THE EFFECTS OF ACUTE AND CHRONIC HYPERCAPNIA

UPON VENTILATION AND ACID-BASE STATUS

IN THE PEKIN DUCK

by

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ABSTRACT

In this study, awake Pekin ducks (*Anas platyrhynchos*) were exposed to periods of acute and chronic hypercapnia (0.05 F_ICO_2). Measurements were made of ventilation and acid-base status in both adult and juvenile male ducks as well as in adult female ducks. All Pekin ducks, regardless of age or sex, responded acutely to inspired CO_2 with a marked hypercapnic-hyperpnea. Inhalation of CO_2 resulted in a significant increase in arterial CO_2 tension ($PaCO_2$) and decrease in arterial pH (pHa). The increase (3 times (x)) in minute ventilation ($\dot{V}_E$), while primarily a function of an increase (2x) in tidal volume (V_T), also involved an increase (1.5x) in breathing frequency (f_b).

The chronic responses of ducks to inspired CO_2, however, did differ depending upon the sex of the animal. In male ducks, the initial increase observed in $\dot{V}_E$ during the first 20 minutes was reduced by 50% after 300 minutes. This partial recovery in $\dot{V}_E$ resulted entirely from the complete return of f_b to its control levels as V_T remained both elevated and constant throughout the period of hypercapnia. In addition, the male ducks also demonstrated a significant recovery (50%) in pHa, a change that was paradoxical to the concomitant increase measured in $PaCO_2$. While a change in strong ion difference (SID) was not detected, the accompanying rise in calculated arterial $[HCO_3^-]$ suggested that metabolic compensatory processes must have alleviated the initial respiratory acidosis. The rate of metabolic compensation seen in the ducks of this study exceeds that reported for any other air-breathing vertebrate.

Female ducks, on the other hand, maintained the initial increase in $\dot{V}_E$ and decrease in pHa throughout the period of CO_2 exposure. The reasons for this remain unclear although it is speculated that the metabolic demands of eggshell formation may have limited the capacity of these birds to mobilize further HCO_3^- stores.

Differences in the changes which occurred in f_b, V_T, pH and P_{CO_2} in male and female ducks during chronic CO_2 exposure strongly suggest that the changes in f_b were a singular function of changes in pHa ($[H^+]$) while changes in V_T were primarily a function of changes in $PaCO_2$. Denervation of peripheral chemoreceptors appeared to have little effect upon the overall ventilatory responses to either acute or chronic hypercapnia, suggesting that central chemoreceptors must have been predominantly responsible for the ventilatory responses observed during this study.

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INTRODUCTION

Carbon dioxide (CO_2) is a powerful respiratory stimulus in all air-breathing vertebrates. It has repeatedly been shown to stimulate respiration in birds that are either awake (Dooley & Koppanyi, 1929; Hiestand & Randall, 1941; Fowle & Weinstein, 1966; Jones & Purves, 1970; Bouverot et al., 1974; Powell et al., 1978; Brackenbury et al., 1982), anesthetized (Fowle & Weinstein, 1966; Richards & Sykes, 1967; Ray & Fedde, 1969; Osborne & Mitchell, 1977; Osborne et al., 1977; Scheid et al., 1978), or decerebrate (Johnson & Jukes, 1966; Tallman & Grodins, 1982a,b). The stimulative effect of CO_2 upon respiration in birds, however, is dependent upon the inspired CO_2 concentration. In the above mentioned studies, the level of inspired CO_2 was always less then 6%. In studies that employ CO_2 concentrations above 6%, respiration is often depressed rather than stimulated (Orr & Watson, 1913; Hiestand & Randall, 1941; Jones & Purves, 1970; Jukes, 1971; Scheid & Piiper, 1986). Such depression could result from several factors including: (1) a CO_2-induced depression of the central nervous system (Jukes, 1971; Scheid & Piiper, 1986), or (2) the result of breathing pattern being severely altered by a CO_2-induced inhibition of intrapulmonary chemoreceptors (Milsom et al., 1981).

In birds, the increases observed in minute ventilation ($\dot{V}_E$), when low levels of CO_2 were inspired, were always accompanied by increases in tidal volume (V_T). The changes observed in breathing frequency (f_b), however, were more variable; f_b either increased (Bouverot et al., 1974; Powell et al., 1978; Milsom et al., 1981; Brackenbury et al., 1982;

Tallman & Grodins, 1982; Scheid & Piiper, 1986), decreased (Richards & Sykes, 1967; Bouverot & Leitner, 1972; Osborne et al., 1977; Osborne & Mitchell, 1978) or remained unchanged (Ray & Fedde, 1969; Jones & Purves, 1970; Colby et al., 1987). Thus, the increase in $\dot{V}_E$ that occurred upon CO_2 inhalation was primarily due to an increase in V_T rather than f_b.

All studies of hypercapnic ventilatory responses in birds have employed relatively short periods of CO_2 exposure (seconds to minutes). It is evident from the mammalian literature, however, that chronic exposure to CO_2 results in ventilatory responses that are markedly different from those observed during acute exposure. Perhaps the most noticeable difference is that following a few weeks of maintained CO_2 exposure, ventilation gradually decreases from the acutely elevated level to a level much closer to that observed prior to the CO_2 exposure. In other words, $\dot{V}_E$ decreases towards normal values even though the imposed respiratory stimulus is maintained. This time-dependent decrease in $\dot{V}_E$ has been defined as an adaptation of the respiratory system to the chronic CO_2 stimulus (Forster & Dempsey, 1981; Dempsey & Forster, 1982). Ventilatory adaptation to a chronically maintained CO_2 stimulus has been examined in several species of mammals including rats (Lai et al., 1981), dogs (Jennings & Chen, 1976; Jennings & Davidson, 1984) and humans (Schaefer, 1949; Chapin et al., 1955; Schaefer et al., 1963; Clark et al., 1969; Guillerm & Radziszewski, 1979).

In an attempt to determine the mechanism(s) responsible for the occurrence of such a ventilatory adaptation, the changes reported in ventilation have been examined against the changes simultaneously measured in arterial CO_2 tension (P_{CO_2}) and arterial pH (for reviews see: Dempsey & Forster, 1982). When the effects of CO_2 inhalation were first

quantitatively described by Haldane and Priestly (1905), they reported that the rise observed in arterial P_{CO_2} was the stimulus responsible for the increase concomitantly observed in ventilation. Since then, however, inhalation of CO_2 has been shown to produce not only an increase in $PaCO_2$, but also an increase in arterial hydrogen ion concentration ($[H^+]$) (or decrease in pH). The independent effects of these two variables upon ventilation have been very difficult to determine as changes in both are inextricably linked through their relationship described by the Henderson-Hasselbach equation. Because of this apparent coupling, Gray (1946) proposed, with his Multiple Factor Theory, that both agents acted together, rather then individually, as the stimulus to the respiratory system.

It was subsequently observed, however, that chronic inhalation of CO_2 resulted in significant increases in both arterial and cerebrospinal fluid (CSF) bicarbonate ion concentration ($[HCO_3^-]$) (Bleich et al., 1964; Clark et al., 1969; Messeter & Siesjo, 1972; Jennings & Chen, 1976; Guillerm & Radziszewski, 1979; Lai et al., 1981; Dempsey & Forster, 1982; Loeschcke, 1982; Jennings & Davidson, 1984; Fencl, 1986; Kazemi & Johnson, 1986). Such an increase was indicative of metabolic compensation of the chronically imposed respiratory acidosis, leading to an increase in pH despite the maintained elevation in $PaCO_2$. Under conditions of chronic hypercapnia, Lai et al. (1981) demonstrated that changes in $\dot{V}_E$ correlated well with changes in arterial pH but not arterial P_{CO_2}. Observations such as this have helped to fuel the debate surrounding the issue of whether P_{CO_2} or pH is a unique stimulus to respiratory chemoreceptors.

Up to this point, most studies involving chronic hypercapnia have been conducted upon mammals rather than birds. In a recent study, however, Dodd and Milsom (1987) also demonstrated that birds produce a significant respiratory adaptation to chronically inspired

CO_2. In addition, they also demonstrated that (1) there appeared to have been a change in the relationship between P_{CO_2} and pH (ie. a significant metabolic compensation), and (2) that changes in $[H^+]$ appeared to have been instrumental in producing the adaptation. Furthermore, the rate and magnitude with which the adaptation occurred far exceeded that previously reported for mammals.

Just as it is not clear to what extent changes in $PaCO_2$ or pHa contribute to the acute and chronic changes in respiration which accompany hypercapnia, it is also not clear at what sites these stimuli act to produce these effects. The avian respiratory system possess three distinct chemoreceptive sites at which P_{CO_2} or H^+ may act either individually or synergistically: (1) the systemic arterial chemoreceptors, (2) the intrapulmonary chemoreceptors, and (3) the central chemoreceptors.

The avian systemic chemoreceptors, located in the carotid bodies, are found in the thoracic cavity in close proximity to both the parathyroid gland and ultimo-branchial body (Jones & Purves, 1970; Fedde, 1976; Bouverot, 1978; Scheid & Piiper, 1986). These bilateral structures are innervated primarily by nerves emanating from the vagus nerve (Fedde, 1970; Bouverot, 1978) although there is some suggestion of sympathetic innervation as well (Burger et al., 1974; Burger & Estavillo, 1978). They receive their arterial blood supply from small branches of the common carotid artery (Fedde, 1970; Bouverot, 1978). Similar in function to the carotid bodies of mammals, carotid body chemoreceptors in birds are stimulated by both low arterial P_{O_2} and high arterial P_{CO_2} (Bouverot et al., 1974; Fedde, 1976; Nye & Powell, 1984; Scheid & Piiper, 1986). Carotid-body chemoreceptors have been suggested as the receptor group responsible for the O_2-chemoreflex observed in birds (Jones & Purves, 1970; Bouverot & Leitner, 1972; Bouverot

& Sebert, 1979; Bouverot et al., 1979). In addition, these receptors have also been demonstrated to aid in evoking respiratory responses to both transient (Jones & Purves, 1970; Bouverot & Leitner, 1972) and steady-state changes in $PaCO_2$ (Bouverot et al., 1974; Milsom et al., 1981). In birds, approximately 20-40% of the total $\dot{V}_E$ response to steady-state hypercapnia has been attributed to carotid-body chemoreceptors (Milsom et al., 1981; Jones et al., 1985), a percentage similar to that reported for mammals (Berger et al., 1977; Berkenbosch et al., 1979; Heeringa et al., 1979; O'Regan & Majcherczyk, 1982; Jennings & Szlyk, 1988).

The second group of peripheral chemoreceptors, found in birds, are the intrapulmonary chemoreceptors (IPCs). These CO_2-specific chemoreceptors are located primarily in the gas exchange regions of the lungs, specifically the neopulmonic and the paleopulmonic parabronchi (Fedde, 1976; Tallman & Grodins, 1982a; Scheid & Piiper, 1986). The activity level of IPCs has been shown to be inversely related to pulmonary CO_2 levels (Fedde, 1976; Osborne et al., 1977; Scheid et al., 1978; Scheid & Piiper, 1986). Thus, unlike either carotid-body or central chemoreceptors, IPCs are inhibited rather then stimulated by increasing levels of inspired CO_2.

The role of IPCs in the control of respiration has been the subject of controversy ever since these receptors were first described (King et al., 1968; Peterson & Fedde, 1968). Several researchers have claimed that IPCs play a very important role in controlling overall levels of ventilation in birds. These reports have all been based upon the observation of an isocapnic-hyperpnea during the inhalation of CO_2 at levels less then 3% (Osborne & Mitchell, 1977; Osborne & Mitchell, 1978; Powell et al., 1978; Scheid et al., 1978). In opposition to these reports, others have maintained that IPCs, exert an influence on

breathing pattern, but contribute little towards the control of overall ventilation (Jones & Purves, 1970; Milsom et al., 1981; Tallman & Grodins, 1982). These studies, and several others, have repeatedly failed to observe an isocapnic-hyperpnea and instead have always observed a hypercapnic-hyperpnea with similar levels of inspired CO_2 (Jones & Purves, 1970; Bouverot et al., 1974; Kuhlmann & Fedde, 1976; Milsom et al., 1981; Tallman & Grodins, 1982a). The lungs of birds, unlike those of mammals, are rigid structures comprised of a series of parallel, narrow tubes called parabronchi. As a result, the avian lung demonstrates very little capacity to either expand or contract during the respiratory cycle. Mammalian-type stretch receptors would serve little function in the avian lung and it has been suggested, based on these latter studies, that the IPCs of birds may provide a functionally analogous receptor to the pulmonary stretch receptors found in mammals (Fedde, 1970).

Since peripheral chemoreceptors contribute relatively little towards the hypercapnic ventilatory response in birds, it is assumed that the major component of the response (60-80%) is due to stimulation of central chemoreceptors located in the brain (Milsom et al., 1981). In birds, the existence of these receptors has only been indirectly deduced from ventilatory-reflex studies (Sebert, 1978; Jones et al., 1979; Sebert, 1979; Milsom et al., 1981) although they are thought to exist on or near the ventrolateral surface of the medulla as they do in mammals (Schlaefke et al., 1970; Bouverot, 1978).

In summary, it was apparent, from Dodd and Milsom (1987), that upon the chronic inhalation of CO_2, birds appeared capable of adapting their initial ventilatory response to hypercapnia. While the exact mechanisms of this adaptation was not determined, a change in the relationship between PaCO_2 and pHa was suspected. Finally, while it is known that

birds possess at least three sets of chemoreceptors that are sensitive to changes in $P_{CO_2}/[H^+]$, both (a) the relative roles of CO_2 versus H^+ as stimuli at each chemoreceptor group, and (b) the relative contributions of each receptor group to the ventilatory response under conditions of either acutely or chronically inspired CO_2, remain unclear.

The purpose of the present study, therefore, was threefold. Firstly, this study set out to better document the phenomenon of ventilatory adaptation in birds. Secondly, it was hoped that the mechanism(s) responsible for such chronic ventilatory changes, particularly the contributions of changes in CO_2 and $[H^+]$, could be better clarified. Finally, it was hoped that this study would provide some insight as to the relative contributions the different respiratory chemoreceptors were making towards the ventilatory responses shown by birds to both acutely and chronically inspired CO_2.

MATERIALS AND METHODS

Experiments were performed on 36 adult and 20 juvenile (6-24 week old) Pekin ducks (*Anas platyrhynchos*) obtained from a breeding colony housed outdoors at the Animal Care Facility of the University of British Columbia. Adult animals ranged in body weight from 2.2 to 3.3 kg while the juveniles were somewhat smaller, ranging in weight from 1.5 to 3.0 kg. Two days prior to any experimentation, the animals were brought indoors, maintained in individual cages (60 x 63 x 91 cm) with free access to food (Buckerfield's Goose and Duck Grow Pellets) and water and allowed to acclimate to room temperature (21-22°C). Calder and Schmidt-Nielsen (1968) have shown that without a period of acclimation, rapid changes in ambient temperature can lead to heat stress and result in considerable hypocapnia and alkalosis.

The experiments that constitute this thesis fall into four separate series of experiments as follows:

i) Series A - Experiments were conducted on adult male birds with fully intact respiratory chemoreceptor groups (n=18) to determine the effects of long term (5 hour) exposure to inspired hypercapnia (5% CO_2) on ventilation and arterial blood gases and pH. Strong ion difference (S.I.D.) in arterial blood was also determined in a subgroup of 8 animals.

ii) Series B - Identical experiments were conducted on adult female birds, again with all chemoreceptor groups intact (n=6) to determine whether the responses measured in

Series A differed between the two sexes.

iii) Series C - Experiments were conducted on adult male birds with carotid body chemoreceptors denervated (n=12) to examine the role of this chemoreceptor group in the responses observed in Series A.

iv) **Series D** - Experiments were conducted on juvenile male birds (n=12) with pulmonary **afferent** nerves denervated to examine the *role of pulmonary* receptors in the responses observed in Series A.

A) SURGERY

All birds undergoing experimentation had a flexible polyethylene cannula (PE-90; Clayton Adams Inc.) implanted in their right brachial artery for both blood sampling and for monitoring mean arterial blood pressure (MAP). Prior to implantation, the tip of each cannula was slightly bevelled. In addition, 1 or 2 small side-holes were cut approximately 0.25-0.75 cm from the bevelled tip. The surgery required to implant the cannula was minor and was conducted under local anaesthesia (Xylocaine®, 20 mg/ml; Astra Pharmaceuticals) administered subcutaneously. With the animal lightly restrained in dorsal recumbency, the right wing was out-stretched and the feathers overlying the humerus were removed. A small incision lateral and parallel to the humerus revealed both the brachial artery and brachial vein. A small incision was made in the artery and the cannula, filled with 1000 IU/ml heparinized saline, was slowly advanced approximately 7 cm. If the use of a general anaesthesia was called for in the protocol (ie. Series C and D), an additional cannula (PE-90 or PE-60) was also implanted in the brachial vein. Each cannula was anchored to the skin and the incision sutured closed with braided nylon surgical silk (00). The portions of

the cannulae that extended from the vessels were sealed and taped to the underside of the birds' wings. Before returning each animal to its cage, the wings were lightly restrained with filament tape to prevent excessive movement from dislodging the implanted cannulae.

