shortening during resting ventilation. Normalized intrabreath VT, costal and crural shortening, during room air ventilation. Y axis shows amplitude of mean shortening, standardized as percent of maximum value per individual breath. X axis shows time scale, with standardized values noted sequentially after each 5% of total breath time (Ttot). All breath profiles averaged per animal; mean results for all animals are shown, with representative variance indicated for each curve as ±SEM. Values for inspiratory flow marked by dotted line and solid triangles, costal shortening marked by solid line and open circles, crural shortening by solid line and closed squares.
As ventilation increased, at equivalent CO₂ and hypoxic stimulated tidal volumes, the breath profile of inspiratory flow was noticeably different (Figures 3a,3b and 4a,4b). Inspiratory flow with hypoxia was significantly greater through early inspiration, with a more protracted peak flow.

As seen in Figures 3a,4a, in both hypercapnic and hypoxic stimulated breathing, crural shortening preceded costal shortening, even though peak shortening of costal then occurred earlier than crural. However, the timing of both segments relative to inspiratory airflow was different; more inspiratory airflow was evident during hypoxia before significant shortening of either costal or crural diaphragm was measurable. Scrutiny of the corresponding EMG profiles in Figures 3b, 4b, confirmed the earlier activity of crural compared to costal segment.
Figure 3a. Costal and crural segmental shortening during CO$_2$ stimulated ventilation. Normalized intrabreath VT, costal and crural shortening, during CO$_2$ stimulated ventilation, mean ETCO$_2$ 49.4 mmHg, VT 0.69 L. Y axis shows amplitude of mean shortening, standardized as percent of maximum value per individual breath. X axis shows time scale, with standardized values noted sequentially after each 5% of total breath time (Ttot). All breath profiles averaged per animal; mean results for all animals are shown, with representative variance indicated for each curve as ±SEM. Values for inspiratory flow marked by dotted line and solid triangles, costal shortening marked by solid line and open circles, crural shortening by solid line and closed squares.
The distinctive character of the segments was seen most clearly in the differing post-inspiratory inspiratory activity (PIIA) of costal and crural during CO$_2$ and hypoxic stimulated breathing. Segmental shortening during CO$_2$ stimulation, in Figure 3a, showed that costal peaked earlier and showed less post inspiratory activity than was seen for the crural segment. Segmental shortening during hypoxic stimulation, in Figure 4a, also showed costal shortening peaking before crural with less costal post inspiratory activity than was seen for the crural segment. When the relative peak shortening and post inspiratory activity of costal and crural were compared between CO$_2$ and hypoxia, in Figures 3a and 4a, segmental differences were apparent. Relative to the inspiratory flow envelope, costal shortening peaked later and showed more persistent post inspiratory activity with hypoxia in Figure 4a, than with hypercapnia in Figure 3a. Crural post inspiratory activity was even
more distinct; as seen in Figure 4a, during hypoxia, crural shortening activity persisted well into expiration, and PIIA was obviously greater than was seen with CO₂ in Figure 3a.

These observations of post inspiratory segmental shortening were underpinned by the observed post inspiratory EMG activity shown in Figures 3b,4b. As seen in Figure 3b during CO₂ stimulation, relative to the inspiratory flow envelope, modest costal EMG was noted in post inspiration with significantly greater crural EMG PIIA activity. Segmental EMG activities were not identical during hypoxia. As seen in Figure 4b, costal post inspiratory EMG activity was greater for a comparable %Ttot than during CO₂ in Figure 3b. And, crural PIIA EMG activity during hypoxia was significantly greater than during CO₂, remaining especially active during post inspiration. Thus both shortening and EMG activities of costal and crural segments were distinctive during CO₂ and hypoxic stimulated ventilation.

Figure 4a. Costal and crural segmental shortening during hypoxic stimulated ventilation. Normalized intrabreath VT, costal and crural shortening, during hypoxic stimulated ventilation, mean SaO₂ 77 %, VT 0.60 L. Y axis shows amplitude of mean shortening, standardized as percent of maximum value per individual breath. X axis shows time scale, with standardized values noted sequentially after each 5% of total breath time (Ttot). All breath profiles averaged per animal; mean results for all animals are shown, with representative variance indicated for each curve as ±SEM. Values for inspiratory flow marked by dotted
DISCUSSION

Data Summary. The ventilatory response curves showed these canines to be moderately responsive to hypoxia and hypercapnia; based solely upon these ventilatory curves, costal and crural segmental responsiveness could not be differentiated for the two chemical stimuli. Examination at matching levels of tidal volume induced by moderate levels of CO₂ and hypoxia, showed some differences in breathing pattern with greater FREQ and lesser TI associated with hypoxic stimulation. Yet, at matching tidal volumes, peak, whole breath, calculations revealed that there was no significant difference in resting length, tidal shortening, or tidal EMG of costal or crural segments at this level of CO₂ or hypoxic
stimulation. The distinctive nature of the segmental response to CO₂ and hypoxia was revealed by examination of intrabreath profiles of flow, shortening, and EMG activity. At equivalent stimulated tidal volumes, the profile of inspiratory flow with hypoxia was significantly greater through early inspiration, with a more protracted peak flow, compared to CO₂. In both hypercapnic and hypoxic stimulated breathing, crural shortening preceded costal shortening, with corresponding early activity of crural EMG activity. Compared to resting, both costal and crural segments showed increased post inspiratory activity with either CO₂ or hypoxic stimulation. However, the PIIA activity of the crural segment was significantly greater. During hypoxia, crural shortening persisted well beyond inspiratory flow and crural post inspiratory shortening during hypoxia was obviously greater than costal shortening, and greater than crural shortening at similar %Ttot during CO₂ stimulation. Similarly, post inspiratory costal EMG activity was greater during hypoxia than hypercapnia, but costal PIIA EMG activity was significantly less than crural EMG activity with either chemical stimulus.

For the computer-generated normalized profiles of within-breath shortening and EMG in Figures 2-4, some additional explanation of computer calculations may be helpful. For example, group mean inspiratory flow during resting breathing sometimes shows a peak inspiratory flow value of less than 100%. This is a logical outcome of standardizing all intrabreath values as percent of the maximum breath value for each animal and then averaging the results for the entire group at each 5% Ttot. Unless every animal presented a maximum inspiratory flow at exactly the same moment, the group mean value would be expected to average to something less than 100%. Similarly, group mean values of segmental shortening are seen to be less than 100% in some instances because the maximum shortening per muscle did not necessarily occur at exactly the same time for each animal. Each breath "begins" with group mean inspiratory flow already at moderate levels, because the first calculations are presented after 5% of Ttot has elapsed. Since the breath profiles are a continuum, events preceding the first 5% are not lost and can be seen on the Y-axis at 100% Ttot, as the breath cycle "wraps around" into the next breath.

Chronic Implantation. These measurements rest upon the reasonable assumption that the fully recovered, awake and spontaneously breathing canine with chronically implanted sonomicrometer transducers and EMG wires presents normal function of the diaphragm segments at rest and when ventilation is stimulated by CO₂ and hypoxia.

In a previous publication, we have described in detail the techniques of implantation and sequence of recovery as the diaphragm returns to normal
breathing pattern and uniphasic segmental shortening of appropriate magnitude synchronous with EMG (9). Identical techniques were employed in this study. Based upon the time sequence of segmental recovery demonstrated in our previous publication, it is important to note that no measurements were made before day 9 and measurements presented here were taken a mean of 18 post-operative days after implantation. The validity of this chronic sonomicrometry implantation technique, including the sequence of segmental recovery with time, robust maximal shortening, and histologic preservation of the diaphragm segments after implantation has been confirmed by other investigators utilizing chronically implanted sheep (35). Between the two chronically implanted preparations, canines and sheep, only the recovery time after implantation differed, and presumably the longer ovine recovery is attributable to an intra-thoracic route of implantation.

**Costal and crural segmental function: awake.** Segmental shortening as recorded from the costal sonomicrometry transducers in these canines may represent shortening throughout the costal segment, if shortening is as homogeneous as suggested in an early sonomicrometer study of the diaphragm in anesthetized canines (21). However, recent evidence suggests that significant regional differences in shortening may occur within the costal segment (37,32), as measured in anesthetized, supine canines. Even if such regional differences are operative in these canines, these recordings of costal shortening were extremely consistent within each canine, and remain representative of the middle region of the costal segment per each animal, corresponding roughly to the costal region innervated by the second phrenic costal branch of the phrenic nerve (14). For the canines in this series, the position of the transducers along the costal segment relative to the origin and central tendinous insertion was consistently placed from animal to animal.

These results are consistent with existing costal and crural segmental data, including shortening and EMG, in intact, awake mammals. Segmental data to date is restricted to resting and hypercapnic stimulated ventilation. Comparable results have been noted in sheep which were chronically implanted with sonomicrometry transducers and EMG wires (35). The sheep showed somewhat larger values of segmental shortening during resting ventilation and during maximum hypercapnic stimulation, than recorded with these canines. Any differences in resting values of segmental shortening between sheep and canines can be attributed to species difference and posture, since the sheep were recorded in a standing position. The greater ovine maximum shortening with hypercapnia is explained by the levels of CO₂ stimulation, which for the sheep extended to 10.5% ETCO₂. Although our
canine measurements were at lesser levels of chemostimulation, segmental chemoresponsiveness was equivalent to, or greater than, the ovine response, since these canines achieved a doubling of costal and crural shortening over a modest range of chemostimulation.

Results presented here are also consistent with data from other canines implanted by the same techniques, for which we reported costal and crural segmental function during hypercapnic stimulation in a previous publication (7). The canines reported here show equivalent segmental results for hypercapnia, with the same costal-crural divergence, especially during post inspiration. An interesting, subtle difference between our previous series and this group is the degree of costal PI2A we noted during CO2 stimulated breathing. In these animals, we noted slightly more costal shortening and EMG activity during post inspiration (Figure 3a,3b) at a lesser level of CO2 stimulation (PaCO2 49.4) than in the previous group (7, Figure 4a,4b), (PaCO2 59.5). This difference in costal PI2A with level of hypercapnia suggests that costal, but not crural, segmental activity during post inspiration may increase with moderate CO2 stimulation then recedes at the highest levels of chemically stimulated breathing.