While arterial cannulation was the only surgery required for birds in Series A and B, the latter two series of experiments (C and D) required additional surgery as described below.

a) Series C - Carotid Body Denervation In addition to the arterial cannulation, birds in this series of experiments underwent further surgery to denervate the carotid bodies. Each of these peripheral chemoreceptor groups, located at the bifurcations of the left and right common carotid arteries (Jones & Purves, 1970), is innervated by a carotid sinus nerve, a direct branch of the ipsilateral vagus nerve arising from the nodose ganglion. Bilateral denervation was performed under intravenously administered general anaesthesia (Somnotol®, sodium pentobarbitol; 65 mg/ml; MTC Pharmaceuticals). The administration of an initial dosage of 16 mg/kg achieved the state of anaesthesia desired. The administration of smaller supplemental doses (3.5 mg/kg), when required, maintained that state of anaesthesia throughout the surgery.

With each animal in dorsal recumbency, feathers were removed from its chest in a triangular patch extending from the apex of the sternal carina to the base of the neck, roughly following the underlying left and right clavicles. A midsagittal incision, approximately 7 cm in length, was made and the skin and subcutaneous fat reflected to expose the clavicular airsac. The airsac was carefully opened and reflected. Marking the cut edges of the airsac with surgical thread allowed for their easier location at the time of closing. The animal was then intubated using an endotracheal tube (4.0 mm I.D.,cuffed;

Mallinckrodt Inc.) and unidirectionally ventilated (inflow via the trachea and outflow via the ruptured inter-clavicular airsac) with a hyperoxic gas mixture (30 % O_2) to ensure sufficient oxygenation of the tissues during surgery. In addition, intramuscularly administered atropine sulphate (2.1 x 10^{-3} mg/kg) was used to prevent the buildup of mucous in the trachea and bronchi, a known side effect of the pentobarbitol anaesthesia. The left and right vagus nerves were identified on the dorsal surface of the cervical airsac and were traced posteriorly to the left and right thyroid glands, respectively. On each side, the thyroid gland was carefully reflected to reveal the underlying nodose ganglion. To ensure complete section of the carotid sinus nerves, the 2 or 3 nerve fibers branching from each vagal nerve trunk in the region of the ganglion (1 cm both posterior and anterior), were sectioned. Performing this on both the left and right side resulted in the bilateral denervation of the carotid body chemoreceptors. Once accomplished, the plane of anaesthesia was then reduced, and when spontaneous breathing movements became apparent, the endotracheal tube was removed and the clavicular airsac tightly sutured to prevent air-leakage. The overlying skin was then sutured and the animal was administered, prophylacticly, Penbritin®-250 intramuscularly (ampicillin, 250 mg/ml; Ayerst Laboratories) and allowed to recover for several days.

The effectiveness of the denervation was determined several days after the surgery by the intravenous injection of sodium cyanide (NaCN, 200 µg/ml), a potent blocker of cellular respiration at the level of the electron transport chain (cytochrome aa_3). Such a small, sublethal dose of cyanide creates a degree of histotoxic hypoxia in the highly O_2-sensitive tissues of the carotid bodies, resulting in their increased activity and thus increased overall respiration. An absence of respiratory increase following cyanide injection

indicated that the carotid body chemoreceptors had been successfully denervated.

b) Series D - Pulmonary Afferent Denervation   In this series of experiments, the CO_2 sensitive, intrapulmonary chemoreceptors (IPC) were surgically denervated. These chemoreceptors, located throughout each parabronchial lung, send afferent input to the higher respiratory centers in the brain by way of fibers travelling in each vagus nerve. Afferent nerve fibers that arise from these chemoreceptors converge into several larger fibers that join the vagus nerve at several points along the length of the lung. Therefore, the bilateral section of both vagi at some level between the lungs and nodose ganglion should result in the removal of all afferent input from IPCs. However, Fedde and Burger (1963) demonstrated that while birds were able to tolerate unilateral vagotomy, the removal of all vagal input to the viscera, following bilateral vagotomy, was mortally damaging. Therefore, in the experiments of this Series, all pulmonary branches of the right vagus nerve were sectioned while the left vagus nerve was completely sectioned. The result of this approach was complete pulmonary denervation but with maintained unilateral visceral innervation. Unfortunately, because it was not possible to physically identify or separate those fibers specific to IPC's from those fibers arising from other lung receptors (ie. mechanoreceptors) also travelling in the vagi, it was necessary to section all vagal afferent branches arising from each lung (ie. total pulmonary afferent denervation).

The surgery required to remove these receptors was extensive and required opening the thoracic cavity to expose the lungs. To aid in the necessary bisection of the sternum, juvenile birds were used in this series of experiments (6-9 weeks old) because they possessed incompletely ossified sternums. All surgery was performed under the same general anaesthetic regime described above. Atropine (2.1 x 10^{-3} mg/kg) was again

administered intramuscularly to prevent tracheal and/or bronchial mucous secretion induced by the general anaesthetic. Each bird was placed in dorsal recumbency and, once fully anaesthetized, was intubated, the clavicular airsac opened, and the animal unidirectionally ventilated in the manner previously described. At this point, the right internal thoracic artery was ligated at a point immediately ventral to the right thyroid gland. This was essential to allow the subsequent bisection of the sternum.

A sagittal incision, extending the entire length of the sternum, was made in the skin to the immediate right of the midline, thus exposing the large pectoralis major muscle. Using an electro-surgical unit (Electrosectilis, Model 770; Birtcher Corp.), a sagittal incision was then made through the pectoralis major extending the full length of the sternum as close to the midline as possible. A second incision was then made through the smaller underlying pectoralis minor muscle. Excessive bleeding was stopped either by clamping the tissue with hemostatic forceps, or by electrocoagulation (Hyfrecator, Model X-712, Birtcher Corp.). Both muscle layers were then laterally reflected to expose the underlying sternum. Because of its predominantly cartilaginous composition, the sternum was quite easily cut with scissors, in a posterior-anterior direction approximately 1 cm to the right of the carina. Extreme care was taken to keep the incision through the sternum shallow to avoid rupturing the underlying pericardium. Once bisected, the sternum was carefully pried apart with a retractor. At all times, it was important to keep the exposed muscle and tissue moist with avian ringers solution to prevent desiccation.

The right vagus nerve was first identified posterior to the right thyroid gland, in an area bordered by the right primary bronchi and the right bracheocephalic vein, and was then traced over the entire length of the right lung. All nerve fibers that branched from the

vagus in the region of the lung were carefully traced. Those that projected dorsally, towards the lung, were sectioned. Only the right lung was denervated in this manner. Earlier attempts to simultaneously denervate both lungs in this fashion usually resulted in death due to the combined respiratory depression following complete lung denervation and the general anaesthesia. Thus, after the right lung had been denervated, each animal was sutured closed in a layered fashion, starting with the sternum. Using a hand-held hobby drill, small holes were bored through the sternum on either side of the incision. The sternum was then laced together with non-absorbable surgical silk (size 0). Secondly, the overlying pectoralis minor and major muscles were individually sewn to the carina of the sternum with absorbable surgical suture (size 1, Vicryl, Ethicon). Finally, the skin was stitched closed using surgical silk (size 00).

With the right lung denervated, the left vagus nerve was prepared for later sectioning (once the animal had recovered from surgery). While still under the general anaesthesia, a small region of skin (1 x 3 cm) on the ventral surface of the neck, approximately 8 cm posterior to the base of the bill, was exposed. A small (3 cm) sagittal incision exposed the trachea. Gentle retraction of the trachea and surrounding tissue exposed the left carotid sheath, a fascial compartment that enclosed the left carotid artery, left jugular vein and left vagus nerve. Without damaging the pulsatile carotid artery, the vagus nerve was gently teased from this fascia and a 1 cm segment of it was isolated and wrapped with latex sheeting to prevent tissue re-growth and allow easy relocation of the nerve at a later time. Once this was completed and the neck incision closed, the plane of anaesthesia was reduced and the endotracheal tube removed once spontaneous breathing movements became apparent. The clavicular airsac and overlying skin were finally closed

as previously described. Penbritin®250 (ampicillin, 250 mg/ml; Ayerst Laboratories) and Demoral® (meperidine hydrochloride, 50 mg/ml; Winthrop Laboratories) were intramuscularly administered every 4-6 hours for two days at dosages of 25 mg/kg and 5 mg/kg, respectively.

After a five to six day recovery period, the pulmonary denervation was completed under local anaesthesia (Xylocaine®) by relocating and sectioning the previously isolated left vagus nerve. Although the completeness of the pulmonary denervation was immediately apparent from the marked change in breathing pattern (breathing became both slower and deeper), all surgical denervations were confirmed post-mortem.

Successfully denervated birds were thus devoid of input from all pulmonary receptors, including intrapulmonary chemoreceptors, and from one carotid-body chemoreceptor. This surgical protocol, although complicated and with a relatively high mortality rate, maintained input from one carotid-body chemoreceptor and also maintained the influence of the parasympathetic nervous system upon cardiovascular, excretory and digestive organ systems, avoiding the problems associated with bilateral cervical vagotomy observed in birds (chickens) by Fedde and Burger (1963).

B) MEASUREMENTS

In all experimental series, body plethysmography was the technique used to measure ventilation. The plethysmograph consisted of two parts, a body compartment and a head compartment (Fig.1). The tubular body compartment (15 L volume), constructed of 6 mm clear plexiglass, was surrounded by a water jacket which allowed the chamber to be maintained at normal avian core body temperature (41 ± 1° C). The head compartment (3

Figure 1.   Schematic diagram of experimental apparatus. See text for details.

blood sampling
cannulae

pneumotachograph

air flow

dental dam
collar

rectal thermometer
(Tb)

water flow

17
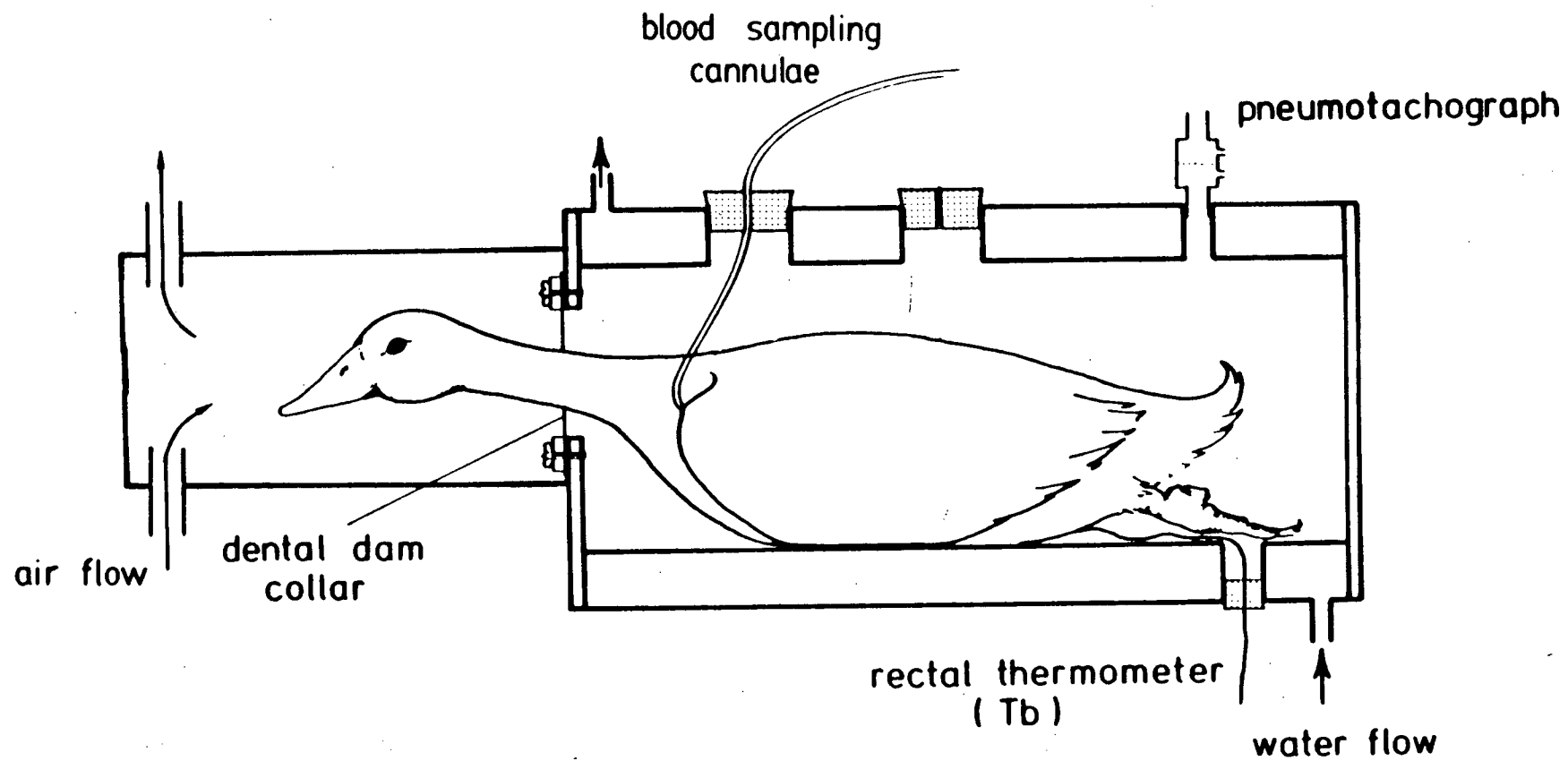
L volume), constructed of dark-colored plastic, served two purposes. Firstly, it enabled specified gas mixtures to easily be administered to the animals to breathe, and secondly it acted as a blind, obscuring activity in the room from the bird resting in the plethysmograph. Respiratory gases entered and exited the head compartment through openings on opposite sides of the compartment. The composition of the gas flowing through the head compartment was altered by mixing pure gases (CO_2, O_2) with compressed air through a series of calibrated flow meters. Prior to their entering the head compartment, gases were bubbled through a humidifier. The composition of both the inflow and outflow gases was monitored with Beckman oxygen (OM-11) and carbon dioxide (LB-2) analyzers (SensorMedic Inc.) that had been calibrated with mixed gases of known composition generated by a gas mixing pump (GMA-2; Radiometer). The flow rate of gas through the head chamber was never less than 20 L/min, thus preventing CO_2 accumulation. The two compartments of the plethysmograph were separated from each other by a flexible latex collar (Dental Dam; Hygenic Corp.) that sealed around the bird's neck.

In the body compartment, changes in body volume, caused by ventilatory movements, resulted in changes in air flowing through a Fleisch #00 pneumotachograph connected to a single port in the body chamber. The air flow, through the pneumotachograph, was measured as a change in differential pressure (Validyne DP 103-16), which was integrated (integrating amplifier; Gould Inc.) to yield a measurement of tidal volume.

Mean arterial blood pressure (MAP) was continuously monitored in all birds by using a physiological pressure transducer (#RP 1500i; Narco Scientific) connected to the

cannula implanted in the brachial artery. MAP was maintained relatively constant throughout the experiment as any volume lost through blood sampling was replaced by the intravenous infusion of avian Ringer's solution (Burton, 1975).

In all experiments arterial blood samples (0.5 ml) were anaerobically drawn and immediately placed on ice to arrest erythrocytic metabolism (Scheid & Kawashiro, 1975). Within five minutes of sampling, arterial blood gases (PO_2 and PCO_2) and pH were determined using a Radiometer blood gas/pH analyzer (pHM 71 Mk2, Radiometer) maintained at avian core body temperature ($41\pm1^\circ$ C). The analyzer was calibrated both before and after each sample using saturated gases from a Radiometer GMA-2 gas mixing pump and commercially prepared pH buffers (Radiometer/Bach-Simpson). In addition, the arterial blood of Series A birds was analyzed for its total CO_2 content using the technique previously described by Cameron (1971). This measurement required only a 25 μL aliquot of blood taken from the original 0.5 ml sampled.

At three times specified in the protocol, a second arterial sample (2.3 ml) was taken and analyzed for the plasma concentrations of strongly dissociated ions. From these changes, the strong ion difference (SID), described by Stewart (1981, 1983), could be calculated as the concentration difference between strongly dissociated cations (ie. Na^+, K^+, Ca^{2+}) and strongly dissociated anions (ie. Cl^- lactate$^-$). From that 2.3 ml, a 2.0 ml aliquot was immediately placed into a test-tube and allowed sufficient time, approximately 20 minutes, to clot. Once the blood had clotted, approximately 800 μL of serum was separated from the initial sample using centrifugation at 4000 rpm. The remainder of the serum was transferred to an evacuated silicone-coated serum tube (Vacutainer; Becton-Dickinson) and immediately frozen at -20°C. Analysis of this serum for the ion concentrations mentioned

above, was performed by Dr. C. Harris and his staff at St. Paul's Hospital (Vancouver, B.C.) using the SMAC (sequential multiple analyzer computer) system. This analysis was completed within two days of the original sampling.

For the lactate analysis, the remaining 0.3 ml of blood was centrifuged (4000 rpm) immediately following sampling and 100 μL of the separated plasma placed in a test-tube with 200 μL chilled 8% (volumetric) perchloric acid ($HClO_4$). Following 30 seconds of vigorous agitation, the mixture was left to stand on ice for five minutes before being re-centrifuged at 1500 rpm. The resultant clear supernatant was then separated from the coagulated proteins (ppt.), sealed in a 400 μL micro-centrifuge tube and stored at 0 to -5°C. for later analysis.

The lactate concentration of the stored supernate was analyzed using a commercially prepared assay kit (Lactic Acid Determination Kit #826-UV, SIGMA Chemicals) and a spectrophotometer (Model SP6-550 UV/VIS, Philips). This technique utilized the prepared enzyme, lactate dehydrogenase (LDH), to catalyze the following reaction:

$$Lactate \quad + \quad NAD \xrightarrow{\text{LDH}} Pyruvate \quad + \quad NADH \qquad (1)$$
$$[Low\ A_{340}] \qquad\qquad\qquad [High\ A_{340}]$$
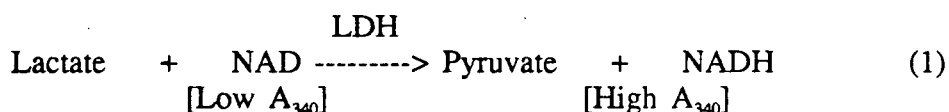
In the presence of LDH and excess nicotinamide adenine dinucleotide (NAD), virtually all of the lactate was converted to pyruvate. Reversal of the reaction was prevented by trapping formed pyruvate with hydrazine. More importantly, the increased absorbency of the solution (at 340 nm), due to the reduction of NAD to NADH, was a measure of the amount of lactate originally present in the supernatant.

In all experimental series, a small amount of blood was drawn at the start and finish of each experiment to determine the hematocrit (Hct) of the animal. This

measurement was particularly important in Series A, where a fairly substantial amount of blood was sampled, and in Series D, where blood was lost during the open-chest surgery. To minimize the change in Hct, as much of the sampled blood as possible was immediately returned to the animal following its analysis. If an animal's HCt was less than 75% of normal, that animal was then allowed to rest for several days, thus allowing the Hct to return to normal levels.

MAP, airflow, tidal volume (V_T), f_b and inspired CO_2 levels (F_ICO_2) were continuously recorded on a Gould multi-channel pen recorder (Series 2400/2600, Gould Inc.).

C) PROTOCOL

The overall experimental protocol used throughout this study varied little between each series of experiments. In each case, the animals were allowed to recover from their respective surgeries before undergoing any experimentation. On the day of an experiment, each animal's wings and feet were lightly restrained with fiber tape to prevent excessive movement and the animal was positioned in the body plethysmograph. The arterial cannula was fed through an opening in the plethysmograph, cleared of any blood clots and the heparin concentration reduced to 10 IU/ml to prevent excessive injection of heparin into the animal. Once the animal had been placed in the plethysmograph, it was allowed 45-60 minutes to adjust to its surroundings. The methods of measuring ventilation, arterial blood gases and pH were identical in all experiments and were always made against a constant hyperoxic (50% O_2) background. The inspiration of this gas mixture represented the control condition for all experiments. The underlying theme of each experimental series was to

examine the kinetics of both ventilatory and acid-base changes when ducks were exposed

to an imposed respiratory acidosis for a prolonged period of time. After each experiment,

the animal used was euthanized with an overdose of sodium pentobarbitol. Four separate

series of experiments were completed and are as follows:

a) Series A: Intact Adult Males   In this first series of experiments, 18 adult (> 18 months

of age) male birds were exposed to an elevated level of inspired carbon dioxide (0.05

F_ICO_2) for a prolonged (300 minute) period of time. The only surgery these birds had

undergone was the implantation of an arterial cannula. Therefore, all respiratory

chemoreceptors were considered fully intact. After the initial 45-60 minutes, ventilatory,

arterial blood gas (PCO_2 and PO_2) and arterial pH measurements were taken from each bird

while it breathed a normocapnic-hyperoxic gas mixture (control conditions). The level of

inspired CO_2 was then quickly (< 30 seconds) elevated, in a single-step, from 0 to 5%.

Additional measurements of ventilation, blood gases and pH were then taken at 20, 60, 180

and 300 minutes (arterial blood gases and pH were measured immediately following all

respiratory measurements in all experimental series). This constituted the standard

experimental protocol used throughout this thesis. In a sub population of 8 animals,

additional blood was sampled for the analysis of strong ion composition of the plasma at 0,

60 and 300 minutes.

As an experimental control, two adult male birds were maintained on 0% CO_2 for

the entire 300 minutes and sampled at the times listed above. Because all four experimental

series used identical apparatus and blood-sampling procedures these two birds acted as an

overall control for all series.

b) Series B: Intact Adult Females   The protocol used in this series was identical to the

standard protocol described above except that the birds used in these experiments were adult (> 18 months) females (n=6).

c) Series C: Carotid Body Denervation   In this series of experiments, adult male birds (n=6), with implanted arterial and venous cannulae, were submitted to the standard protocol described in Series A. Each of the six birds then underwent surgery to bilaterally denervate carotid body chemoreceptors. The presence or absence of carotid body chemoreceptors was tested both before and after denervation surgery using sodium cyanide injection (see Surgery). If the denervation of carotid body chemoreceptors was confirmed by the cyanide injection, then a four to five day recovery period was allowed before the animal was resubmitted to the standard experimental protocol listed above.

d) Series D: Pulmonary Afferent Denervation   The ducks used in this series of experiments were juvenile (6-9 weeks) male ducks. Prior to the denervation surgery, these animals were exposed to 5% inspired CO_2 as outlined by the protocol described above. This allowed the responses of the same animals to be compared with intact and denervated pulmonary receptors (n=5). It also allowed the responses of intact juvenile (n=12) and intact adult birds to be compared.

D) CALCULATIONS AND STATISTICS

Minute ventilation ($\dot{V}_E$) was calculated as the product of both tidal volume (V_T) and breathing frequency. The bicarbonate ion concentration of the arterial blood was calculated by two methods. First, it was calculated from the Henderson-Hasselbach equation, shown below, assuming the constants of 6.10 for pK^1 (Heisler, 1984) and 0.0282 (mmol.L^{-1}.mm Hg^{-1}) for CO_2 solubility (αCO_2) in plasma (Helbacka et al., 1964).

$$pH = pK' + \log\frac{[HCO_3^-]}{\alpha PaCO_2} \qquad (2)$$

Secondly, in Series A experiments only, $[HCO_3^-]$ was also calculated from total CO_2 content (C_{CO_2}) using the formula:

$$[HCO_3^-] = C_{CO_2} - \alpha.PaCO_2 \qquad (3)$$

The strong ion difference (SID) was calculated as the difference between strongly dissociable cations ($[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$) and strongly dissociable anions ($[Cl^-]$, $[lactate^-]$). The calcium ion concentration was calculated from the total serum calcium content assuming that ionic calcium comprised 47.5 percent of total plasma calcium (Bianchi, 1968).

Unless otherwise indicated, all values reported are mean values $\pm$ standard errors (S.E.). Statistical analysis of the data was carried out using either a Student's t-test or an analysis of variance (ANOVA), combined with Tukey's multiple comparison test (Zar, 1984), at a significance level of 0.05. Regression analysis was used to determine if slopes of lines were different from zero while an analysis of covariance (ANCOVA) was used to determine if two or more slopes were significantly different from each other.

RESULTS

A. Responses of Adult Male Ducks to Prolonged Hypercapnia

All ducks in Series A experiments increased minute ventilation ($\dot{V}_E$) within the first 20 minutes of exposure to 5% CO_2 (Fig. 2a). This approximately 3-fold increase in $\dot{V}_E$ was the result of increases in both tidal volume (V_T; 2 fold) and breathing frequency (f_b; 1.5 fold) (Fig. 2b,c). Changes in arterial CO_2 tension ($PaCO_2$) and arterial pH (pHa) were also recorded within the first 20 minutes with $PaCO_2$ increasing approximately 5 mm Hg (from 34.49 to 39.51 mm Hg) and pHa decreasing approximately 0.05 units (from 7.46 to 7.41 pH units) (Fig. 3a,b).

Despite the maintained level of inspired CO_2, most respiratory and blood variables did not remain constant between 20 and 300 minutes. Minute ventilation demonstrated a progressive and significant ($p < 0.05$) decline back towards control levels after the first 20 minutes (Fig. 2a). This decrease, of approximately 50%, resulted entirely from a significant ($p < 0.05$) decrease in f_b (Fig. 2c) as V_T remained elevated and unchanged after the first 20 minutes (Fig. 2b). In the two male birds not exposed to inspired CO_2, $\dot{V}_E$, V_T, and f_b never varied from the control levels recorded at the start of the experimental run (Fig. 2a,b,c).

Although $\dot{V}_E$ and f_b, both returned towards control levels, the level of $PaCO_2$ continued to rise after the initial 20 minutes. In fact, the level of $PaCO_2$ reached after 300 minutes was significantly ($p < 0.05$) greater then that recorded after 20 minutes (Fig. 3a). This approximately 3 mm Hg increase in $PaCO_2$, recorded between 20 and 300 minutes,

Figure 2. The changes observed in (A) $\dot{V}_E$, (B) V_T and (C) f_b in adult male ducks

during 300 minutes following a step increase in inspired CO_2 from 0 to

5%. Data are expressed as a percentage of control (0% CO_2) values (dashed

line). Open symbols represent animals breathing 0% CO_2 for an equivalent
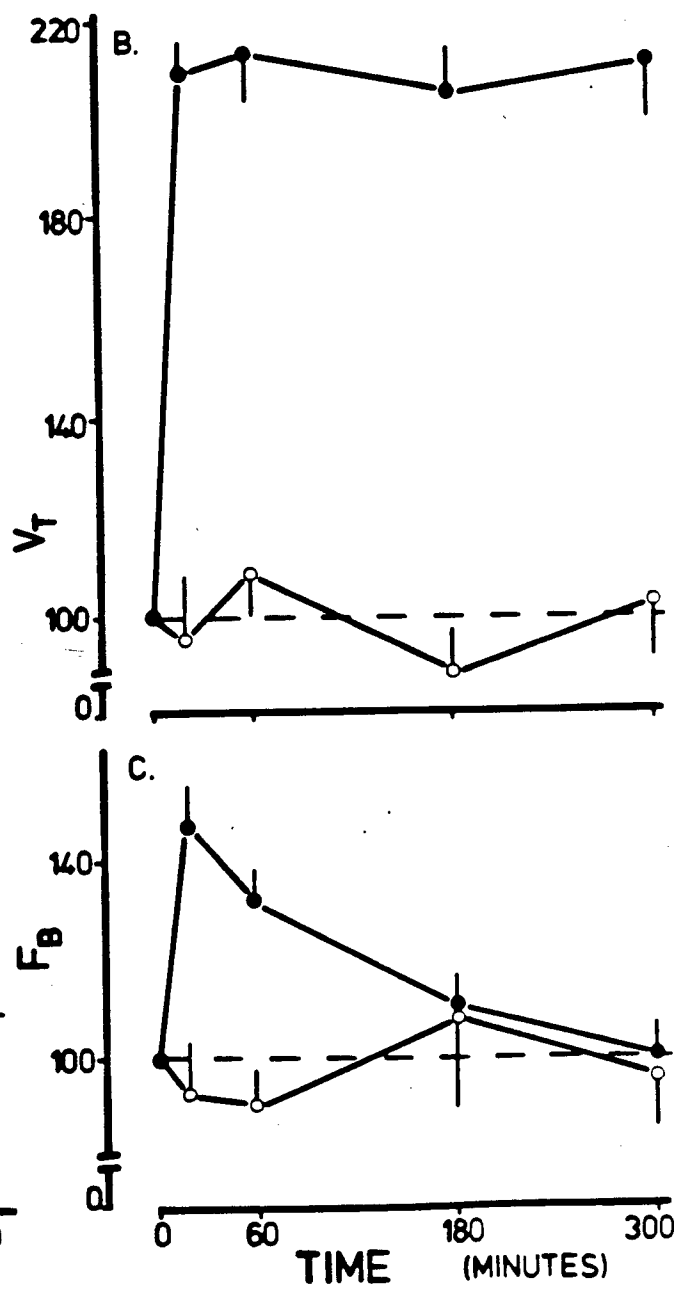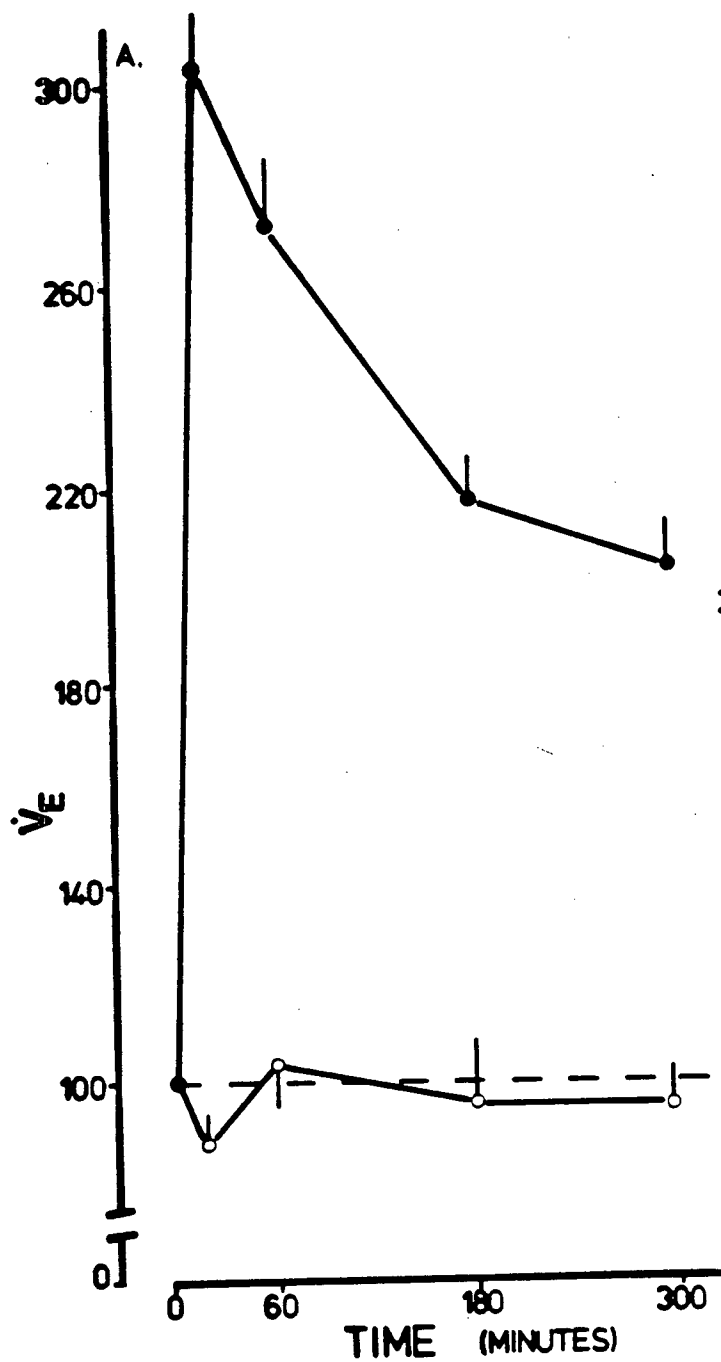
300 minute period.