Some additional information about the differential function of the diaphragm segments has been provided by recent investigations utilizing 6 intact, awake, canines implanted with bipolar EMG wires in costal and crural segments and studied in the standing position two weeks post implantation (30,31). These studies suggested that costal PI2A was more extensive than crural, and that with hypercapnia or normocapnic hypoxia, PI2A of crural and especially costal tended to remain unchanged or decrease. These studies also suggested that diaphragm EMG activity lagged behind the onset of inspiratory flow, by 23% of TI during hypercapnic stimulated breathing. These studies do agree generally with our assertion here that chemical stimulation can alter PI2A, and that such alteration is not equivalent for all muscles. However, our results in these and other animals (7), suggest a crural predominance in PI2A. Although our animals were studied in the lateral decubitus position rather than standing, this difference in posture probably cannot fully reconcile our results with the reported lag of diaphragm EMG after the onset of inspiratory flow (31). And, an EMG lag was not seen in sonomicrometry and EMG implanted sheep in the standing position (35). Given the probable heterogeneity of the canine costal segment (32,37), relative differences in costal segmental recording may contribute to these differing observations regarding costal PI2A. More complete reconciliation will have to await studies of the effect of
Costal and crural diaphragm: Results
Progressive hypoxia and hypercapnia

Costal and crural segmental function: anesthetized. Even in the first measurements of costal and crural diaphragm length by sonomicrometry in the acute anesthetized preparation, Newman et al. suggested that costal and crural shortening was not identical during normocapnia (21). Subsequently, Van Lunteren, et al. (36), suggested that electrical stimulation elicited by CO₂ rebreathing had a nonuniform effect upon the two diaphragm segments. Crural diaphragm was noted to have a greater relative increase in EMG activity with hypercapnia than was noted for costal EMG. Measurements of costal and crural segmental length by sonomicrometry along with EMG in the anesthetized canine (27) provided further information that the segments were not identical during hypoxia and hypercapnia. In these supine anesthetized canines, crural diaphragm shortened more than costal with both types of chemostimulation and the velocity of shortening of both costal and crural diaphragm was greater during hypoxia than hypercapnia. In addition, increasing degrees of hypoxic stimulation resulted in a reduction in resting length of both diaphragm segments, and a slight relative increase in recruitment and shortening of costal diaphragm (27). Measurements in acutely anesthetized canines by other investigators have shown similar results. Anesthetized canines with bipolar EMG wires demonstrated a greater peak and rate of rise of crural EMG compared to costal, at all levels of hypoxia (4), as well as an earlier onset of electrical activity of the crural diaphragm over much of the range of hypoxia (4). These findings in acute anesthetized are not identical to our chronic results. Presumably the measured differences in outcome are related at least in part to the presence of anaesthetic and supine position of the canines in the aforementioned studies. In addition, the level of chemostimulation delivered to the acutely anesthetized animals considerably exceeded the degree of hypoxia and hypercapnia that could be induced in our awake, chronically implanted canines. In these studies, our lack of changes in segmental resting length and equivalence of costal and crural segmental shortening may be typical of segmental function during the degree of chemostimulation which is available in the awake canine, rather than the more extensive, theoretic response which can be elicited in an unconscious preparation with extreme chemostimulation.

Differential respiratory muscle activity during hypercapnia or hypoxia. These measurements of costal and crural function during hypercapnic and hypoxic stimulation must also be integrated with current information regarding other respiratory muscles during chemical stimulation.
Compared to hypercapnia, there is a traditional and controversial claim that ventilatory stimulation by hypoxia is characterized by a disproportionately greater increase in breathing rate than in total volume. Hypercapnia is believed to be related to an alternative breathing pattern which is more tidal volume dependent, and less frequency dependent (13,24,6,19). Our results suggested for these canines, that at equivalent levels of VI there was a different breathing pattern based upon a relatively greater respiratory rate.

A large and expanding body of evidence suggests that there are distinctive, fundamental differences in respiratory muscle recruitment with hypoxia and hypercapnia. In this study, even the overall shape of the inspiratory flow envelope suggested that net differential respiratory muscle function was significantly different for the two chemostimulants for these canines. Clear differential effects of hypoxia and hypercapnia on upper airway muscles have been shown. In humans (10,2) during expiration, the usual expiratory narrowing of the glottis is decreased substantially during hypercapnia but not during hypoxia. With hypoxia, the vocal cords actually move closer to midline than with equivalent ventilation stimulated by hypercapnia (19). There is one recent observation that at comparable tidal volumes, there is a relatively greater decrease in phasic activity of the thyroarytenoid (TA) muscle, a vocal cord adductor, with hypoxia compared to hypercapnia (20). These studies suggest that hypoxia tends to increase upper airway expiratory resistance and influence "braking" of expiratory outflow compared to hypercapnia.

The relation of diaphragm, intercostals, and other lower respiratory muscles to the changes in resistance effected by the upper airway muscles requires further study. For example, in one experiment in awake, intact, chronically implanted cats, Haxhiu et al. (17) has shown that hypercapnia preferentially increased EMG of genioglossus compared to posterior cricoarytenoid (PCA) or diaphragm.

Segmental activity and braking. Other investigations have demonstrated that hypoxia and hypercapnia have a measurable effect upon post inspiratory activity of certain inspiratory muscles (PIIA), and the interaction between inspiratory and expiratory muscle activities. Years ago it was suggested that hypercapnia decreases the post inspiratory activity and inhibits the braking effects of inspiratory muscles (26,12), while more recent studies suggest that hypoxia may have the opposite effect, to prolong post inspiratory activity (PIIA) (15,16). In anesthetized cats, isocapnic hypoxia was found to increase PIIA of diaphragm, whereas hypercapnia shortened the duration of PIIA (22). Our results extend these
observations by quantifying the relative effect of hypoxia and hypercapnia on diaphragm shortening and the differential effect on PIIA of the two segments.

Finally, the adjustments in inspiratory braking with hypoxia compared to hypercapnia do not occur in isolation but in conjunction with changes in expiratory muscle activity. Measurements of efferent activity from the cranial ilohypogastric nerve in decerebrate, paralyzed, vagotomized and ventilated cats showed that hypercapnia enhanced abdominal expiratory activity while isocapnic hypoxia caused inhibition of abdominal nerve discharge (11). Similarly, in awake humans, one report suggested that there was a greater peak diaphragmatic EMG activity for a given minute ventilation for hypoxia than hypercapnia, and that hypercapnia caused a more consistent recruitment of abdominal expiratory muscles at lower minute ventilation, than did hypoxia (34). In the context of this study of costal and crural function, it is of interest that the aforementioned study (34) measured diaphragm EMG using an esophageal electrode and presumably reflects essentially the crural diaphragm. In awake ponies EMG was measured from implanted wires in crural diaphragm and transversus abdominis, and showed that hypoxia increased diaphragm, and decreased transverses abdominis EMG (3). Finally, recent preliminary work with our chronically implanted canines, in which we have included EMG and length measurements of transversus abdominis in addition to the diaphragm segments, suggests that compared to hypercapnia, hypoxia elicits relatively more greater PIIA of crural segment compared to costal, and relatively greater inhibition of transversus abdominis shortening (18). Altogether, evidence to date seems to suggest that compared to hypercapnia, hypoxia elicits a relatively greater degree of braking of expiration through effects on upper airway, costal and especially crural diaphragm, with associated inhibition of abdominal expiratory muscle function.

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Segmental participation in reflexic events

Costal and crural diaphragm function during panting in awake canines. Manuscript

Thematic overview.

During some reflexic or involuntary events in which the respiratory system is a participant, the recruitment pattern of the respiratory muscles appears centrally preprogrammed and "automatic". In this study we examined the diaphragm, by direct measurement of length and EMG, to determine if the individual segments were recruited in a unique or automatic pattern within the coordinated respiratory muscle actions of thermal panting.

We found that in this centrally orchestrated, partially "non-respiratory" activity, of thermal regulatory panting, the segments were subject to unique differential activation. During normal respiration, shortening of costal and crural segments was synchronous with inspiratory airflow; during panting, costal segment continued to shorten with inspiratory airflow, while crural shortening occurred in expiration, out of phase with costal motion. The divergence in shortening activity of the segments during panting was accompanied by a shift in timing of costal and crural segmental EMG. This costal and crural segmental asynchrony could contribute significantly to gas mixing in a manner akin to high frequency ventilation (HFV).
Costal and crural diaphragm function during panting
in awake canines.