Figure 3. The changes observed in (**A**) $PaCO_2$, (**B**) pHa and (**C**) arterial $[HCO_3^-]$ in adult male ducks during 300 minutes following a step increase in inspired CO_2 from 0 to 5%. Open symbols represent animals breathing 0% CO_2 for an equivalent 300 minute period.

A. PaCO$_2$ (mm Hg)

B. pHa (Units)

C. [HCO$_3^-$] (mM/L)

TIME (MINUTES)
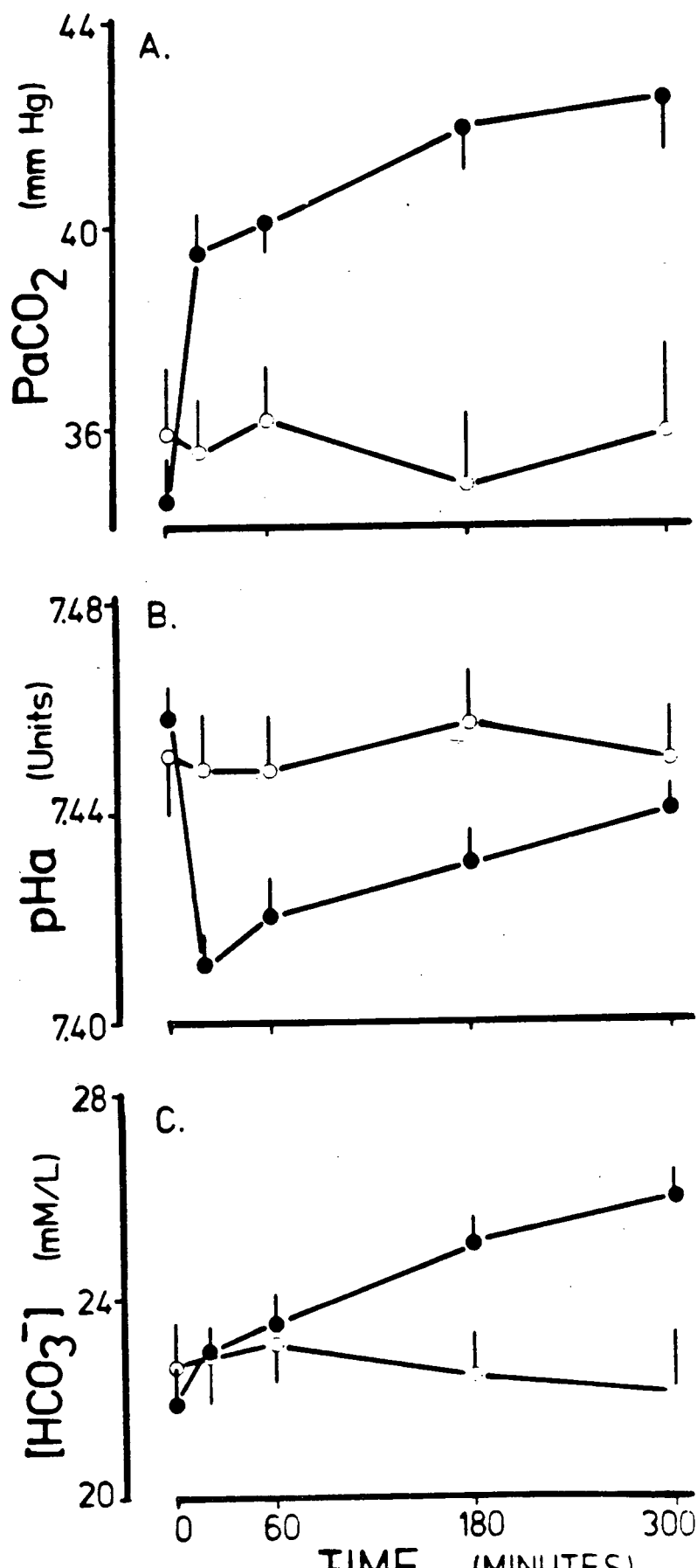
was accompanied by a significant, but paradoxical, increase in pHa from 7.41 to 7.44. Such an increase represented an approximately 50% recovery of arterial pH towards its control level (Fig. 3b). For such a change in pHa to have occurred, given the change in $PaCO_2$, the arterial bicarbonate ion concentration ($[HCO_3^-]$) is calculated to have increased approximately 4 mmol/L (Fig. 3c). However, despite these changes in $PaCO_2$, pHa and $[HCO_3^-]$, no significant changes were detected in the plasma concentrations of any of the major strong cations (Na^+, K^+, Ca^{++}) or anions (Cl^-, lactate$^-$) following either 60 or 300 minutes of 5% CO_2 administration (Table 1). Therefore, no significant change was noted in the strong ion difference (SID) between measurements calculated at 0, 60 and 300 minutes of 5% CO_2 inhalation (Table 1).

B. Responses of Juvenile Male Ducks to Prolonged Hypercapnia

Intact juvenile male birds (Series D) were also exposed to 5% inspired CO_2 for a 300 minute period. These birds exhibited very similar ventilatory responses to those previously described for adult male birds. Again, $\dot{V}_E$ initially showed a marked increase during the first 20 minutes, predominantly due to an increase in V_T (Fig. 4b), which was followed by a progressive and significant ($p < 0.05$) decrease between 20 and 300 minutes (Fig. 4a). The changes in f_b and V_T, observed between 20 and 300 minutes, were similar in both juvenile and adult male ducks, thus the fall in $\dot{V}_E$ was again the singular function of a near complete return of breathing frequency to control levels.

Although the relative changes described for $\dot{V}_E$, V_T and f_b were similar between juvenile and adult male ducks, the absolute levels of $\dot{V}_E$ were considerably lower in the younger birds (Table 2) (all respiratory variables are normalized for body weight). This was

TABLE 1

Mean concentrations ($\pm$ SE) of the major strong cations (mmol/l) and anions (mmol/l) found in the plasma of adult male birds (n=8) at rest (0 minutes; 0% CO_2) and during 300 minutes of breathing 5% CO_2.

	TIME (minutes)					
	0 (mean $\pm$ SE) (mmol/l)		60 (mean $\pm$ SE) (mmol/l)		300 (mean $\pm$ SE) (mmol/l)	
Cations:						
Na^+	145.50	1.68	142.75	1.16	142.00	1.41
Ca^{++}	3.83	0.41	3.71	0.46	3.64	0.45
K^+	2.56	0.11	2.64	0.10	2.79	0.13
A. Total Cations:	151.90	1.66	149.10	1.24	148.81	1.52
Anions:						
Cl^-	110.13	1.22	107.88	0.91	107.38	1.50
lactate$^-$	1.61	0.17	1.55	0.11	1.40	0.08
B. Total Anions:	111.74	1.31	109.42	0.93	108.78	1.44
C. S.I.D.: (A - B)	40.16	1.08	39.67	0.94	40.02	0.82

(■) Significantly different (P<0.05) from value at 0 minutes

TABLE 2

Mean values ($\pm$ SE) of ventilatory and acid-base variables for adult male ducks, juvenile male ducks and adult female ducks at rest (0 minutes; 0% $\cdot$ CO$_2$) and during 300 minutes of breathing 5% CO$_2$.

	Time (min)	Adult Male (n=18) mean $\pm$ SE		Juvenile Male (n=12) mean $\pm$ SE		Adult Female (n=6) mean $\pm$ SE	
$\dot{V}_E$	0	314.92	20.81	229.12 ▲	14.39	256.12	25.64
(ml/min/kg)	20	936.23	56.48	667.62 ▲	47.21	611.61 ▲	65.44
	60	847.62	44.47	692.13	61.91	594.71 ▲	58.33
	180	670.92 □	43.59	576.45 □	49.07	576.33	56.88
	300	635.33 □	40.08	530.39 □	42.67	547.06	51.57
V_T	0	29.14	2.61	19.68 ▲	1.43	21.48	1.62
(ml/kg)	20	59.19	3.83	41.29 ▲	3.03	38.47 ▲	2.50
	60	60.34	4.00	42.40 ▲	2.95	37.51 ▲	1.98
	180	55.08	5.27	42.27 ▲	3.04	37.20 ▲	2.12
	300	61.07	4.28	40.97 ▲	2.65	35.13 ▲	3.03
f_b	0	11.22	1.02	12.33	1.29	12.33	1.22
(min^{-1})	20	16.06	1.09	16.46	1.01	15.92	1.39
	60	14.31	0.97	16.29	0.93	15.92	1.61
	180	11.84 □	1.01	13.71 □	0.69	16.34	1.76
	300	10.53 □	0.42	13.00 □	0.69	16.08 ▲	2.00
pHa	0	7.46	0.005	7.46	0.009	7.45	0.015
(units)	20	7.41	0.006	7.42	0.010	7.40	0.220
	60	7.42	0.007	7.42	0.010	7.41	0.019
	180	7.43	0.007	7.43 □	0.009	7.41	0.015
	300	7.44 □	0.005	7.44 □	0.009	7.41	0.015
PaCO$_2$	0	34.49	0.78	32.60	1.38	36.63	0.64
(mm Hg)	20	39.51	0.71	37.90	0.85	42.30	1.00
	60	40.12	0.65	38.46	0.68	42.05	1.24
	180	41.96	0.87	39.88	1.00	43.05	1.12
	300	42.47 □	0.81	41.54 □	0.74	43.78	0.94
[HCO$_3^-$]	0	22.09	0.52	20.91	0.76	23.27	0.79
(mmol/L)	20	22.89	0.51	22.27	0.40	23.87	1.01
	60	23.62	0.41	22.59	0.53	24.21	0.68
	180	25.14 □	0.49	24.29	0.47	24.68	0.76
	300	25.99 □	0.50	25.96 □	0.43	25.44	0.84

(▲) Significantly different (P<0.05) from intact adult male ducks
(□) Significantly different (P<0.05) from value at 20 minutes

Figure 4. The changes observed in (A) $\dot{V}_E$, (B) V_T and (C) f_b in adult male ducks (●), juvenile male ducks (▲) and adult female ducks (■) during 300 minutes following a step increase in inspired CO_2 from 0 to 5%. Data are expressed as a percentage of control (0% CO_2) values.
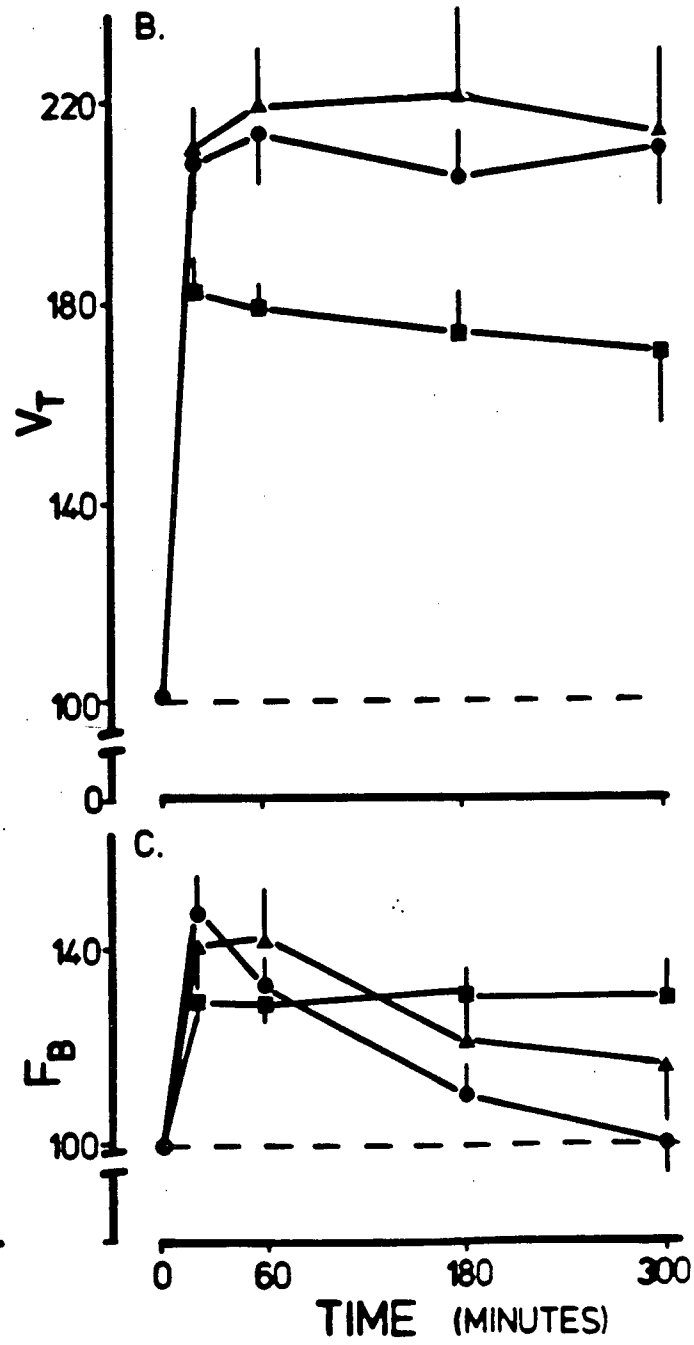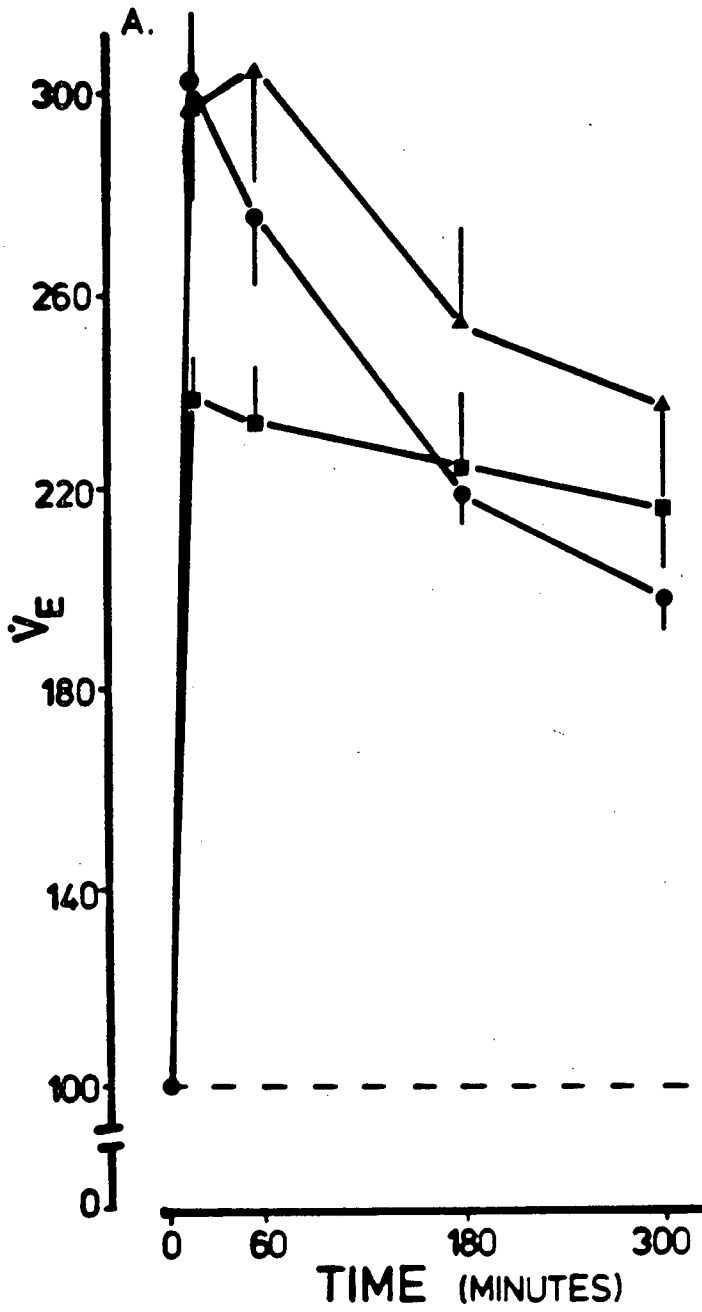
the result of significantly lower levels of V_T recorded in the younger birds. Breathing frequencies, however, were similar between both age groups (Table 2).

The changes measured in $PaCO_2$, pHa and $[HCO_3^-]$, during exposure to prolonged hypercapnia, were also similar in both the juvenile and adult male birds. Again arterial P_{CO2} showed a significant (p< 0.05) 5 mm Hg increase during the first 20 minutes (Fig. 5a) followed by a further significant (p< 0.05) increase over the next 280 minutes (Fig. 5a) while arterial pH demonstrated a significant (p< 0.05) return towards control levels following its initial decrease of approximately 0.06 units (Fig. 5b). As was the case with adult male ducks, it was calculated that juvenile male ducks also demonstrated a significant (p< 0.05) increase in arterial $[HCO_3^-]$. (Fig. 5c).

C. Responses of Female Birds to Prolonged Hypercapnia

Like their male counterparts, female ducks exposed to 5% inspired CO_2 also demonstrated an immediate increase in $\dot{V}_E$ (Fig. 4a), the result of both increased V_T (Fig. 4b) and f_b (Fig. 4c). Although significantly (p< 0.05) greater than their respective control levels, the levels of $\dot{V}_E$ recorded in female ducks, after 20 minutes, were significantly (p< 0.05) less than those recorded in either juvenile or adult male ducks (Fig. 4a). This blunted V_E response, shown by the females, resulted from a smaller increase in V_T than was shown by male ducks; changes in f_b were not significantly different between the two sexes (Fig. 4b,c).

Unlike male birds, however, female birds failed to show any change in $\dot{V}_E$ after the first 20 minutes (Fig. 4a), and instead maintained this level of $\dot{V}_E$ over the entire 300 minutes. This resulted from the fact that female birds maintained the initial increases of V_T

Figure 5. The changes observed in (A) $PaCO_2$, (B) pHa and (C) arterial $[HCO_3^-]$ in adult male ducks (●), juvenile male ducks (▲) and adult female ducks (■) during 300 minutes following a step increase in inspired CO_2 from 0 to 5%.
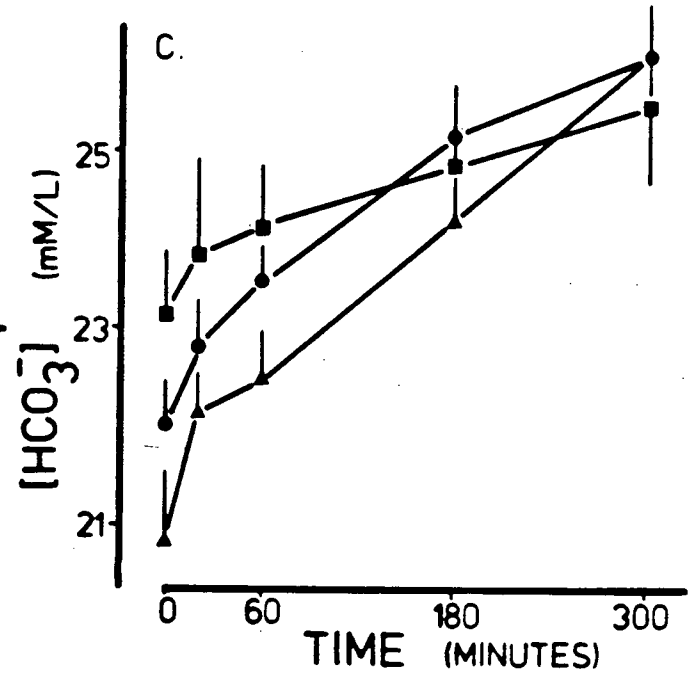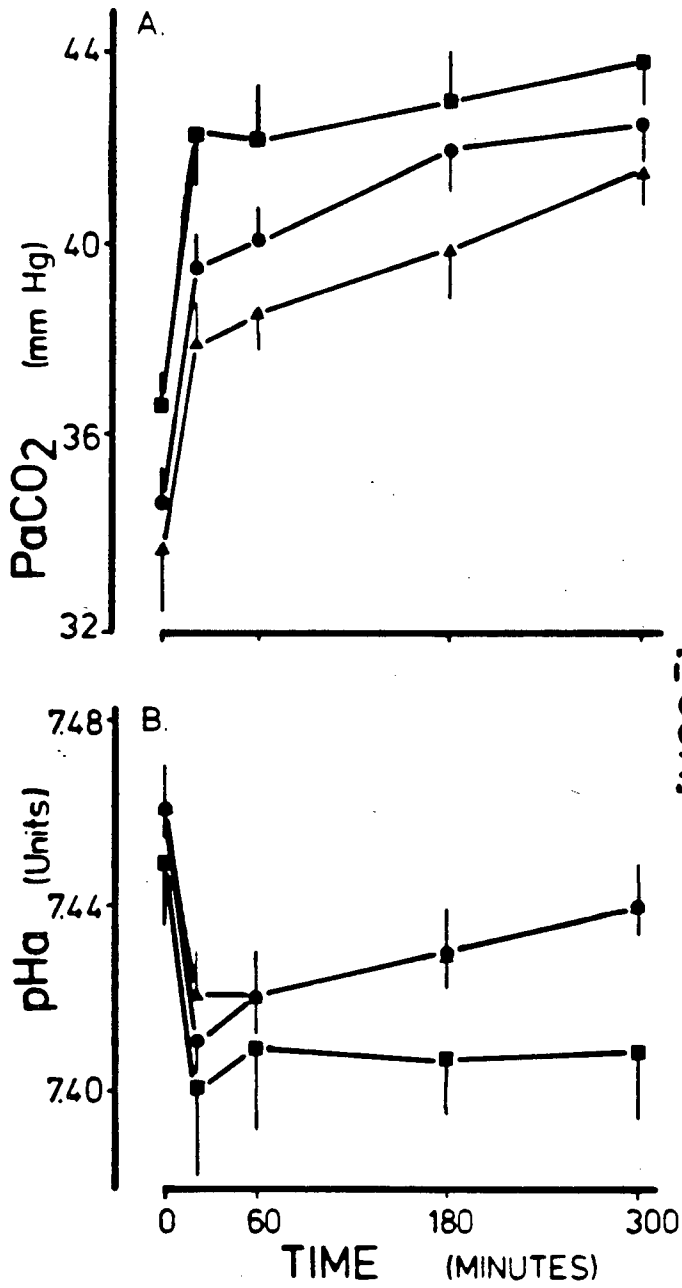
throughout the period of hypercapnic exposure (Figure 4b). While female birds had slightly higher resting levels of $PaCO_2$ than did male birds, they still showed similar increases in $PaCO_2$ when given CO_2 to breathe (Fig. 5a). However, while female ducks showed similar changes in pHa over the first 20 minutes as did male ducks, they failed to show the recovery in pHa observed in all male ducks between 20 and 300 minutes (Fig. 5b). The female ducks had higher resting levels of arterial $[HCO_3^-]$ than did male ducks and showed a much smaller increase in bicarbonate concentration during the 300 minute exposure (Fig. 5c). Thus, the observed absence of a pHa recovery in female ducks was accompanied by a significantly ($p < 0.05$) smaller increase in $[HCO_3^-]$ (Fig. 5b,c).

D. Comparison of the Responses of Adult and Juvenile Male and Adult Female Birds to Prolonged Hypercapnia.

Figure 6 expresses the changes previously described for $PaCO_2$, pHa and $[HCO_3^-]$ in all three groups, in the form of a $[HCO_3^-]$/pH diagram. The slope ($\beta = -\Delta[HCO_3^-]/\Delta pHa$) of the line joining the 0 and 20 minute points on the diagram represents the in vivo buffer line of the extracellular fluid, also referred to as the apparent plasma buffer value (Glass & Heisler,1986). Regardless of the fact that the absolute levels of the three variables may differ between the three groups of birds, they all exhibited similar slopes and therefore, similar apparent plasma buffer values. The additional increases observed in $[HCO_3^-]$ after the first 20 minutes, presumably due to ionic exchange processes between the plasma compartment and other body-fluid compartments and/or renal base retention, were relatively

Figure 6. pH/[HCO$_3$] diagram showing the effects of the sustained increase in inspired CO_2 on $PaCO_2$, pHa and HCO_3 concentration in adult male ducks (●), juvenile male ducks (▲) and adult female ducks (■). Time (minutes) of each measurement is listed in brackets.
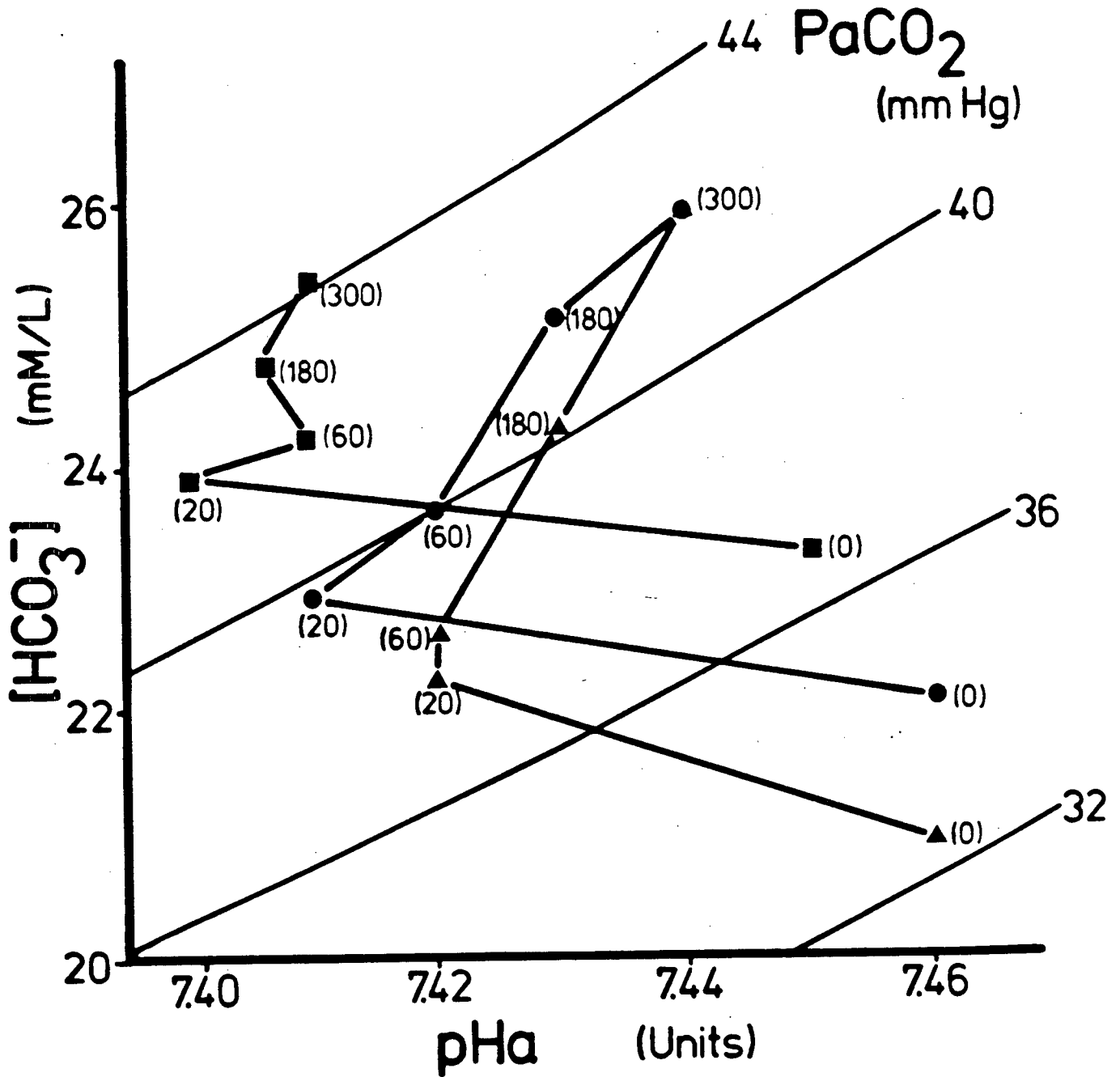
large in juvenile and adult male birds (3-4 mmol/L). Despite the fact that $PaCO_2$ levels continued to rise throughout the experiment, male ducks demonstrated a concomitant rise in $[HCO_3^-]$ that was obviously sufficient to not only stop any further decrease in pHa, but also to return pHa towards its control levels. On the other hand, figure 6 also illustrates that while the small increase in $[HCO_3^-]$, shown by female ducks was sufficient to prevent pHa from decreasing further, it was not sufficiently large to elicit any significant increase pHa.

Figure 7 summarizes, for all 3 groups, the changes calculated in $\dot{V}_E$, V_T, f_b, $PaCO_2$, pHa and $[HCO_3^-]$ (expressed as the percentage of the initial change observed between 0 and 20 minutes) between 20 and 300 minutes of breathing 5% CO_2. The negative values in the figure indicate changes towards control values (ie. recovery) while the positive values represent changes away from control values. Both adult and juvenile male ducks showed similar recoveries in pHa and $\dot{V}_E$ (due to reductions in f_b) while female ducks showed very little recovery of any of these variables during the 300 minutes of hypercapnic exposure.

E. Responses of Carotid Body Denervated Adult Male Ducks to Prolonged Hypercapnia.

Carotid-body chemoreceptor denervation (CBX) in adult male ducks resulted in a decrease in the absolute level of $\dot{V}_E$ under normocapnic (control) conditions (Table 3). It also reduced the absolute increase in $\dot{V}_E$ observed upon exposure to 5% inspired CO_2 for either an acute (20 minute) or a prolonged (300 minute) period of time (Fig. 8a, Table 3). This was due to a smaller absolute increase in breathing frequency in CBX birds compared to intact birds (Fig. 8c, Table 3). However, regardless of the absolute differences observed in levels of ventilation between CBX and intact male ducks, both groups exhibited the same relative changes in ventilation when exposed to inspired CO_2 for prolonged periods of

TABLE 3

Mean values ($\pm$ SE) of ventilatory and acid-base variables for adult male ducks and carotid body chemoreceptor denervated ducks (CBX) at rest (0 minutes; 0% CO_2) and during 300 minutes of breathing 5% CO_2.

	Time (min)	Intact Adult (n=18) (mean $\pm$ SE)		CBX Adult (n=6) (mean $\pm$ SE)	
$\dot{V}_E$	0	314.92	20.81	269.31	16.01
(ml/min/kg)	20	936.23	56.48	706.71	99.76
	60	847.62	44.47	637.88 ▲	59.20
	180	670.97 ■	43.59	506.53	56.04
	300	635.33 ■	40.08	445.62 ▲■	48.89
V_T	0	29.14	2.61	28.95	3.41
(ml/kg)	20	59.19	3.83	57.58	6.37
	60	60.34	4.00	58.75	6.60
	180	55.08	5.27	55.70	6.32
	300	61.07	4.28	56.88	5.67
f_b	0	11.22	1.02	10.00	1.34
(min^{-1})	20	16.06	1.09	12.50	1.52
	60	14.31	0.97	11.25	1.08
	180	11.84 ■	1.01	9.33	0.96
	300	10.53 ■	0.42	8.00 ▲	0.83
pHa	0	7.46	0.005	7.45	0.005
(units)	20	7.41	0.006	7.41	0.006
	60	7.42	0.007	7.42	0.010
	180	7.43	0.007	7.43	0.007
	300	7.44 ■	0.005	7.44 ■	0.005
$PaCO_2$	0	34.49	0.78	35.63	1.23
(mm Hg)	20	39.51	0.71	39.67	1.25
	60	40.12	0.65	41.38	1.02
	180	41.96	0.87	43.97	1.49
	300	42.47 ■	0.81	46.15 ▲■	1.22
$[HCO_3^-]$	0	22.09	0.52	22.61	0.91
(mmol/L)	20	22.89	0.51	23.03	0.83
	60	23.62	0.41	24.30	0.71
	180	25.14 ■	0.49	26.39	0.84
	300	25.99 ■	0.50	28.16 ▲■	0.77

(▲) Significantly different (P<0.05) from intact adult male ducks
(■) Significantly different (P<0.05) from value at 20 minutes

Figure 7. Changes in $\dot{V}_E$, V_T, f_b, $PaCO_2$, pHa and arterial $[HCO_3^-]$ that were observed between 20 and 300 minutes, expressed as percentages of the changes initially observed between 0 and 20 minutes. A negative value indicates the return of that variable towards its control level.