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ABSTRACT

During natural panting for thermal regulation, the pattern of activation of the major respiratory muscles is not known. The diaphragm segments are known to exhibit distinctive function during some respiratory maneuvers, but the role of costal and crural diaphragm in panting is not known. We report measurements of diaphragm segmental length, shortening, and EMG activity from five canines chronically implanted with sonomicrometry transducers and bipolar EMG electrodes, then studied a mean of 16 days post-implantation, while awake and breathing spontaneously. The animals were measured at rest, and then during the presentation of a mild, dry heat stress. During panting, the animals increased minute ventilation four-fold from 5.07 L/min, and increased respiratory rate from 16.9 breaths per minute to panting frequencies of 192.8 breaths per minute, or 3.2 HZ. From resting values of 0.32 and 0.62 L/sec, TI/Ttot and VT/TI increased to 0.56 and 0.59 respectively. During panting, end expiratory length of both costal and crural segments decreased significantly. From resting end expiratory length of 15.75 and 16.86 mm, costal and crural length decreased to 13.99 and 15.21 mm respectively. This was associated with a concurrent increase in end expiratory moving average EMG. With the onset of panting, tidal shortening of costal segment decreased from 6.29% of end expiratory length to 3.54%, while crural shortening decreased from 6.04 to 2.46%. While segmental shortening during panting decreased, the amplitude of segmental tidal EMG difference increased. Costal EMG difference increased from 1.48 to 1.69 volts (V), while crural segmental EMG increased from 1.51 to 2.06 V. Examination of costal and crural segmental function within each breath revealed distinctive differential activation during panting. Compared to the synchronous shortening of costal and crural segments with inspiratory airflow during normal respiration, during panting the costal segment continued to shorten in concert with inspiratory airflow, while peak crural shortening occurred in expiration, almost 180° out of phase with costal. The divergence in shortening activity of the segments during panting was accompanied by a shift in timing of costal and crural segmental EMG. During panting, onset and peak of crural EMG occurred earlier, relative to costal. These results suggest that in the awake, spontaneously breathing canine, during panting: 1) there is an active decrease in end expiratory length of both costal and crural diaphragm segments, which is likely to reflect an increase in FRC, 2) tidal shortening of both costal and crural segments decreases despite constant or increased electrical activation of the segments, 3) there is a shift in timing of activation, so that crural EMG activity comes to precede activity of costal segment, 4) costal shortening occurs...
synchronously with inspiratory airflow, while crural shortening shifts out of phase to become maximal during expiration, 5) this asynchrony of costal and crural shortening may significantly increase gas mixing in a manner analogous to high frequency ventilation (HFV).

INDEX TERMS: shortening, sonomicrometry, EMG, nonanesthetized canine, pant, temperature regulation, high frequency ventilation, HFV, HFOV.
INTRODUCTION

In panting, an overriding need for thermal regulation drives the respiratory system to a rapid shallow pattern of movement which is less than optimal for gas exchange (21,28,39). During panting the mechanical capabilities of the respiratory system are devoted to efficient movement of airflow through the upper airway to maximize evaporative cooling for thermal regulation. This function is aided by deliberate patterns of movement of oropharyngeal and laryngeal structures in the upper airway (which effectively "valve" the system) to promote a highly efficient unidirectional flow of air over the evaporative surfaces (18,6,37). To assist with this thermoregulation, the diaphragm and other muscles of the lower respiratory system are obliged to generate a rhythmic motion which may approach 5 HZ, or roughly the resonant frequency of the respiratory system (27,10). At first glance this extreme heat-induced tachypnea appears likely to be expensive and inefficient, with an increased oxygen cost of breathing (39), and a disadvantageous ratio of deadspace to tidal volume (29). However, during panting, gas exchange is adequate and arterial blood gases are defended; in severe heat stress, the panting mechanism can even sustain a significant respiratory alkalosis for a brief period of time (27,1). It has been proposed that gas exchange during panting may depend upon mechanisms of gas transport other than the usual bulk convective movement of inspired gas to the alveolus. Instead, gas exchange during panting may proceed as a natural system of gas transport akin to contemporary techniques of high frequency ventilation (29,38,9).

Little is known of the role in panting of the diaphragm and other respiratory muscles below the upper airway. Indirect evidence arising from non-thermal panting-style movements employed during plethysmographic measurement of lung volume, suggests that quite different patterns of muscle recruitment can achieve the panting motion (19). In natural, thermal regulatory panting, the pattern of respiratory muscle recruitment presumably operates to maximize gas transport and exchange and minimize oxygen cost of breathing, within the constraints of extreme tachypnea. But, what is the strategy of respiratory muscle recruitment during panting? What is the role of the diaphragm in panting? Since evidence continues to accumulate in support of costal and crural diaphragm segments as separate muscles (12,35), then what is the relative function of costal and crural segments in the respiratory movements of panting?

To address these questions, we measured length, shortening and EMG activity of the left hemidiaphragm during panting induced by mild heat stress, in
awake and spontaneously breathing canines, who had previously been chronically implanted and recovered to apparently normal diaphragm function.

METHODS

Surgical Preparation. Each tracheostomized mongrel dog had sonomicrometry transducers and EMG wires implanted in costal and crural diaphragm. Animals were studied after diaphragm segmental shortening had recovered. The technique of chronic sonomicrometry and EMG implantation, and the 7-10 day progressive recovery of diaphragm segmental shortening, has been described fully elsewhere (15). Briefly, implantation was performed under general anesthesia with thiopentol sodium induction and halothane. The left diaphragm was exposed through a mid-abdominal incision and ultrasonic transducers were implanted between muscle fibres on a flat portion of each of the costal and crural segments of the hemidiaphragm. Costal transducers were implanted in the mid-portion of the costal segment approximately midway between central tendon and chest wall in the region corresponding roughly to the SC2 branch of the phrenic nerve (24,40). Crural transducers were inserted posteriorly in the mid-perivertebral aspect of the muscle. On each segment immediately adjacent to each pair of transducers, a fine wire stainless steel bipolar electromyogram electrode was attached. All implants were secured by fine, synthetic, non-fibrogenic sutures (Prolene, Ethicon Ltd.). All wires were externalized by a subcutaneous skin tunnel and the animals were recovered.

Measurement techniques. Measurements of baseline resting and panting ventilation were performed with the animals awake and lying in the right lateral decubitus position. The animals were familiar with the location, routine and personnel of the recordings. During the measurements, the animal on its recording platform and one experimenter were enclosed within a large cloth tent. Baseline measurements were at ambient room temperature of 18-22°C, at 30-35 % relative humidity. Intra-tent temperature was raised to 28-30°C gradually over 25-40 minutes with a dry, fan driven space heater. Panting measurements were recorded specifically throughout the initial period of transition from regular ventilation to panting, and then for an additional 10-15 minutes after panting became continuous.

All measurements were made with the animals breathing through the tracheostomy which was attached through a unidirectional valve to a low resistance open breathing circuit (<1 cm H2O/L/S), connected to a pneumotachograph (Fleish #2) for measurement of airflow. Continuous measurements were made of inspiratory airflow, costal and crural segmental length and EMG.
Dynamic measurement of the changing distance between the sonomicrometer transducers of each pair was provided by the sonomicrometer (Model 120, Triton Technology, San Diego, CA). Measurement of diaphragm length by sonomicrometry has been described in detail (31). Briefly, when electrically excited, the piezoelectric transducer resonates, radiating ultrasonic waves into the surrounding muscle where some waves strike and deform the receiving transducer to produce a measurable voltage. A quartz crystal clock also measures the transit time of the waves and because the conduction of velocity in muscle is known, the sonomicrometer provides the inter-transducer distance. EMG signals from the wire electrodes were amplified (Mark III, TECA, White Plains, NY) and band pass filtered (16 Hz-1.6 KHz). The output signals were then rectified and processed by passage through resistance-capacitance, leaky "integrators" with a time constant of 50 ms, to provide moving averages of the electromyograms of costal and crural diaphragm.

Analysis of Ventilation. All signals were recorded on a strip chart recorder. Simultaneously, signals were monitored on-screen and sampled at 200 Hz by a single board analog-to-digital system (Model 2801-A, Data Translation, Marlboro, MA) directly to hard disk on a microcomputer (PC, IBM, Boca Raton, FL) for subsequent examination using a series of dedicated acquisition and analysis programs written by one of the authors (PE). The flow signal was evaluated for respiratory timing and digitally integrated; minute ventilation (VT), respiratory frequency (FREQ), tidal volume (VT), inspiratory time (TI), mean inspiratory flow (VT/TI), and inspiratory fraction of respiration (TI/Ttot) were calculated breath-by-breath.

Whole Breath Analysis. Using the sonomicrometry data from implanted muscle, the computer algorithm identified the muscle length for each breath which corresponded to the onset of inspiratory flow. In each breath, the computer compared this value to the data samples of muscle length in the final third of the preceding expiration and identified the maximum resting end expiratory muscle length. This baseline, resting length at end expiration in mm was titled LBL, i.e. baseline length prior to the onset of muscle shortening, in inspiration. This length has been titled L_{REC} in some of our previous reports (14,15), but LBL is utilized here and preferred since LBL can be extended to any inspiratory or expiratory muscle. From this baseline, end expiratory length, the shortening for each breath was expressed as a percentage change from resting length entitled %LBL. EMG activity was quantified arbitrarily per breath as baseline value in volts (EMG_{BL}), and the maximum difference in volts (V) between baseline EMG and the peak height of the moving average EMG signal expressed as EMG_{DIFF}.
These measurements defined whole breath or "tidal" breath activity of inspiratory flow and respiratory timing, as well as costal and crural diaphragm length, shortening, and EMG activity. These tidal breath-by-breath values were calculated for resting baseline and for panting ventilation, and compared.

**Intrabreath Analysis.** After calculating peak tidal, whole breath values, the computer algorithm calculated the intrabreath development or "shape" of inspiratory airflow, diaphragm shortening, and EMG activity for each breath. Briefly, for each whole breath, the peak inspiratory airflow, maximum segmental shortening and peak EMG activity were identified. Then throughout the duration of each breath (Ttot), the percent of peak segmental activity was determined after each successive 5% "slice" of the total time of the breath (%Ttot). That is at each 5% increment, inspiratory flow, segmental shortening and EMG activity were expressed as a percent of the maximum tidal value for the breath. This created a profile for each breath of segmental shortening and EMG standardized as a percentage of the peak whole breath value, against time standardized as a percent of Ttot. The breath profiles were calculated for baseline resting and panting ventilation for each canine and then averaged for the group.

**Statistical Analysis.** After calculation, mean values were exported for review to spreadsheet software (Microsoft Excel, Microsoft, Redmond, WA), to graphic software to output figures 1-3 and to the PC version of SAS (36), for statistical analysis. Mean values for parameters of breathing pattern, segmental length and shortening, and EMG activity were tested between the two conditions of baseline and panting by paired t test (42). Intrabreath results were examined graphically by plotting the group mean standardized breath profiles for baseline and panting, with inter-animal variability expressed as SEM.

**RESULTS**

Measurements were made during panting in 5 awake, chronically instrumented canines, mean weight 23.6 kg. The studies were conducted an average of 16 days after chronic implantation of sonomicrometer transducers and EMG electrodes, range 8-25 days post-implantation.

**Breathing pattern.** For these canines, awake and breathing spontaneously after full recovery from implantation, mean baseline resting VI was 5.07 L/min (std. dev. 1.52 L/min), VT 0.31 (0.08) L, and FREQ 16.9 (5.6) b/min. During panting, FREQ increased to 192.8 (55.5) b/min, VT decreased to 0.11 (0.02) L, and VI was 19.79 (3.88) L/min; all changes p<0.001. From resting values of TI 1.19 (0.18) s and TE 2.18 (1.26) s, timing during panting breaths decreased to TI 1.19 (0.18) s and TE
2.18 (1.26) s, respectively (both \( p < 0.001 \)). Concomitantly, \( TI/T_{tot} \) and \( VT/TI \) increased from 0.32 (0.08) and 0.26 (0.03) L/s at rest, to 0.56 (0.09) and 0.59 (0.11) L/s during panting, respectively, (both \( p < 0.003 \)).

**Segmental function during tidal breathing.** The end expiratory length in mm during resting ventilation, from which the costal diaphragm segment began to shorten during subsequent panting, mean \( L_{BL} \), was 15.75 (1.25) mm. Costal \( L_{BL} \) decreased to 13.99 (2.15) mm during panting (\( p < 0.11 \)). Similarly, crural segmental baseline, pre-panting length, crural \( L_{BL} \), decreased from 16.86 (2.75) mm during resting breathing, to 15.21 (3.28) mm during panting (\( p < 0.13 \)). These changes in resting length with panting did not occur gradually; the transition from a regular non-panting pattern of breathing to panting was marked by an abrupt adjustment in resting length over the space of a few breaths. The change from segmental, pre-panting \( L_{BL} \) to panting \( L_{BL} \) of both costal and crural segments was accompanied by an increase in baseline segmental EMG from inspiratory, pre-panting levels. During panting, costal and crural \( EMG_{BL} \) mean values increased 1.05 (0.33) and 1.64 (0.58) V respectively, increases significant \( p < 0.04 \) and \( p < 0.05 \).

During resting breathing, mean tidal shortening was 6.29 (2.44) \( \%L_{BL} \) and 6.04 (3.64) \( \%L_{BL} \) for costal and crural segments, respectively. With the onset of panting, tidal shortening decreased to 3.54 (2.59) and 2.46 (1.23) \( \%L_{BL} \) for costal and crural; changes \( p < 0.007 \) and \( p < 0.10 \) respectively. The lesser segmental shortening with panting was not accompanied by a fall in tidal EMG; \( EMG_{DIFF} \) tended to increase during panting. Costal \( EMG_{DIFF} \) increased from 1.48 (0.43) to 1.69 (1.11) V, crural \( EMG_{DIFF} \) from 1.51 (0.44) to 2.06 (1.63) V; differences NS.

**Intrabreath segmental function.** Shortening and EMG activity of costal and crural segments were examined dynamically within each tidal breath. In contrast to the tidal, peak analysis per whole breath cited in previous paragraphs, this analysis concentrated on the shape profile of inspiratory flow, segmental shortening, and segmental EMG that occurred within each breath. Compared to tidal values, profile analysis offered additional insight into the differential actions of the diaphragm segments.
Costal and crural diaphragm: Results

Figure 1 shows a computer printout of raw signals from a single animal panting at 3 Hz. As seen in the upper panel, inspiratory airflow during panting was synchronous with costal shortening. However, crural shortening was noted to be distinctly out of phase with inspiratory flow. Corresponding segmental EMG during panting is illustrated in the lower panel. Activity of crural EMG preceded costal EMG at the initiation and over the early portion of each panting inspiration.

Intrabreath costal segmental shortening is illustrated during both resting breathing and panting in figure 2a. The curves present normalized shortening data from all resting and panting breaths in all animals, averaged for the group, and expressed against a time base normalized as %Ttot. From resting ventilation to panting, intrabreath costal shortening changed. With panting, costal segmental
shortening in earliest inspiration was increased and shortening persisted relatively longer, consistent with the previously cited increase in TI/Ttot calculated from airflow. Costal segmental shortening during panting presented a more biphasic, sine wave-like profile, in contrast to the usual shortening profile at rest.

Figure 2a. Costal shortening during resting ventilation and panting. Normalized intrabreath costal shortening during baseline room air ventilation and panting. Y axis shows amplitude of mean shortening, standardized as percent of maximum value per individual breath. X axis shows time scale, with standardized values noted sequentially after each 5% of total breath time (Ttot). All breath profiles averaged per animal; mean results for all animals are shown, with representative variance indicated for each curve as ±SEM. Values for costal shortening during resting ventilation marked by solid line and open circles, during panting by solid line and closed circles.

Intrabreath crural segmental shortening is illustrated during both resting breathing and panting in figure 2b. This averaged normalized data for all animals confirmed the phase change in crural shortening suggested in the raw trace. From the resting crural segmental curve, typically showing more late inspiratory and post inspiratory (PIIA) activity than costal, there was a distinct change with panting. During panting, peak crural shortening was shifted forward to occur in end expiration. Crural shortening is apparently 180° out of phase in panting compared to crural shortening during resting ventilation. And, comparing figures 2a and 2b,
crural shortening during panting was similarly out of phase with costal shortening during panting.

![Graph showing crural shortening during resting ventilation and panting.](image)

**Figure 2b.** Crural shortening during resting ventilation and panting. Values for crural shortening during resting ventilation marked by solid line and open squares, during panting by solid line and closed squares. Other symbols and conventions as in figure 2a.

An intrabreath EMG profile of both costal and crural segments during panting is illustrated in figure 3. This figure expresses mean normalized values from all breaths in a single animal. This profile indicates that during panting, timing of costal and crural segmental EMG was not identical. Crural segmental EMG activity developed earlier than costal, predominated in earliest inspiration, and reached maximal values sooner. Thereafter, costal EMG activity persisted through inspiration as crural EMG decayed. There was an apparent shifting or asynchrony in timing of costal and crural segmental EMG activity during panting. This change in EMG corresponded to the differences in segmental shortening during panting illustrated in figures 2a, 2b, and suggested a fundamental alteration in the activity of segments during panting.
DISCUSSION

Data Summary. In response to increased ambient temperature induced by dry heat, panting was induced in these 5 canines, with respiratory rate during panting increasing to 3 Hz. Although tidal volume decreased slightly during panting, net minute ventilation increased significantly. Whole breath tidal measurements of costal and crural segmental function showed that panting was associated with a significant decrease in end expiratory length of both diaphragm segments. This end expiratory length decrease was accompanied by a concurrent sustained increase in end expiratory values of segmental EMG. Tidal shortening of both costal and crural segments decreased slightly during panting although tidal EMG values of both segments increased. Differential activity of the segments during panting was apparent by intrabreath analysis of the shape profile of shortening and EMG within each breath. During panting, costal shortening assumed a more sine wave-like character with earlier inspiratory shortening, while crural shortening

Figure 3. Costal and crural segmental EMG activity during panting. Normalized intrabreath costal and crural EMG activity during baseline panting in a single animal. Y axis shows amplitude of mean EMG, standardized as percent of maximum value per individual breath. X axis shows time scale, with standardized values noted sequentially after each 5% of total breath time (Ttot). All breath profiles averaged for one animal, with representative variance indicated for each curve as ±SEM. Values for costal EMG during panting marked by solid line and closed circles, for crural EMG by solid line and closed squares.
during panting moved distinctively out of phase from costal, so that peak crural shortening was present during expiration. This asynchrony of costal and crural segmental shortening was accompanied by a concurrent shift in timing of segmental EMG with crural EMG leading costal during panting.

Chronic implantation and normal segmental function. These measurements rest upon the reasonable assumption that the fully recovered, awake and spontaneously breathing canine with chronically implanted sonomicrometer transducers and EMG wires represents normal diaphragm function. In a previous publication, we have described in detail the techniques of implantation and sequence of recovery that provided: a return to normal breathing pattern, uniphasic segmental shortening of appropriate magnitude synchronous with EMG, expected values of maximal segmental shortening with supramaximal phrenic stimulation, and histologic evidence of healthy, nonfibrotic muscle between the sonomicrometer transducers (15). Identical techniques were employed for this canine study group; in accordance with our documentation of post laparotomy/implantation recovery sequence the earliest measurement was made on post operative day 8 and, on average, measurements were made 16 days after implantation.

Segmental shortening as recorded from the costal sonomicrometer transducers in these canines may represent shortening throughout the costal segment, if costal segmental shortening is relatively homogeneous as suggested in an early sonomicrometry study of the diaphragm in anesthetized canines (31). However, recent evidence suggests that significant regional differences in shortening may occur within the costal segment (40). If such regional differences are operative in these canines, then these recordings of costal shortening are most representative of the middle region of the costal segment (40). It should be noted that for the canines in this series, the position of the transducers along the costal segment, relative to origin and central tendinous insertion, was consistent from animal to animal.

Presentation and interpretation of the data from these chronically implanted canines depends upon sufficient measurement resolution to justify both the usual whole breath, tidal breathing parameters, as well as the bin style, standardized intrabreath profiles to graphically illustrate within-breath events. Since all signals were gathered and permanently stored direct-to-digital, with 12 bit resolution at a sampling rate of 200 Hz, the computerized recording and storage of muscle length and moving average EMG occurred with ample resolution and precision for this analysis.
For the computer-generated normalized profiles of within-breath shortening and EMG of figures 2 and 3, additional explanation of computer calculations may be helpful. For example, group mean costal shortening during resting breathing shows a peak inspiratory flow value which is less than 100%. This is a logical outcome of standardizing all intrabreath values as a percent of the maximum value within each breath, then averaging the results for each animal, and for the entire group, at each 5% Ttot. Unless each breath presented a maximum intrabreath shortening at exactly the same moment, then the group mean value would be expected to average to something less than 100%. Each breath "begins" with group mean shortening or EMG values already at moderate levels, because the first calculations are presented after 5% of Ttot has elapsed and the breath events are timed according to inspiratory flow, so that significant EMG and shortening activity is expected to occur before airflow. Since the breath profiles are a continuum, events preceding the first 5% are not lost and occur on the Y-axis between 100% Ttot and 5% Ttot, as the breath cycle "wraps around" into the next breath.