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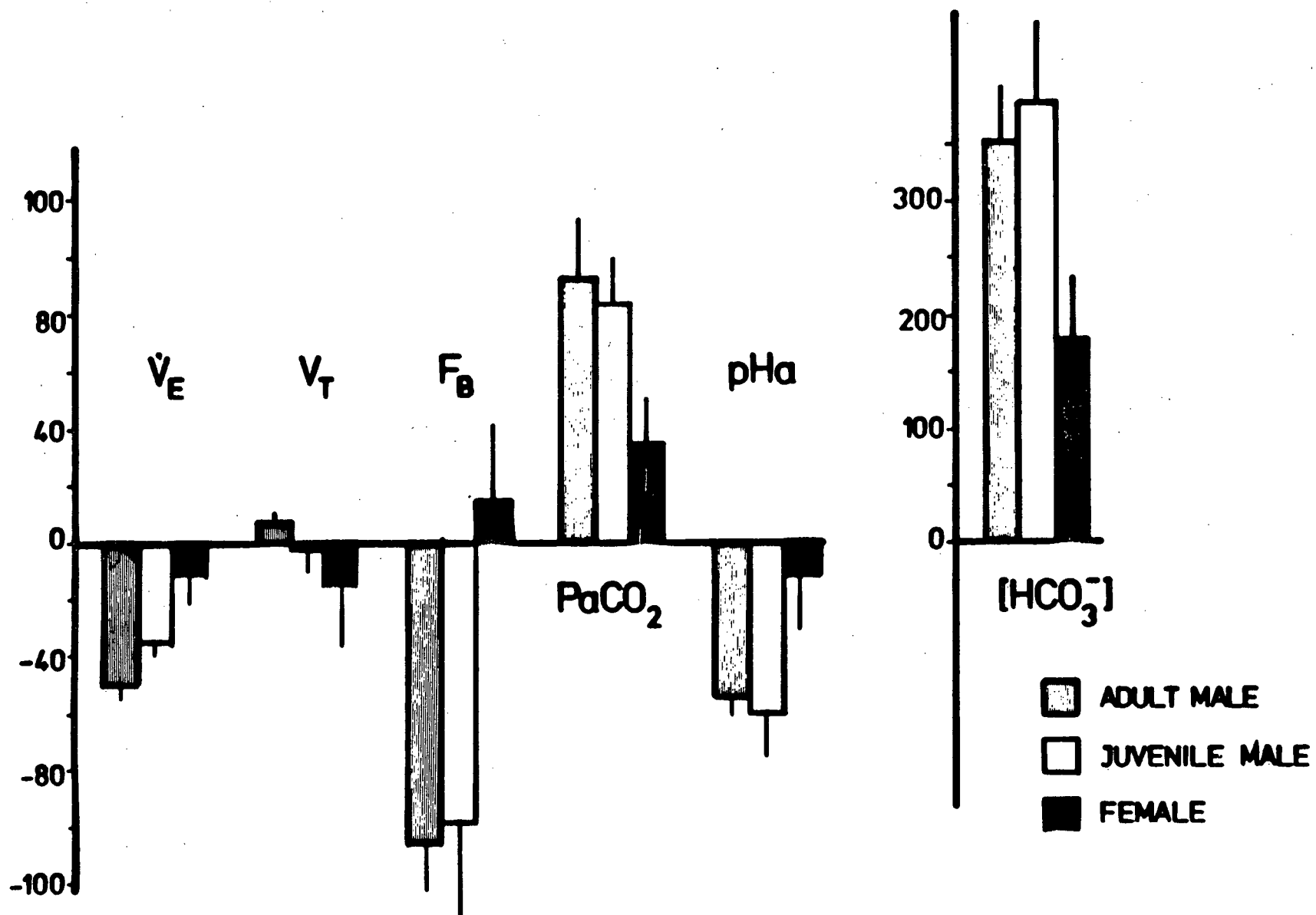
Figure 8. The changes observed in (A) $\dot{V}_E$, (B) V_T and (C) f_b in chemoreceptor intact adult male ducks (●) and following bilateral carotid body denervation (○) during 300 minutes following a step increase in inspired CO_2 from 0 to 5%. Data are expressed as a percentage of control (0% CO_2) values.
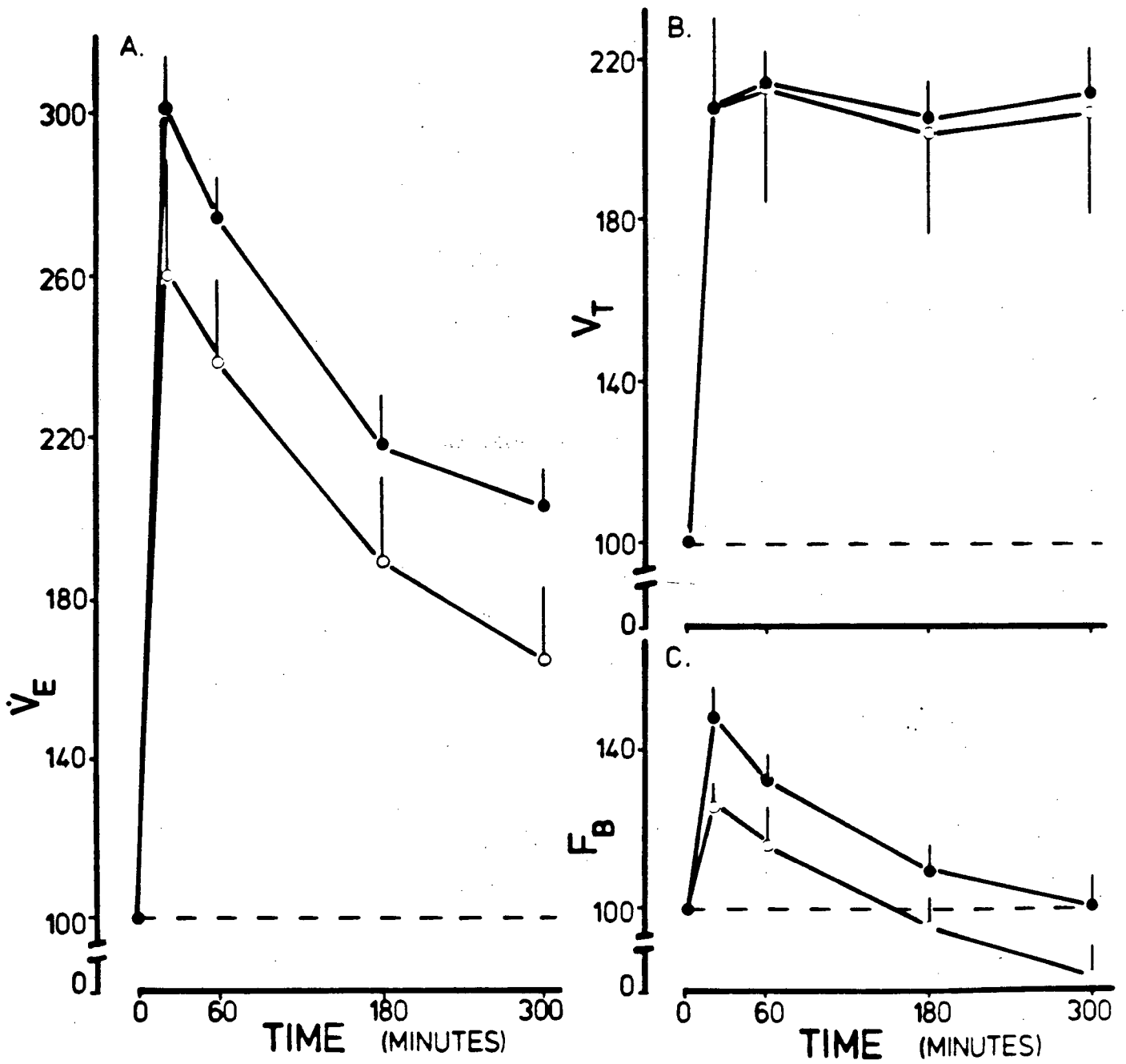
time; the relative changes observed in $\dot{V}_E$, V_T, and f_b were identical in both CBX and intact male ducks over equivalent 300 minute exposures to elevated levels of inspired CO_2 (Fig. 8a,b,c).

The relative changes measured in arterial P_{CO2}, pH and [HCO_3^-] in CBX ducks were also similar to the relative changes measured in their intact counterparts. Arterial P_{CO2} showed an initial, significant ($p < 0.05$) increase (4 mm Hg) after the first 20 minutes followed by a further significant ($p < 0.05$) increase (6.5 mm Hg) over the subsequent 280 minutes (Fig. 9a). Although the rise in $PaCO_2$ was significantly ($p < 0.05$) greater in CBX ducks compared to intact male ducks (Fig. 9a), the changes in pHa, measured in both groups, were identical (Fig. 9b) indicating that CBX birds produced a larger bicarbonate response then did intact adult birds (Fig. 9c).

Once again, a pH/[HCO_3^-] diagram is used to summarize the changes observed in measured blood variables between the two groups of birds (Fig. 10). Both CBX and intact, adult, male ducks demonstrated similar apparent blood buffer values (slopes between 0 and 20 minute points) and similar abilities to compensate for an imposed respiratory acidosis with an increased plasma bicarbonate concentration.

F. Responses of Pulmonary Denervated Juvenile Male Ducks to Prolonged Hypercapnia.

In series D, pulmonary afferent information arising from pulmonary receptors, including intrapulmonary chemoreceptors, was eliminated as a consequence of surgical denervation in juvenile male ducks. These pulmonary denervated birds (PAX), however, maintained a single intact carotid body whose presence was confirmed by a positive ventilatory response to intravenously injected sodium cyanide (NaCN). As these PAX birds

Figure 9. The changes observed in (A) $PaCO_2$, (B) pHa and (C) arterial [HCO_3^-] in chemoreceptor-intact adult male ducks (●) and following bilateral carotid body denervation (○) during 300 minutes following a step increase in inspired CO_2 from 0 to 5%.
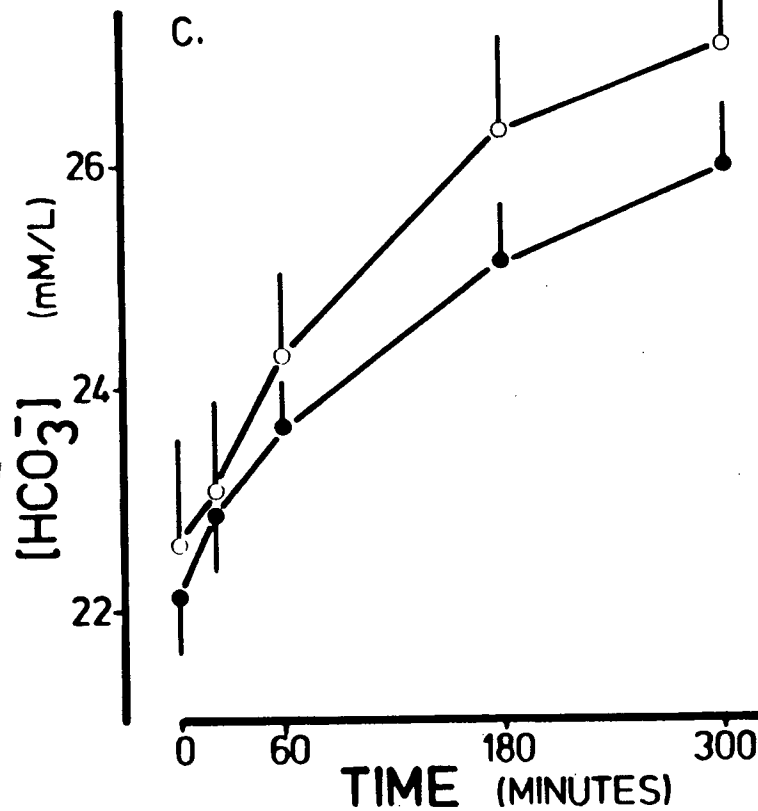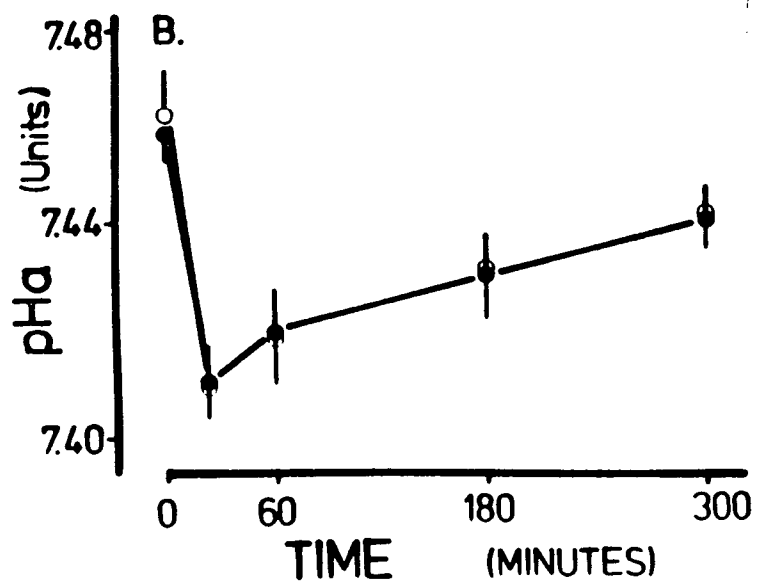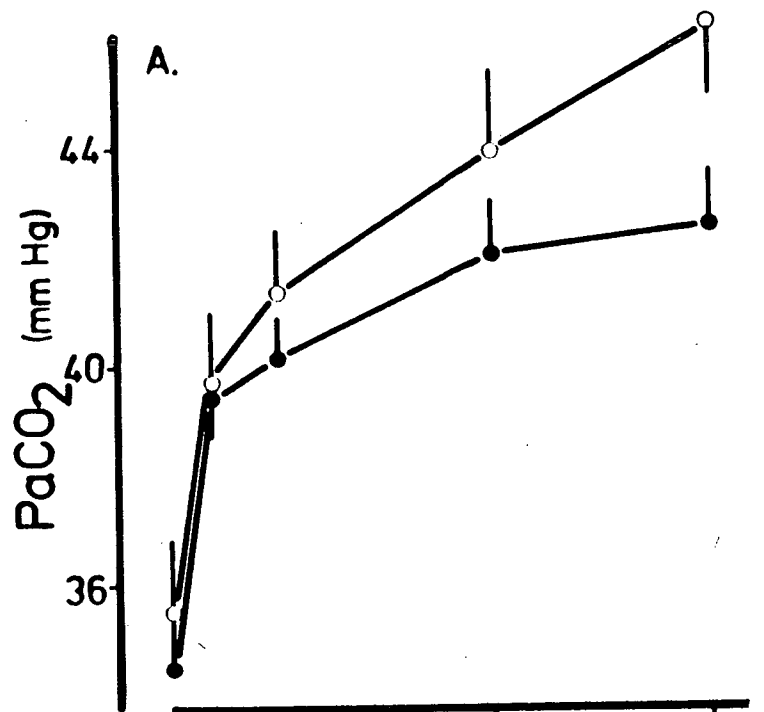
Figure 10. pH/[HCO_3^-] diagram showing the effects of the sustained increase in inspired CO_2 on $PaCO_2$, pHa and HCO_3^- concentration in chemoreceptor intact adult male ducks (●) and following bilateral carotid body denervation (○). Time (minutes) of each measurement is listed in brackets.
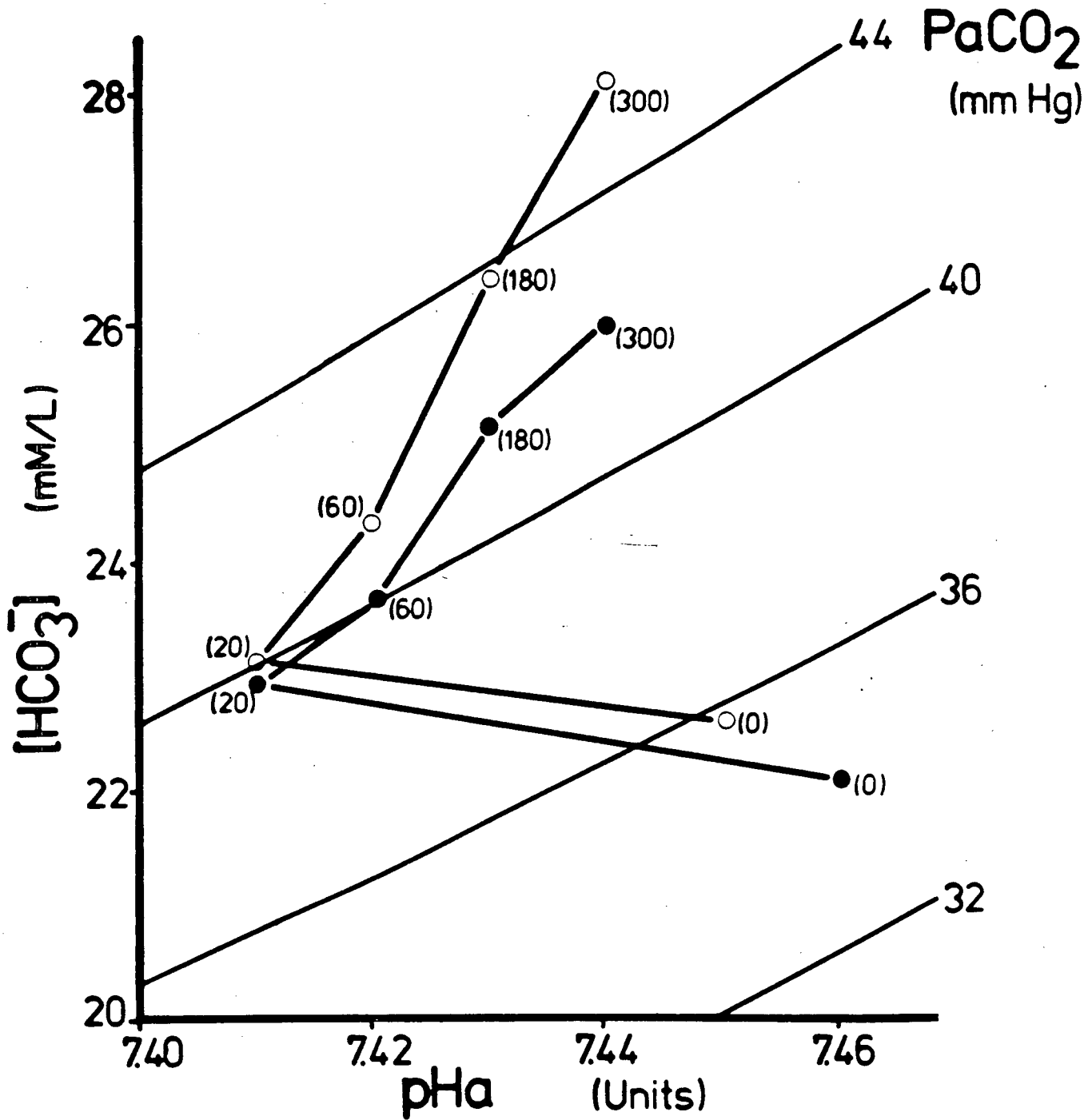
were juvenile birds, the observed ventilatory and acid-base responses were compared to the responses observed in intact juvenile male birds previously described in section 2 of Results.