Control and mechanics of panting. Since sweating is not available to the canine as a practical means of temperature control, panting is the principal mode of thermal regulation (23). Control over the panting mechanism is centered in the hypothalamus (25,26). Panting is a complex mechanism which involves much more than simple tachypnea. During panting, the hypothalamus also regulates a significant vasodilation of the mucosal vasculature of the upper airway to assist in heat loss. Although somewhat independent of the central thermal regulatory control, heat loss is also facilitated by up to a five-fold increase in tracheobronchial blood flow during panting (3,4). The most visible expression of the thermal regulation of panting, is the tachypnic panting motion of the thorax. This panting motion need not be precipitated by heat stress, however, since canine panting is also elicited by behavioral stresses, and can occur in apparently non-stressed animals even in cool ambient temperature (27). Humans commonly simulate thoracic motions of panting during plethysmographic measurement of lung volume. In natural thermal panting in canines the frequency of the panting motions is highly variable (27), but typically ranges from approximately 180 to 350 thoracic motions per second (27,29,21,1). Commonly, a visible, abrupt transition in breathing rate occurs to initiate panting at rates of up to 200 breaths per minute. Thereafter, with a stable moderate heat stress the thoracic motion stabilizes gradually at rates of up to 300 breaths per minute if the heat stress is constant and moderately severe (28). This stable rate in excess of 300 breaths per minute may be a convenient approximation of the resonant frequency of the respiratory system which has been
estimated to be very similar at 5.28 HZ (10). In the event of extreme heat stress, e.g., 40°C with high relative humidity, panting rates may approach 8 HZ but ultimately collapse to a slower, deeper pattern of breathing which has been titled "second phase panting", and seems to be less effective in thermal regulation (6,20).

Our measurement of the breathing pattern and timing of panting are in agreement with other investigators. These animals began to pant with a sudden switch from a mean respiratory rate of 16.9 breaths per minute to 192.8 breaths per minute, or slightly more than 3 HZ. This is the response that would be predicted from the moderate, dry, heat stress of approximately 30°C we imposed. Since we were focussed upon the function of diaphragm in panting and any adjustments in resting length that occurred in the abrupt transition to panting, our measurements were made within a few minutes of the onset of panting. Presumably, if the heat stress had been maintained most animals would have gradually increased their rate of motion to approximately 3-5 HZ (27,29).

Differential activation of respiratory muscles during panting. Although thermal regulatory drive may take precedence over the usual chemical and nonchemical influences that control respiration and determine recruitment pattern, thermal regulatory panting is a sophisticated pattern of neuromuscular activation. The visible, extreme tachypnea is only a superficial first observation. To maximize evaporative heat loss, canines circumvent the usual countercurrent system for preservation of heat and water vapor by panting with unidirectional airflow. Typically, canine thermal panting is characterized by inhalation through the nose and exhalation through the open mouth. Less than one-quarter of the air volume inhaled through the nose is measurably exhaled through the nose (6). Although this mechanism was first proposed by Negus (19) based solely upon his anatomical studies, the control of airflow and heat loss during panting has been shown to be a dynamic process reliant upon differential recruitment of upper airway muscles and defacto "valving" of the respiratory airflow (18,6,5). In canines three patterns of panting have been observed with increasing demand for respiratory evaporative heat loss; initially inhalation and exhalation occurs through the nose, then at intermediate or high rates of evaporative heat loss the animal alternates between inhalation through the nose plus exhalation through the nose and mouth, as well as inhalation through the nose and mouth plus exhalation through the nose and mouth (18). The alteration in airflow according to these three patterns is not related to any measurable change in the frequency of panting. Cineradiographic studies of movement patterns of oropharyngeal and laryngeal structures during panting demonstrate that alterations in inspiratory and expiratory airflow patterns are
related to coordinated movements of soft palate as well as dorsal tongue and epiglottis. The net effect of this specific recruitment of upper airway structures is effectively a "valving" of airflow to achieve the observed nasal or oral dominant route of flow (6). During panting, glottic aperture increases and shows minimal variability between inspiration and expiration, as a result of marked widening during the expiratory phase. This glottic opening in expiration of panting is the result of a distinctive activation of the posterior cricoarytenoid muscles for abduction of the vocal cords during panting (7). For similar flow rates, the glottic aperture is measurably wider during panting than during a vital capacity expiration (41) suggesting that glottic width is dependent upon differential activation of the laryngeal muscles which is at least partly specific to the respiratory maneuver which has been undertaken (8).

The pattern of activation of lower respiratory muscles acting on chest wall and abdomen, has not been extensively examined during panting. Indirect evidence from studies of the effects of breathing patterns on plethysmographic lung volume measurement suggests that the relative order and pattern of recruitment of diaphragm and other respiratory muscles in the lower respiratory system could have an important role in the effectiveness of panting for both heat and gas exchange. In the simulated, nonthermal panting during plethysmographic measurements, panting motions generated predominantly by the intercostal muscles, resulted in a significantly larger measured value for thoracic gas volume than attempts to pant exclusively with the diaphragm (19). Some indirect evidence of the role of diaphragm and abdominal muscles in panting is suggested by measurement of respiratory muscle blood flow. Values of diaphragmatic blood flow during panting are noted to be greater than those measured in awake dogs during moderate exercise (17), while measured abdominal muscle blood flow has been found to be low and unchanged with panting, suggesting a lack of participation of abdominal expiratory muscles in panting (17,3). The limitations of current knowledge regarding respiratory muscle use, mechanics, and panting are clearly expressed in the lack of consensus regarding the energy cost of panting. In fact, some authors even suggest that the value of panting as a thermal regulatory activity is limited by heat production and undue oxygen consumption of the respiratory muscles at these high rates of ventilation (34,39). However, most investigators suggest that oxygen consumption of the respiratory muscles during thermal panting is minimally increased and indeed is less than that observed for a comparable level of ventilation produced by hypercapnia (22).
Differential function of costal and crural segments in panting. Evidence continues to accumulate in support of the concept that costal and crural segments have the potential to function differentially as separate muscles. The two segments are innervated by different phrenic nerve roots and when stimulated the two segments exert a different mechanical action on the rib cage (12,35). Certain actions reveal clear divergence of costal and crural function, such as the crural diaphragm activation during gastroesophageal reflexes (2). A recent investigation applying EMG and glycogen depletion methods has suggested that the diaphragm may be observed as four identifiable, well-delineated, motor unit territories corresponding to four primary branches of each phrenic nerve; three costal areas and a single crural area (24). In theory, diaphragm function could be differentiated even more discretely than simply as costal and crural segments.

Previous investigations which measured costal and crural segmental EMG and/or length by sonomicrometry in acutely anesthetized cats and canines, suggested unique activity during respiration for each of the two segments. Overall, existing evidence suggests that the onset of crural EMG activity precedes costal EMG activity in early inspiration, the associated onset of crural segmental shortening precedes costal shortening in early inspiration, crural EMG and shortening is greater than corresponding costal segmental values, and with CO₂ stimulation there is a proportionally greater increase in crural segmental activity compared to costal (14,11,32,43). A recent investigation using the same chronically implanted canines reported here, demonstrated that crural segmental activity predominated in early inspiration, and showed more persistent end inspiratory and early expiratory (PIIA) activity (13).

Our results in these awake and spontaneously panting canines show a small but consistent, and significant decrease in the end expiratory length of both costal and crural diaphragm segments. These changes should not be underestimated. These resting levels are certainly well within the resolution of this system, and if the end expiratory length were expressed as a percent change (as was done in our previous publication) (15), the relative magnitude of this adjustment in end expiratory length would be striking. That is, the sustained decrease in costal and crural end expiratory length amounted to 1.76 and 1.65 mm respectively, changes equivalent to 11.18 and 9.79 %L₆₉. In some animals, this adjustment in end expiratory length of the diaphragm segments occurring with the onset of panting was twice the magnitude of the changes in segmental length which occurred with each tidal breath. In theory, these changes in end expiratory length could be "passive"; that is, end expiratory segmental length changes might reflect only an
alteration in relative activity of abdominal and chest wall muscles as well as the
effect of increased respiratory rate, for an overall net effect on FRC unrelated to any
change in baseline electrical activity of the respective diaphragm segments. This
was not the case. Both costal and crural segments had a significant measurable
increase in end expiratory EMG which occurred at the onset of panting. Thus,
exclusive of any changes in other chest wall or abdominal respiratory muscles,
initiation of panting motions was associated with a deliberate increase in baseline
electrical activity and concurrent shortening of the diaphragm. Since FRC is
unlikely to have much direct influence upon the thermal regulatory mechanisms of
the upper airway, then presumably this evidence of increased lung volume and FRC
is related to minimizing respiratory work or improving gas exchange during panting.
Since the resting length of costal and crural segments is probably less than optimal
length (L0) during room air breathing to begin (16,33), this further shortening of end
expiratory length is unlikely to optimize length-force characteristics of the segments
or improve the efficiency of pressure generation of the diaphragm during panting.

The decrease in costal and crural segmental shortening with the onset of
panting was of particular interest because of the lack of correlation between
changes in segmental shortening and concurrent EMG or tidal volume. Although
costal tidal shortening decreased to approximately half the value measured during
resting ventilation, this occurred while tidal volume in panting decreased to only
one-third of its baseline resting value. Although superficially the relative decrement
in crural tidal shortening correlated more closely with a fall in tidal volume, this
relationship is obviously spurious since peak crural shortening during panting
actually occurred out of phase with inspiratory flow. And, for both costal and
crural segments, even as tidal segmental shortening during panting decreased
significantly, corresponding tidal EMG actually increased. Without direct
measurements of other lower thoracic muscles, we cannot synthesize the relative
contributions of the major contributing respiratory muscles during panting, but this
evidence suggests that shortening of the diaphragm segments and tidal volume has
diverged as the segments are integrated into a different overall recruitment pattern
of respiratory muscles for panting.

The most distinctive differences between costal and crural segmental
function were revealed by intrabreath examination of panting. The relative timing
of the electrical activity of costal and crural segments was changed unequivocally
between resting ventilation and panting. Although our moving average
measurements were made with a hardware time constant of 50 msec, this would
have affected the relative timing of both segmental EMGs equally. This averaging
effect may have artefactually delayed the timing of both costal and crural EMG by a few msec compared to segmental shortening, without altering the relative timing of costal versus crural EMG. The distinct shift of crural EMG to an earlier, end-expiratory, pre-inspiratory onset and earlier peak activity compared to costal was a consistent accompaniment of the more striking divergence in phase exhibited by segmental shortening. Clearly the nearly 180° phase shift of peak crural shortening relative to inspiratory airflow and costal shortening, was the result of both the aforementioned change in timing of segmental EMG, as well as some difference in elastance loads faced by the two segmental "pumps". Until we have additional information regarding the relative activity of other respiratory muscles of chest wall and abdomen during panting, the change in timing of peak segmental shortening, out of proportion to changes in timing of segmental EMG, cannot be fully explained.

**Gas exchange, segmental function, and panting.** Even though panting is associated with increased deadspace in proportion to total minute or alveolar ventilation, with worsening of alveolar-arterial differences representative of alveolar deadspace ventilation (28,29), in general, over a range of heat stress and relative humidity, arterial blood gases and estimates of intracellular hydrogen ion are remarkably well defended (1). Only after prolonged exposure to extreme (40°C) heat stress do some animals exhibit deterioration of their blood gases; and in the extreme, heat exhaustion comes finally to be associated with a severe respiratory alkalosis. In response to such extreme stress, the extreme tachypnea of panting seems to provide for adequate gas exchange as well as required thermal regulation. But how can an apparently inefficient rate and pattern of respiration effectively transport gas to the alveolus?

In recent years, high frequency, low tidal volume modes of mechanical ventilation have been shown to be effective methods of gas transport and gas exchange in clinical medicine (38). The obvious similarities between the low tidal volume, high frequency respiration of panting with clinical methods of high frequency ventilation has been noted (29). The magnitude of the tidal volume during panting, which remains slightly greater than deadspace, suggests that the clinical modes of high frequency positive pressure ventilation (HPPV) or high frequency jet ventilation (HFJV) are more analogous to panting that true high frequency oscillatory ventilation (HFOV) which is characterized by tidal volumes less than deadspace delivered at higher frequencies (38). If panting is a natural analogue to high frequency jet ventilation (HFJV), then the mechanisms proposed for convective gas transport during high frequency ventilation may also apply to gas transport and mixing during panting (38,9).
of pendumuft or other mode of lateral mixing is an expected component of gas transport in high frequency ventilation. The observation in this study that tidal shortening of costal and crural segments during panting is asynchronous supports the notion that panting is a natural form of HFV. While the shortening activity of the costal segment coincides in time with inspiratory airflow and bulk convective gas transport, the second, out-of-phase crural segmental shortening event may contribute to gas mixing at least in the basilar, peridiaphragmatic regions of the lung. In those lower peridiaphragmatic regions, where bulk transport is likely to have less impact, the additional shortening activity of the crural segment may add an element of pendumuft or lateral mixing, and provide a rate of basilar lung HFV which is effectively double the observed, global, respiratory rate in panting.

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REFERENCES


CONCLUSION

Overview
Overview.

From the group of investigations reported in the preceding chapters, we conclude generally that costal and crural diaphragm are segment-muscles, exhibiting distinctive individual function and differential activity under certain conditions of respiration. We conclude that the chronically implanted canine preparation, providing for direct, computerized, simultaneous measurement of length and EMG of the segments, is a valid mode of study of respiratory muscle function and control.

From individual investigations, we propose additional specific conclusions.

From the study of canine diaphragm segmental recovery following upper abdominal surgery, we confirm that direct chronic measurement of diaphragm segmental length is reliable. We conclude that there is a reproducible pattern of postoperative recovery of the segments after operative dysfunction, and we note that EMG alone is not satisfactory as an indicator of diaphragm activity.

From our investigation of diaphragm contraction during airway occlusion, we conclude that diaphragm contraction is not isometric against occlusion. And, by examining muscle function over a defined portion early in the breath cycle, we confirm that the relative action of the segments can be distinct.

We conclude that the basic relation between segmental velocity of shortening, and mean inspiratory flow, was constant, linear, and altered as predicted in contrast to the relationship with muscles of the chest wall.

In response to posture change, we note rough equivalence of segmental tidal shortening despite changing posture. We conclude that an essential interaction exists between the intrinsic diaphragm response to maintain tidal excursion and the control of phasic expiratory activity of the abdominal musculature.

With induction of anesthesia, a distinctive alteration in the resting length of crural compared to costal segment was noted. We conclude that two segments differ in their inherent tonic activity and that this divergence is exposed by anesthetic.

From the response of the segments to chemical stimulation, we conclude that relative crural actions during both early inspiration and PIIA are distinctive from costal. We deduce from the singular pumping action of the costal, persistent through changing chemical stimulation, compared to the differential actions of
crural, that the crural segment has an additional role in adjustment and coordination within the system of respiratory muscles.

We conclude that, during hypoxia compared to hypercapnia, there is a fundamentally different pattern of recruitment of respiratory muscles. We note that the relative participation and interaction of the segments within this stimulant-specific recruitment differs; hypoxia is characterized by prominent crural PIIA in concert with diminished abdominal expiratory activity, compared to hypercapnia.

We note that segmental responses during thermal panting is distinctive. We observed that during panting, costal and crural segmental shortening move significantly out of phase. We conclude that the capacity for differential function of the segments is exploited to maintain gas exchange during the low tidal volume respiratory motions of panting; a natural analog of HFV.
CLAIMS FOR ORIGINAL RESEARCH

The investigations and results embodied within this thesis are evidence of original scholarship, as required of the candidate for Doctor of Philosophy. This concluding section, entitled Claims for Original Research, summarizes these contributions in accordance with the Guidelines Concerning Thesis Preparation of McGill University.

From this work, claims of contribution to original knowledge include:

1) Evidence that costal and crural diaphragm segments can function as individual segment-muscles, exhibiting distinctive differential activity under certain conditions of respiration.

2) The first direct measurement of length and electromyographic activity (EMG) of the costal and crural diaphragm segments in any awake, intact animal.

3) The first successful chronic implantation of sonomicrometry transducers and bipolar EMG wires for chronic recordings from any respiratory muscle.

4) The first direct measurements of diaphragm dysfunction after upper abdominal surgery. Specifically, this work confirmed, by direct measurement, abnormal function of costal and crural diaphragm segments after upper abdominal surgery, and elucidated a consistent pattern of post-operative recovery of segmental function in the 7-10 days after surgery.

5) Evidence that under some conditions of respiration, e.g. the period immediate post-operative after upper abdominal surgery, that direct measurement of respiratory muscle activity expressed by shortening diverges from an apparently normal phasic EMG. This repudiates interpretation of respiratory muscle EMG as a valid sole indicator of respiratory muscle function, without some additional measurement of respiratory muscle function.
6) Demonstration that during the earliest moments of each inspiration, activity of costal and crural diaphragm segments were not equal. The crural diaphragm segment was found to be recruited preferentially in earliest inspiration. Both diaphragm segments were shown, by direct measurement, to contract non-isometrically during earliest inspiratory efforts against an occluded airway.

7) With change in posture in the awake canine, total shortening of costal and crural diaphragm segments was not changed significantly. Although directly measured end expiratory length was noted to shorten differentially in moving from the decubitus to sitting position, an increase in the segmental EMG activity was inconsistent.

8) Evidence that with general anesthesia, the end expiratory length of the crural diaphragm segment increased significantly while costal end expiratory length did change appreciably. This first direct confirmation of differential change in end expiratory segmental length with anesthetic provides evidence for a difference in resting tone in the diaphragm segments during wakefulness.

9) The relationship between inspiratory flow and velocity of shortening of external intercostal and costal and crural diaphragm segments was first measured in this work. We documented a regular increase of EMG activity and peak tidal shortening of costal and crural diaphragm segments with increasing ETCO₂. By contrast, for the external intercostal, no consistent relationship was found between inspiratory flow and either EMG activity or tidal shortening.

10) The initial delineation of function of the diaphragm segments during CO₂ rebreathing, which revealed distinctive differences in segmental function with chemical stimulation. Crural segmental activity was found to be more prominent than costal in both early inspiration and post inspiratory inspiratory activity (PIIA) at rest during CO₂ stimulation. With increasing CO₂ stimulated breathing, crural segmental prominence in early inspiration was accentuated and its predominant post inspiratory activity (PIIA) increased further.

11) Evidence of relative function of costal and crural diaphragm segments during hypoxic compared to hypercapnic stimulated breathing. The overall CO₂ and hypoxic responsiveness of the two segments was not measurably different, demonstrating the limitations of whole breath, tidal measurements to discern
activities of individual respiratory muscles. By utilizing normalized intrabreath analysis of inspiratory flow and muscle activity, the relative post inspiratory activity (PIIA) expressed both by EMG and shortening of the two diaphragm segments was noted to be unique per each chemical stimulant. Compared to rest and ventilation, both costal and crural diaphragms segments showed increased post inspiratory inspiratory activity (PIIA) with chemically stimulated ventilation, but PIIA of crural segment was significantly greater. During hypoxia, crural shortening PIIA was greater than costal, and greater than crural PIIA at a similar intrabreath time during CO2 stimulation.