The bilateral removal of lung receptor information resulted in a significant ($p< 0.05$) reduction of f_b and a significant ($p< 0.05$) increase in V_T under normocapnic conditions (Table 4). These changes did not perfectly offset each other, however, and led to an increase in the average level of $\dot{V}_E$ in PAX birds (Table 4). Relative to intact birds, $\dot{V}_E$ increased significantly less in PAX birds upon inspiration of 5% CO_2 (Fig. 11a). This resulted from comparably smaller increases observed in both V_T and f_b in PAX birds (Fig. 11b,c). Despite these initial differences, PAX male birds exhibited the significant and progressive decline in both $\dot{V}_E$ and f that was commonly observed in all male ducks when exposed to 300 minutes of 0% CO_2 (Fig 11a,b,c). Although arterial pH remained unchanged following pulmonary denervation, $PaCO_2$ and [HCO_3^-] were both somewhat lower under normocapnic (control) conditions than in intact juvenile animals under similar conditions (Fig. 12a,b,c; Table 4). Upon inhalation of 5% CO_2, however, PAX birds demonstrated comparably larger increases in $PaCO_2$ and comparably larger decreases in pHa than were recorded in intact juvenile male ducks (Fig. 12a,b). Continuous exposure of PAX birds to 5% CO_2 resulted in changes to $PaCO_2$, pHa, and [HCO_3^-] that paralleled those previously measured in intact juvenile male birds (Fig. 12a,b,c).

The pH/[HCO_3^-] diagram (Fig. 13) further illustrates that even though absolute values differed, the relative changes observed in $PaCO_2$, pHa and [HCO_3^-], which were measured during prolonged exposure to the hypercapnic gas were similar regardless of whether pulmonary afferent receptors were present or not.

TABLE 4

Mean values ($\pm$ SE) of ventilatory and acid-base variables for juvenile male ducks and pulmonary receptor denervated ducks (PAX) at rest (0 minutes; 0% CO_2) and during 300 minutes of breathing 5% CO_2.

	Time (min)	Intact Juvenile (n=12)		PAX Juvenile (n=5)	
		mean $\pm$ SE		mean $\pm$ SE	
$\dot{V}_E$	0	229.12	14.39	293.00	37.07
(ml/min/kg)	20	667.62	47.21	625.37	87.83
	60	692.13	61.91	654.67	67.27
	180	576.45 ■	49.07	598.49	63.37
	300	530.39 ■	42.67	512.27 ■	75.59
V_T	0	19.68	1.43	35.65 ▲	6.04
(ml/kg)	20	41.29	3.03	64.86 ▲	3.47
	60	42.40	2.95	66.18 ▲	2.49
	180	42.27	3.04	62.16 ▲	2.24
	300	40.97	2.65	63.52 ▲	0.84
f_b	0	12.33	1.29	9.47	2.00
(min^{-1})	20	16.46	1.01	9.70 ▲	1.45
	60	16.29	0.93	9.90 ▲	0.97
	180	13.71 ■	0.69	9.70 ▲	1.11
	300	13.00 ■	0.69	8.10 ▲■	1.25
pHa	0	7.46	0.009	7.46	0.014
(units)	20	7.42	0.010	7.38	0.007
	60	7.42	0.010	7.39	0.006
	180	7.43 ■	0.009	7.41 ■	0.010
	300	7.44 ■	0.009	7.42 ■	0.009
$PaCO_2$	0	32.60	1.38	30.53	1.15
(mm Hg)	20	37.90	0.85	41.24 ▲	2.32
	60	38.46	0.68	41.44	1.03
	180	39.88	1.00	42.78	1.64
	300	41.54	0.74	44.58	1.85
$[HCO_3^-]$	0	20.91	0.76	19.80	0.69
(mmol/L)	20	22.27	0.40	21.90	0.94
	60	22.59	0.53	22.57	0.45
	180	24.29	0.47	24.89	1.17
	300	25.96 ■	0.43	26.43	1.00

(▲) Significantly different (P<0.05) from intact juvenile male ducks
(■) Significantly different (P<0.05) from value at 20 minutes

Figure 11. The changes observed in (A) $\dot{V}_E$, (B) V_T and (C) f_b in chemoreceptor intact juvenile male ducks (■) and following bilateral pulmonary receptor denervation (□) during 300 minutes following a step increase in inspired CO_2 from 0 to 5%. Data are expressed as a percentage of control (0% CO_2) values.
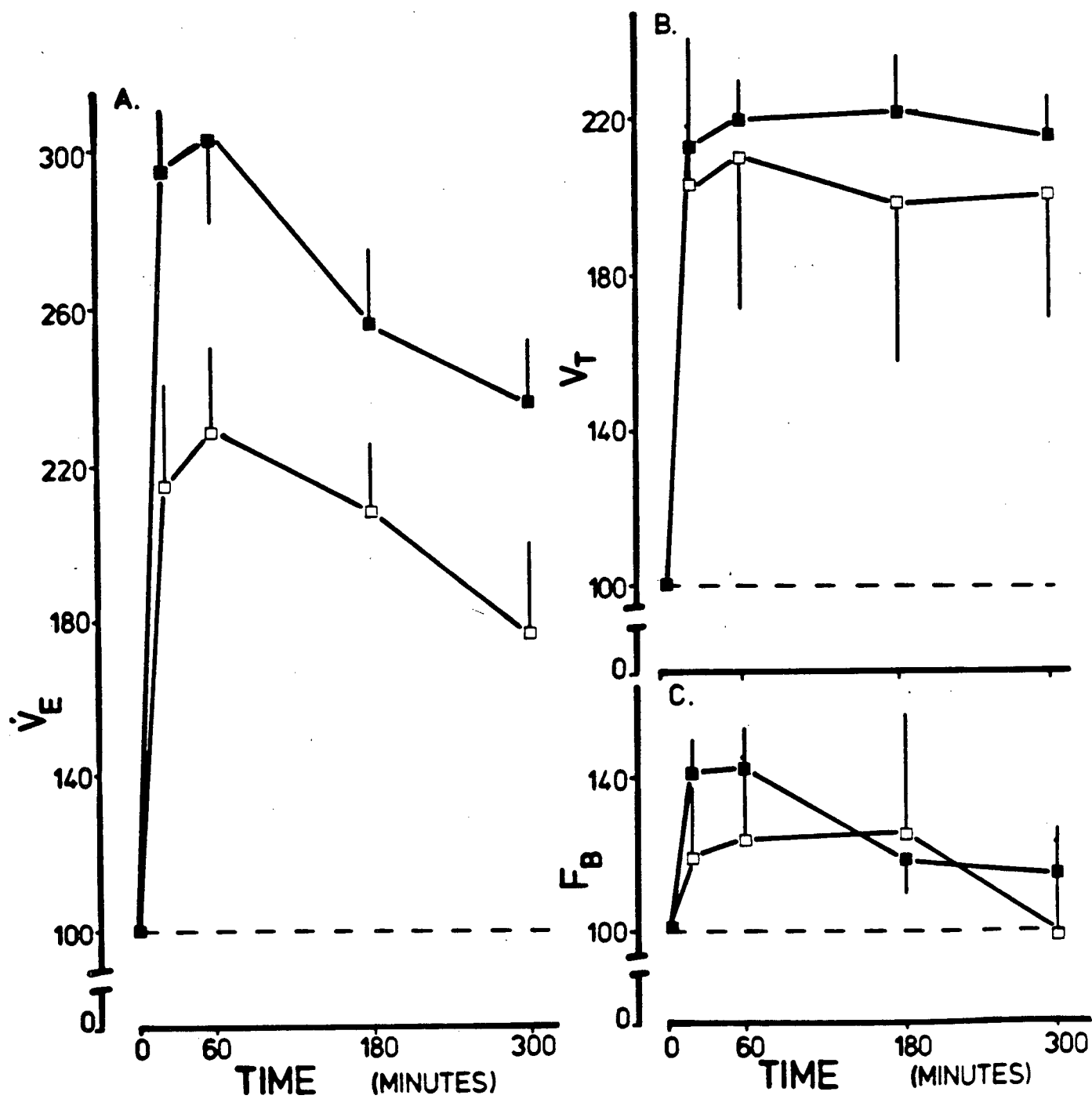
Figure 12. The changes observed in (A) $PaCO_2$, (B) pHa and (C) arterial $[HCO_3^-]$

in chemoreceptor-intact juvenile male ducks (■) and following bilateral

pulmonary receptor denervation (□) during 300 minutes following a step

increase in inspired CO_2 from 0 to 5%.
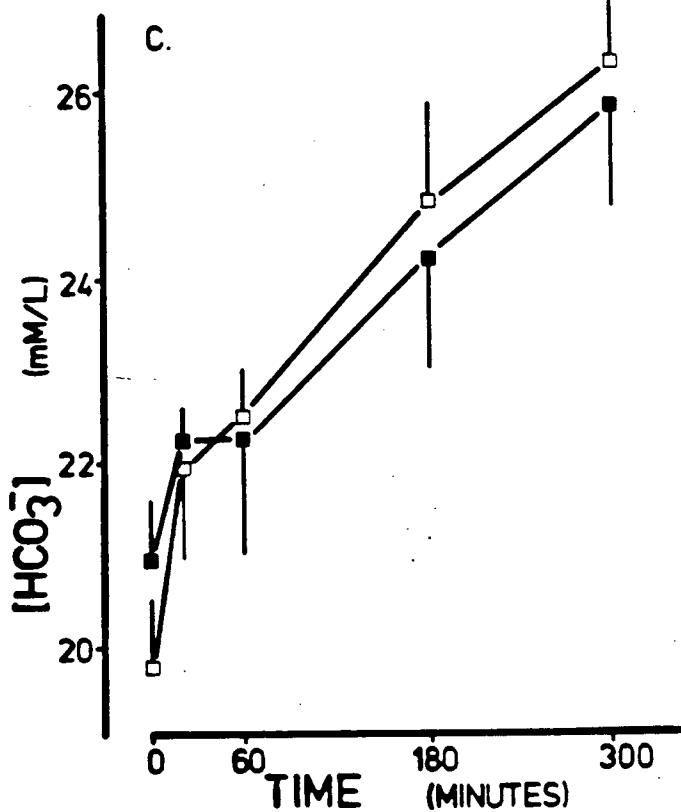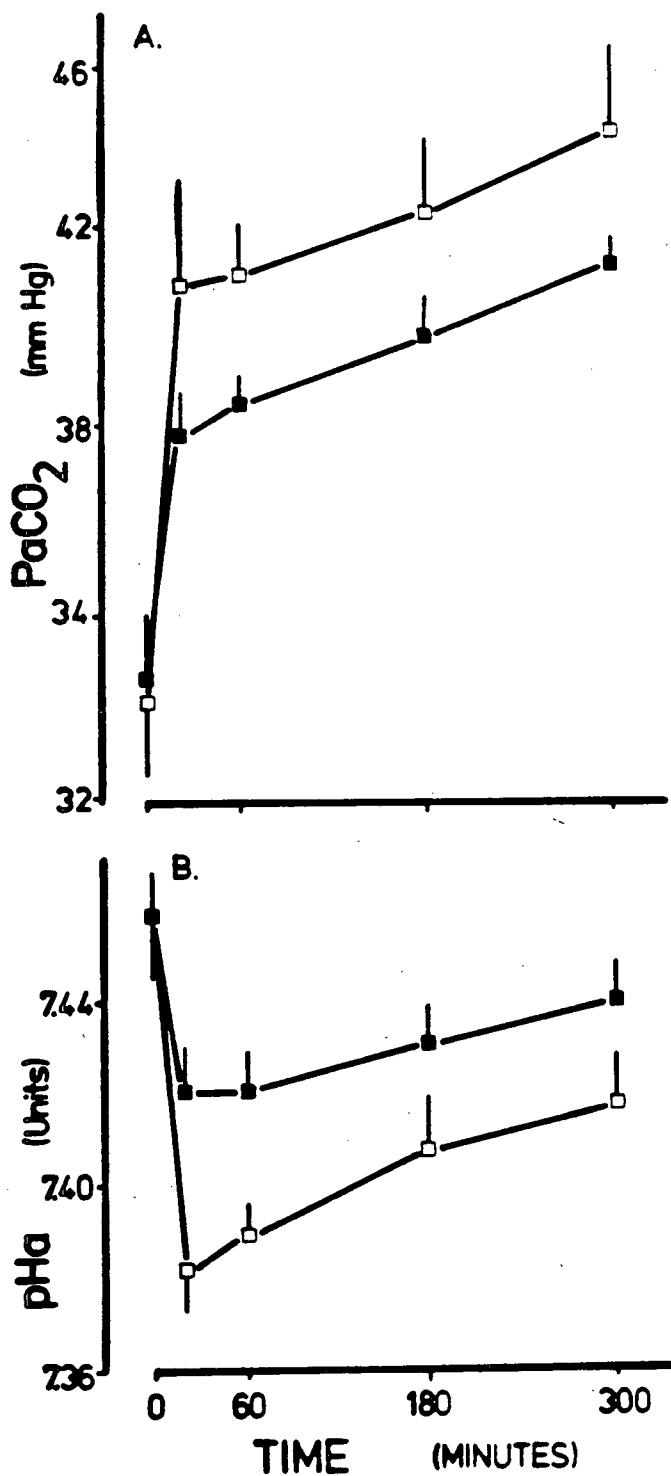
Figure 13. pH/[HCO_3^-] diagram showing the effects of the sustained increase in inspired CO_2 on $PaCO_2$, pHa and HCO_3^- concentration in chemoreceptor intact juvenile male ducks (■) and following bilateral pulmonary receptor denervation (□). Time (minutes) of each measurement is listed in brackets.
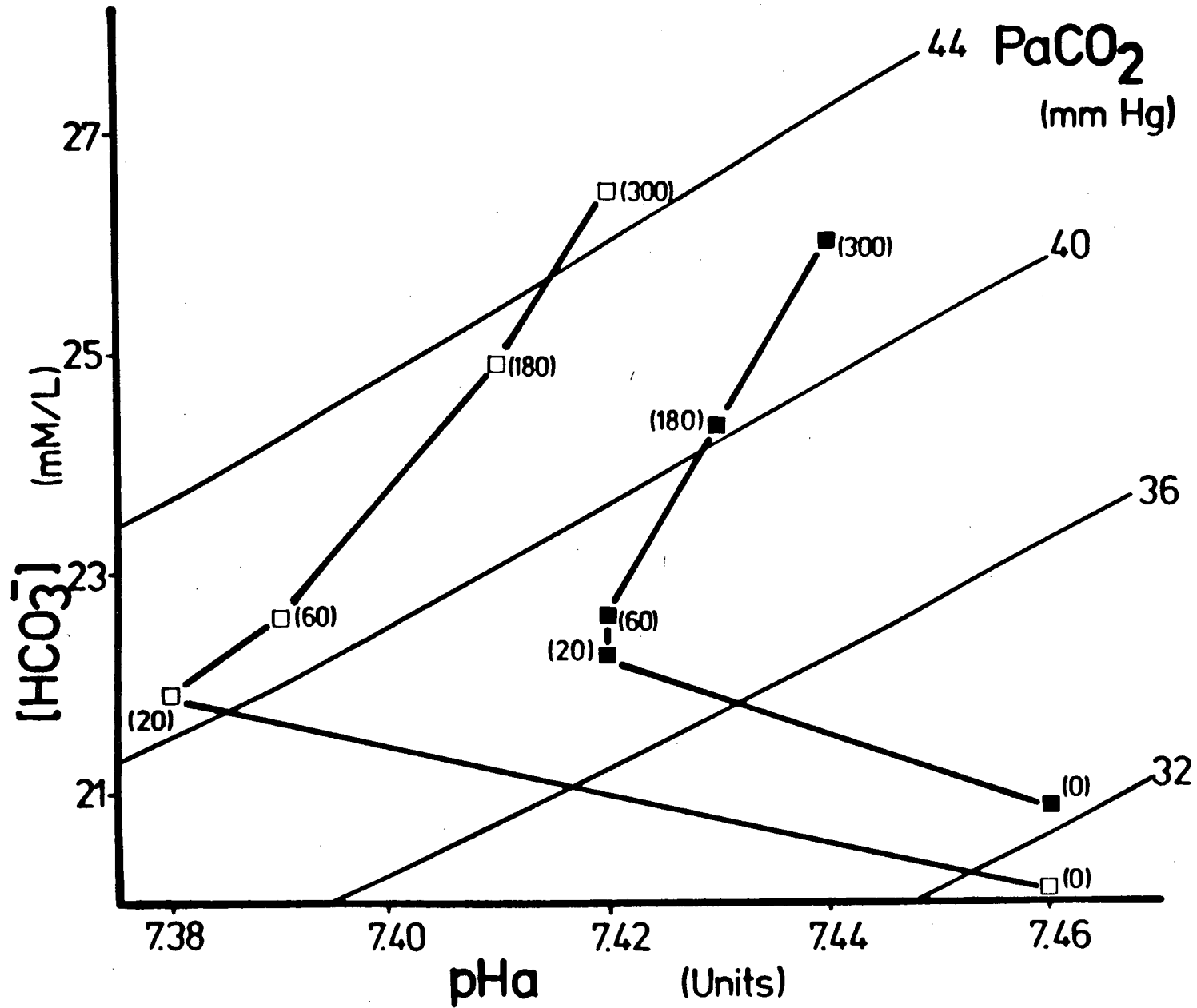
DISCUSSION