12) The first direct recording of relative activity of costal and crural diaphragm segments during thermal panting. Compared to the synchronous shortening of costal and crural diaphragm segments with inspiratory airflow during normal respiration, during panting, costal segment continued to shorten synchronously with inspiratory airflow, while peak crural shortening shifted to expiration, moving out-of-phase with costal. This divergence in the timing of segmental shortening during panting was accompanied by a shift in phase of segmental EMG. These results indicate that during panting the differential activity of costal and crural diaphragm may increase gas mixing by a mechanism which is a natural analogue to high frequency ventilation (HFV).
APPENDIX: FUTURE STUDIES

Overview

Investigations

- Activity of diaphragm, parasternal intercostal, and transversus abdominus during progressive hypoxia or hypercapnia.
- Differential function and coordination of the respiratory muscles with posture change and resistive loading.
- Respiratory muscle activity during emesis in awake or anesthetized canines.
- Respiratory muscle length and EMG spectral analysis in the awake canine.
- Effects of REM and nonREM sleep on differential function of the respiratory muscles.
- Respiratory muscle compensation for diaphragm paralysis in the awake canine.

Human studies of respiratory muscle function.

- Differential respiratory function of the abdominal muscles.
- Costal and crural function after abdominal surgery.

References from future studies
Overview

In the interim since the investigations recorded in the preceding chapters, this candidate has relocated to the University of Calgary. At that site, a laboratory has been created to continue studies of respiratory muscle control and function, utilizing a chronically implanted canine preparation derived from the model developed in these studies. The implanted canine in current use is a "superset" of the model described here. Besides costal and crural diaphragm, included from the outset, the preparation has been expanded to include parasternal intercostal, transversus abdominis, and most recently, lateral intercostal and geniohyoid muscles, as well. A new combined transducer for simultaneous point measurement of sonomicrometry and EMG has been developed. The techniques of computerized data review and analysis have been greatly expanded. And, in a personal effort independent of this science, a commercially capable data acquisition program has been developed which provides real time display of multiple incoming physiologic signals with high resolution printed output, completely obviating the need for a strip chart recorder in this type of investigation.

Traditionally, the final chapter in a document of this type is devoted to inspired imaginings of potential future studies and illusory results. However, there is at least one pleasant benefit of my tardiness in completing this thesis. Because of the potential of the computerized chronically implanted canine preparation, several investigations which were imagined for the future have already been undertaken or completed. Under the guise of Future Studies, this section summarizes briefly the continuing line of investigation that sprang from the earlier studies in this thesis. To date, these Future Studies have attracted several coworkers and generated original data sufficient for more than a dozen abstracts with a similar number of manuscripts following along in various states of completion and publication.

1 Activity of diaphragm, parasternal intercostal, and transversus abdominis during progressive hypoxia or hypercapnia.

As a result of our interest in the effects of chemical stimulation on costal and crural diaphragm, we promptly undertook investigations to examine differential activity of other muscles with hypoxic and hypercapnic stimulation. Figure 1 is a normalized breath profile similar to those presented throughout this thesis, but expanded to present intrabreath activity of four respiratory muscles. Figure 1 illustrates the EMG activity of costal, crural, transversus abdominis, and parasternal
during moderate hypercapnic stimulation. Of note in this figure, are the modest
PIIA activity of the crural segmental EMG shown by the solid line and open
squares, the lesser PIIA of costal segment, the relative absence of PIIA for
parasternal, and the large phasic expiratory EMG activity of the transversus
abdominis with significant continuation into the subsequent period of inspiration,
as postexpiratory expiratory activity (PEEA).

Figure 1. Inspiratory airflow and EMG per breath during hypercapnic ventilation.
Normalized intrabreath values averaged for all respirations during hypercapnic
ventilation, group mean end tidal CO₂ 52 mmHg, tidal volume 0.65 L. Y axis
shows standardized values for EMG as percent of maximum EMG per individual
breath. X axis marks mean values for each muscle standardized per 5% of total
breath time (% Ttot). Mean results for all animals are shown. Values for
inspiratory flow marked by solid line and solid circles, costal segment by dashed
line and solid squares, crural segment by solid lines and open squares, transversus
abdominis by dashed line and open squares, and parasternal intercostal by dotted
line and solid triangles.

By comparison, in figure 2, EMG activity of the same four muscles is
illustrated during moderate hypoxic stimulation equivalent to 78% SaO₂, with a
tidal volume of 0.62 litres. Thus the two conditions of hypoxia and hypercapnia
which are shown in these two figures represent data which is matched for tidal
volume. In figure 2, it can be noted that with hypoxia there is significantly more
PIIA EMG activity of all 3 inspiratory muscles with a particularly notable increase
of crural and parasternal PIIA. In contrast the transversus abdominis EMG activity is much less phasic and there is no evidence of transversus EMG activity spilling into the subsequent inspiration as PEEA. Since this is a normalized figure, it actually overrepresents the real activity of transversus; mean tidal transversus EMG decreased by more than 50% of its tidal value during hypercapnic stimulation. Corresponding tidal shortening of transversus abdominis was 1.7 %LBL during baseline resting ventilation, which increased to 5.1 %LBL during hypercapnic stimulation as shown in Figure 1, and decreased to 2.9 %LBL during hypoxic stimulated ventilation as shown in Figure 2 (for n=9, canines, difference significant p<0.001).

![Figure 2](image_url)

**Figure 2.** Inspiratory airflow and EMG per breath during hypoxic ventilation. Group mean end tidal hypoxia 78% SaO\(_2\), with a tidal volume of 0.62 L. Y axis shows standardized values for EMG as percent of maximum EMG per individual breath. Other symbols and conventions as in Figure 1.

These investigations have resulted in a pair of abstracts (2,3) and manuscripts under review (15,18).
Differential function and coordination of the respiratory muscles with posture change and resistive loading.

The differential response of the respiratory muscles with two examples of natural mechanical loading have been studied, namely the responses to inspiratory flow resistance, and to changes in posture.

Some posture data is excerpted here in figures 3,4 which show group mean values of resting length and tidal shortening for costal, crural, transversus, and parasternal muscles in 3 postures; right lateral decubitus (RLD) standing (STAND), and sitting (SIT).

In figure 3, moving from RLD to SIT, both costal and crural diaphragm shortened their resting length; although not shown, this occurred in conjunction with a significant increase in resting baseline EMG. Also, it can be seen that transversus resting length, although decreased during STAND, was not significantly different between RLD and SIT. This occurred despite a corresponding, large, increase in baseline EMG of transversus in both SIT and STAND. In fact, transversus EMG corresponding to these resting lengths, was actually greater during STAND. Finally, although the absolute magnitude was small, parasternal resting length was significantly changed as the animal shifted from RLD to STAND or SIT. This occurred with a significant increase in baseline parasternal EMG for both STAND and SIT compared to RLD.

Figure 3. Group mean resting length for costal, crural, parasternal, and transversus muscles, in three postures. All lengths expressed as percent of baseline RLD values.
In Figure 4, tidal shortening is illustrated for the four muscles. Both parts of the diaphragm showed an increase in tidal shortening in STAND posture although this was matched by an increase in tidal EMG only for the crural diaphragm. For both the expiratory transversus, and the chest wall inspiratory parasternal, there was a significant increase in tidal shortening with movement to a more vertical posture, as shown in the figure. This was underpinned by quite different degrees of increase in tidal EMG. That is, the transversus significantly increased shortening with vertical posture with an approximately equivalent increase in tidal EMG. By contrast, parasternal showed modest but significant increases in shortening as seen in figure 4, in contradistinction to very large increases in tidal EMG activity (not shown).

To date these investigations have generated a pair of presentations (1,4), and two papers underway (17,19).

Respiratory muscle activity during emesis in awake or anesthetized canines.

Similar to the investigations noted in this thesis of costal and crural function during the reflexive act of panting, we have investigated differential activities of the major respiratory muscles during the act of emesis in the awake canine. Obviously, emesis requires a coordinated differential recruitment of the major respiratory muscles with gastrointestinal smooth muscle and upper airway muscles, and this
coordination is under the guidance of the specific interaction between brain stem respiratory control and vomiting centers. Our studies, in which we induced emesis with apomorphine, showed that during emesis resting EMG of costal, crural, transversus, and parasternal all increased to produce a marked decrease in resting length prior to expulsion. To actually produce an emetic maneuver, both inspiratory and expiratory muscles acted synchronously, in a reversal of their usual phase during ventilation, with crural and transversus showing the most significant shortening. In fact, activity of the crural segment was distinctive compared to all other respiratory muscles.

![Graph](image)

**Figure 5.** Inspiratory airflow and shortening of respiratory muscles during emesis. Crural shortening is shown by the solid lines and open squares, inspiratory flow marked by solid line and solid circles, costal segment by dashed line and solid squares, transversus abdominis by dashed line and open circles, and parasternal intercostal by dotted line and solid triangles.

In Figure 5, which summarizes "intrabreath" or at least intraevent activities of the four muscles, the striking biphasic behaviour of crural is evident. Crural activity is noted to begin with a brief pre-emetic shortening, followed by a very large relaxation during emesis, which presumably assists the function of the HPZ sphincter. In contrast, both transversus and the other two inspiratory muscles, costal and parasternal, show coordinated, uniphasic, shortening corresponding to the emetic event which occurs in this figure over the range of 40-70% Ttot. Our findings are generally in agreement with Monges (cited in the Background section), although the addition of the length measurements in awake canines greatly extends
our insight into the relative mechanical interactions of the muscles, as well as the apparent discordance between crural motion and its measured EMG. Unlike Menges, we did not find that the crural segment became electrically silent during emesis. This might reflect a larger regional sampling effected by the sonomicrometry, showing a general relaxation even though we still measured increased EMG activity in the nonhiatal fibres. Or, it may be that in our canines, with a possible exception of a very few crural fibres exactly on the hiatus, that crural EMG really was generally increased during emesis and the crural segmental lengthening reflected the net effect of surrounding forces to cause an "opening" of crural and passage of gastric contents.

This work has generated a presentation (5), and manuscripts (21,22).

© Respiratory muscle length and EMG spectral analysis in the awake canine.

As we have become more experienced with direct measurements of length of various respiratory muscles, we have become disenchanted with the use of moving average EMG as an indicator of electrical activity of the respiratory muscles. It seems incongruous to strive for maximum precision in muscle length and then correlate length with a deliberately approximate moving average EMG value, with the one dimensional focus on amplitude, and loss of frequency information that EMG treatment entails.

We have developed a new combined sonomicrometry and EMG transducer for conjoint measurement of the respiratory muscle length and EMG from exactly the same point sources within the muscle. Along with this device, we have developed a suite of custom programs which cleanly gate EKG artifact, digitally filter sonomicrometry interference, and then do spectral analyses by fast fourier transform of selected slices of the inspiratory and expiratory cycle. With these innovations, we have been able to explore the basic relationship between centroid frequency (F0c) and length of the respiratory muscles. Even during tidal breathing, we have identified a modest but highly significant relationship between length and F0c. We are extending these measurements to other conditions of respiration and exploring the intrabreath "profile" of the EMG spectrum through the respiratory cycle.
Figure 6. Schematic of centroid frequency calculation per crural segmental length. See text for details.

Figure 7. Crural segmental length and Fc. A representative animal.

In Figure 6, we show a schematic overview of our analysis of the EMG. In this trace an Fc value is calculated by fourier transform at each of the specific
lengths in the inspiratory cycle which are marked by the vertical stripes. A series of such FFT calculations at a sequence of lengths in many breaths generates the data presented in figure 7, which relates Fc to % shortening for the crural segment in this single canine. It can be noted in this representative animal, that the magnitude of the relation between crural length and Fc is measured to be 1.4 Hz/% shortening (a significant slope, P< 0.001).

To date our interest in Fc and length has resulted in two abstracts/presentations (6,8) and two manuscripts under review (16,20).

5 Effects of REM and nonREM sleep on differential function of the respiratory muscles.

We have expanded our investigations to consider the influence of state change upon the differential activities of the major respiratory muscles. This work has its roots in the studies described in this thesis; we review nonREM sleep as a natural, imposed, physiological, upper airway resistive load, while REM is a unique natural disability of non-diaphragmatic muscles within an additional upper resistive load, for which costal and crural diaphragm must compensate.

We have some preliminary measurements of respiratory muscle activity during REM and non REM sleep. We have expanded our techniques to include sleep staging according to usual canine techniques, utilizing electro-oculogram (EOG), electroencephalogram (EEG), neck EMG, and observation.
Figure 8. Transversus abdominis shortening during wakefulness, REM and nonREM sleep. Mean values from a single canine, normalized over time as %Ttot.

In Figure 8 we show mean values from a single canine normalized for time but with absolute shortening on the Y axis, during wakefulness (AWAKE), nonREM (NREM), and phasic REM (PREM) sleep. It can be seen that shortening activity of the abdominal muscle transversus is significantly decreased during either nonREM or phasic REM periods. Meanwhile, parasternal shortening and EMG were similar between wakefulness and nonREM sleep, decreasing in phasic REM but not tonic REM. Costal activity increased from wakefulness, through nonREM, phasic REM, and tonic REM, showing significantly greater shortening and corresponding tidal EMG. However, crural diaphragm activity differed significantly from that of costal. Crural diaphragm activity significantly decreased from wakefulness through phasic REM as shown in figure 9. Figure 9 is representative of a single animal showing intrabreath shortening of crural segment during wakefulness, nonREM and phasic REM. To expand our sleep investigations, we have begun to implant the upper airway musculature beginning with geniohyoid.

To date we have generated an abstract for the upcoming federation meeting (9) and a manuscript is in preparation (24).
Respiratory muscle compensation for diaphragm paresis in the awake canine.

We have begun to extend our investigations of respiratory muscle function to conditions of abnormal respiratory function and respiratory muscle compensation. We have begun by studying the successful compensation by the major respiratory muscles for sudden, reversible loss of one or both hemidiaphragms, induced by unilateral or bilateral phrenic anestheisia. Since respiratory failure is unknown in instances of unilateral hemiparalysis, and may not even occur with bilateral paralysis, it is clear that there is a significant capability for compensation, to allow the remaining respiratory muscles to maintain adequate ventilation. In these investigations, we wished to characterize this successful compensation, with reversible hemidiaphragm paralysis and then extend the studies to chronic diaphragm paralysis. In the final two figures, we demonstrate mean results from a representative canine.

Figure 10. Transversus abdominis shortening during: normal diaphragm function (NOFRZ), reversible hemiparalysis of the diaphragm segment on the side opposite to the recording electrodes induced by local anesthesia of the phrenic nerve (CONTFRZ), and bilateral hemiparalysis induced by anesthesia of both phrenics (FRZ). Y axis shows absolute shortening as %LBL; time is normalized as %Ttot.
Figure 11. Costal segmental shortening during: normal diaphragm function (NOFRZ), reversible hemiparalysis of the diaphragm segment on the side opposite to the recording electrodes induced by local anesthesia of the phrenic nerve (CONTRZ), and bilateral hemiparalysis induced by anesthesia of both phrenics (FRZ). Y axis shows absolute shortening as %LBL; time is normalized as %Ttot.

Figure 10, illustrates data for transversus abdominis in conditions of normal diaphragm function, unilateral diaphragm paralysis on the side opposite to the measurements (contralateral freezing or CONTRZ as noted in the figure), and bilateral diaphragm paralysis (FRZ). It can be seen that, as diaphragm function was lost progressively, that transversus activity increased in a corresponding stepwise fashion. Measurements from the costal diaphragm are illustrated in the final figure 11. These are mean intrabreath values from a single animal. From mean normal costal shortening with no disability of approximately 2.5 %LBL, it is seen that left costal segmental shortening approximately doubled, and altered early inspiratory shape and development in response to loss of the contralateral hemidiaphragm. Thereafter, the complete paralysis in this canine is demonstrated by the total loss of shortening of the costal segment after the second phrenic nerve was anesthetized. In fact costal motion becomes frank lengthening.

While this investigation has provided significant experience in locating, isolating, and manipulating the phrenic nerve and phrenic nerve branches and roots, it may be that our most interesting data will arise where phrenic anesthesia is incomplete. For example, if the lowest C7 phrenic root is omitted, then crural shortening may be partially spared and the pattern of compensation measurably
different. This line of investigation has also generated interest in direct measurement of intercostal musculature in addition to the parasternal; we have begun to implant lateral external intercostal.

To date, this work has generated an abstract for the upcoming meeting (10) and a manuscript in preparation (25).

Human studies of respiratory muscle function.

There has been an inevitable extension of these canine investigations and investigative techniques to the study of normal physiology and clinical pathophysiology of respiratory muscles in humans.

Obviously the technology of value in human studies is not chronically implantable sonomicrometry transducers. However, there is an interesting anecdotal report that an "unsuccessful" attempt has been made in another part of the world, against our advice, to leave sonomicrometry crystals in place for a time after human upper abdominal surgery. Details of the lack of success are not known, but presumably the crystal heads fractured at the soldered junction with the lead-in wire, to remain permanently imbedded in the diaphragm when removal was attempted by traction on the extruding wires. If transducers could be left in consenting human subjects after routine surgery, extraordinary measurements could be made. However, to achieve that end, existing lead-in wires would need to be replaced by telemetric signal transmission, and transducers would need to be manufactured of absolutely inert or entirely dissolvable material. With existing materials, the latter requirement is not possible. For the moment, other mammals must continue as our surrogates in the study of human respiratory muscle function.

As inspired by the studies in this thesis, data acquisition programs that effectively replace a strip chart recorder and are commercially capable have now been developed. Experience in this whole area of study, as well as computerized acquisition capability, and the entire suite of dedicated programs for breath-by-breath analysis of respiration and muscle function, are all immediately transferable to human studies.

Differential respiratory function of the abdominal muscles. The first foray into human clinical studies occurred in collaboration with Dr. Tadashi Abe, at Kitasato University in Tokyo. Our experience and analysis techniques were mated with his expert exploitation of another form of ultrasound, rather than sonomicrometry. Using high resolution, grey scale, diagnostic ultrasound and a
specially visible insertable fine wire electrode, we were able to implant under direct vision and study, the differential respiratory function in normal humans of the abdominal muscles: transversus abdominis, rectus abdominis, internal oblique, and external oblique. These are the first investigations of normal function and interaction of the complete abdominal musculature using fine wire electrodes, during resting and stimulated ventilation, in various postures.

To date, a pair of abstracts (7,12) and one manuscript in press (23), have resulted from this work.

© Costal and crural function after upper abdominal surgery. The latest extension of these canine studies has been in a collaborative effort with Dr. Gordon T. Ford, at the Calgary General Hospital. Our canine analysis techniques, specific even for the respective diaphragm segments, were mated with his innovative techniques for postoperative measurement of the costal and crural diaphragm in humans. Using specially modified cardiac electrophysiologic recording catheters which were implanted at routine upper abdominal surgery to measure EMG, the postoperative function of costal and crural diaphragm segments could be studied directly in the intact human. These investigations have produced the first evidence in clinical medicine of differential dysfunction of the diaphragm segments. After cholecystectomy, the crural segment is preferentially inhibited compared to costal. In addition, although cholecystectomy by laparoscopy is now in vogue, this innovation in surgical technique may not deliver the expected benefits in decreasing postoperative atelectasis and pneumonia. The postoperative inhibition of the diaphragm segments that follows open incisional laparotomy, is not different even after laparoscopic surgery. These studies continue.

To date this work has resulted in three abstracts (11,13,14), and two manuscripts are in preparation (26,27).
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