The respiratory responses to chronic CO_2 exposure have been well described for several species of mammals (for review see: Forster & Dempsey, 1981; Dempsey & Forster, 1982). Perhaps because of the similarities believed to exist between birds and mammals in regard to the chemical control of breathing (Jukes, 1971; Bouverot, 1978), there was little reason to suspect that these two different classes of vertebrates should differ in their responses to a chronically imposed CO_2 stimulus. The data from this study, however, supports our earlier work (Dodd & Milsom, 1987) and suggests that not only do birds differ from mammals in both the speed and magnitude of their ventilatory and acid-base responses to chronic hypercapnia, but that the responses exhibited by birds are unequalled by any other vertebrate species that has been examined.

I. RESTING CONDITIONS

All values recorded for resting ventilation, blood gases and pH in this study fell within the ranges reported by Powell et al. (1978) for ducks under similar normocapnic-hyperoxic resting conditions.

The birds of this study were given 50% O_2 to breathe because the purpose of the present study was to examine the effects of CO_2, and not O_2, upon respiration. The level of inspired O_2 was increased from 21 to 50% at least one hour prior to the start of the

experimental protocol. Such a step-increase in inspired O_2 resulted in an average 15-20%

decrease in the level of resting minute ventilation ($\dot{V}_E$). Similar examples of O_2-

chemoreflexes in birds have been previously reported in the literature (Jones & Purves,

1970; Bouverot & Leitner, 1972; Fedde, 1976; Bouverot & Sebert, 1979; Bouverot et al.,

1979). Under hyperoxic conditions, all O_2-chemoreflex drive was removed and thus the

denervation of carotid body chemoreceptors resulted in little change in either the levels of

ventilation (Table 3) or the levels of arterial blood gases and pH (Fig. 9a,b; Table 3).

These data also suggest that because ventilation did not change, carotid body

chemoreceptors appeared to contribute little towards the CO_2/H^+ drive under normocapnic

conditions.

The removal of all vagally-associated pulmonary receptors (PAX) had little affect

upon the overall level of respiration, but had a significant effect upon the pattern of

respiration in the birds of this study. Table 4 shows that under resting conditions, the

removal of pulmonary receptors resulted in a near doubling of tidal volume (V_T), an

increase which was almost completely offset by a concomitant decrease in breathing

frequency (f_b). Thus, $\dot{V}_E$ was only slightly elevated in ducks devoid of pulmonary afferent

information. These data are in agreement with several previous studies that have suggested

the involvement of pulmonary receptors in the regulation of breathing pattern rather than

respiratory drive (Jukes, 1971; Milsom et al., 1981; Tallman & Grodins, 1982a,b). Carotid-

body chemoreceptors, on the other hand, appeared to have contributed 15 to 20% of the

respiratory drive under resting conditions. With no other peripheral chemoreceptors known

to exist, the remainder of the respiratory drive presumably was a function of the central

chemoreceptors located in the medulla and the interaction between CO_2 and H^+ at those

chemoreceptive sites.

II. ACUTE CO$_2$

The level of inspired CO$_2$ used in this study was carefully chosen to produce a near maximal respiratory response. Beginning with the early studies of Stehlik (1922) and Dooley and Koppanyi (1929), it has repeatedly been shown that the inspiration of low levels of CO$_2$ (< 5-6% CO$_2$) stimulates ventilation in birds (for reviews see: Jukes, 1971; Fedde, 1976; Bouverot, 1978; Scheid & Piiper, 1986). Conversely, the inhalation of CO$_2$ at levels greater then 6% tends to depress breathing below the levels seen at 5% CO$_2$ (Orr & Watson, 1913; Hiestand & Randall, 1941; Jones & Purves, 1970; Jukes, 1971; Scheid & Piiper, 1986) albeit the exact mechanisms responsible for such depression are still unclear. It has been suggested that high levels of inspired CO$_2$ may: (1) irritate non-specific receptors in the respiratory tract, thus inhibiting breathing (Jones & Purves, 1970), (2) depress the central nervous system and thus, the respiratory centers in the brain (Jukes, 1971; Scheid & Piiper, 1986), and (3) inhibit intrapulmonary chemoreceptors, disrupting the breathing pattern sufficiently to depress V$_E$ (Milsom et al., 1981).

The inspiration of CO$_2$ resulted in immediate increases in all respiratory variables ($\dot{V}_E$, V$_T$ and f$_b$) in all ducks used during this study, regardless of either their age or gender. These data qualitatively agree with those collected in earlier studies on unanesthetized (Hiestand & Randall, 1941; Fowle & Weinstein, 1966; Jones & Purves, 1970; Bouverot et al., 1974; Powell et al., 1978; Brackenbury et al., 1982), anesthetized (Fowle & Weinstein,

1966; Richards & Sykes, 1967; Ray & Fedde, 1969; Osborne & Mitchell, 1977; Osborne et al., 1977; Scheid et al., 1978) or decerebrate birds (Johnson & Jukes, 1966; Tallman & Grodins, 1982a,b).

In this study, adult male ducks exhibited a 3-fold increase in $\dot{V}_E$ within twenty minutes of being exposed to 5% inspired CO_2 (Fig. 2a). This large increase resulted from significant increases in both V_T (2-fold) and f_b (1.5 fold) (Fig. 2b,c). As has been shown previously (Bouverot et al., 1974; Powell et al., 1978; Milsom et al., 1981; Brackenbury et al., 1982; Tallman & Grodins, 1982a; Scheid & Piiper, 1986), although f_b increased significantly, the respiratory response to inspired CO_2 was predominantly one of increased V_T. The contribution of changes in f_b to the respiratory response to inspired CO_2 has been the subject of some debate in the literature. Several reports exist demonstrating that f_b either remained unchanged or even decreased when the level of inspired CO_2 was increased (Richards & Sykes, 1967; Jones & Purves, 1970; Bouverot & Leitner, 1972; Osborne et al., 1977; Osborne & Mitchell, 1978; Colby et al., 1987). The reasons for such decreases in f_b remain unresolved. However, Milsom et al. (1981) suggested that high levels of CO_2 acted upon intrapulmonary chemoreceptors to depress f_b and increase V_T in a manner analogous to that seen following bilateral pulmonary vagotomy.

The presence of a significant respiratory acidosis was apparent in all the ducks used in this study after twenty minutes of breathing 5% CO_2. These increases in arterial P_{CO_2} and concomitant decreases in arterial pH qualitatively agree with data collected previously under similar conditions (Jones & Purves, 1970; Bouverot et al., 1974; Kuhlmann & Fedde, 1976; Milsom et al., 1981; Tallman & Grodins, 1982a; Dodd & Milsom, 1987). There exist, however, several reports of isocapnic rather than hypercapnic responses to low levels of

CO_2 inhalation (Osborne & Mitchell, 1977; Osborne & Mitchell, 1978; Powell et al., 1978; Scheid et al., 1978). It has been argued (Dodd & Milsom, 1987), however, that such isocapnic responses are little more then artifacts of the methods employed in each study.

The responses of juvenile males ducks acutely exposed to elevated levels of inspired CO_2 were similar to those of adult male ducks. The absolute values recorded for $\dot{V}_E$ and V_T were slightly smaller in the juveniles (Figs. 4,5,6,7; Table 2), however, suggesting that the respiratory control system of juvenile male ducks may exhibit a lower CO_2-sensitivity than that found in adult male ducks.

These data suggest that the contribution of carotid body chemoreceptors, to the acute hypercapnic response, is statistically insignificant. While this observation is contrary to that seen in previous studies that have examined ventilatory responses to transient changes of arterial P_{CO_2} (Jones & Purves, 1970; Bouverot & Leitner, 1972; Bouverot et al., 1974), it does agree with some mammalian studies conducted under similar hyperoxic-hypercapnic conditions (Whipp & Wasserman, 1980; O'Regan & Majchercyzk, 1982). However, this study demonstrated that, in the absence of carotid body chemoreceptors, birds exhibit less of an increase in f_b but an identical increase in V_T to an acute elevation in inspired CO_2 stimulus (Fig 8b,c; Table 3). Despite the lack of significance, the observed changes in ventilation, in these carotid-body denervated birds, followed a trend that was similar to that previously described by Bouverot et al. (1974).

The inspiration of CO_2 also significantly increased ventilation, regardless of whether or not birds possessed pulmonary receptors. The absence of pulmonary receptors did, however, result in a significant change in breathing pattern, similar to that already seen under normocapnic conditions. In other words, breathing was significantly slower and

deeper then that observed in the intact birds under conditions of both normocapnia and hypercapnia (Table 4). The conclusions that were drawn from these data were that pulmonary receptors, while important in establishing the pattern of breathing under both conditions, appeared to contribute little towards the overall ventilatory response to hypercapnia.

Several previous studies have concluded that IPCs are the dominant receptor group involved in the generation of the hypercapnic ventilatory response. These conclusions were based upon observations of elevations in ventilation at constant levels of arterial P_{CO_2} or pH during inhalation of low levels of CO_2. Such a state of isocapnic hyperpnea suggested that arterial chemoreceptors could not have been involved (Osborne & Mitchell, 1977; Osborne et al., 1977; Osborne & Mitchell, 1978; Powell et al., 1978; Scheid et al., 1978; Mitchell & Osborne, 1979). However, this study and several others have repeatedly failed to demonstrate that such an isocapnic state exists and have instead reported the presence of a hypercapnic hyperpnea during low level CO_2 inspiration (Jones & Purves, 1970; Bouverot et al., 1974; Kuhlmann & Fedde, 1976; Milsom et al., 1981; Tallman & Grodins, 1982a; Dodd, 1985).

Thus, the data from this study suggest that under conditions of acute hypercapnia, central chemoreceptors are primarily responsible for eliciting the respiratory response (most of the increase in f_b and all of the increase in V_T) observed in Pekin ducks (Sebert, 1978; Jones et al., 1979; Sebert, 1979; Milsom et al., 1981). The data from this study agree with those of Milsom et al. (1981) in attributing central chemoreceptors with at least 60-80% of the overall respiratory response to inspired CO_2.

III. CHRONIC CO$_2$

Respiratory adaptation has been defined variously in the literature. On one hand, the process of adaptation has been construed simply as the respiratory changes observed when animals are exposed to respiratory stimuli. The increased $\dot{V}_E$ that was observed when ducks were acutely exposed to inspired CO_2 is an example of such a definition of respiratory adaptation. Classically, however, the process of respiratory adaptation (or acclimatization) has referred to the time-dependent changes observed in ventilation during prolonged exposure to a respiratory stimulus. In other words, adaptation described the process by which the initial respiratory response was modified as that stimulus was prolonged. It is this latter definition of respiratory adaptation that is used throughout this study.

While the avian literature abounds with studies that have described the acute consequences of hypercapnia, the study of Dodd and Milsom (1987) appears to be the first to deal directly with the consequences of chronic hypercapnia upon both respiration and acid-base homeostasis in birds.

A. The Effect of Chronically Inspired CO$_2$ Upon Ventilation

Chronic (300 minute) exposure of adult male Pekin ducks to 5% inspired CO_2 resulted in a progressive decrease in $\dot{V}_E$ (after the 3x increase observed in the first 20 minutes) over the latter 280 minutes of the experiment. The average respiratory adaptation that was exhibited by these ducks was approximately 50% (range: 42-76%) after five hours. This decrease resulted entirely from the complete return of f_b to its pre-hypercapnic (control) levels (Fig. 2c). On the other hand, the level of V_T remained unchanged after the

first twenty minutes and thus contributed nothing to the progressive decline in V_E that was observed.

Respiratory adaptation was also observed in juvenile male birds chronically exposed to the elevated CO_2 (Fig 11). However, the degree of adaptation was somewhat smaller (although not significantly) then that which was demonstrated by their adult counterparts (average: 42%; range: 21-66%). The manner by which this adaptation was achieved was similar between the two age groups (Fig. 11b,c). Therefore, it is possible that the reduced degree of respiratory adaptation that was shown by these younger birds resulted from a reduced degree of CO_2-sensitivity, similar to that observed earlier during the acute phase of the hypercapnic exposure.

The phenomenon of ventilatory adaptation to chronic exposure to CO_2 has been previously documented for a variety of different vertebrate species, both aquatic and terrestrial. Most of these reports, however, list only the changes observed in overall respiration; not the changes which occurred in respiratory pattern. Randall et al. (1976) reported that the larger spotted dogfish (*Scyliorhinus stellaris*), which initially exhibited a 175% increase in gill ventilation ($\dot{V}_G$) upon exposure to hypercapnic-water, exhibited a complete (100%) return of $\dot{V}_G$ to control values after only four hours. The rainbow trout (*Oncorhynchus mykiss*; formerly *Salmo gairdneri*) also demonstrated a remarkable degree of ventilatory adaptation upon chronic exposure to CO_2-rich water. Within 5-10 hours $\dot{V}_G$ had already recovered 50%, from an initial five-fold increase, towards control levels. Complete recovery was apparent after 2-3 days (Janssen & Randall, 1975). Not all fish, however, show such rapid adaptation to hypercapnic conditions. The bimodally breathing spotted gar (*Lepisosteus oculatus*) demonstrated no adaptive changes in ventilation after 72 hours of

breathing hypercapnic water (Smatresk & Cameron, 1982). The reason for such differences will become more apparent in the following section on acid-base homeostasis.

Ventilatory adaptation has not been examined in amphibians and has only been examined in a single species of reptile, the western painted turtle (*Chrysemys picta bellii*) (Silver & Jackson, 1985). Like the gar, the turtle showed no respiratory adaptation during chronic hypercapnic exposure.

Ventilatory adaptation has been observed in several species of mammals but the process is always significantly slower and smaller in magnitude than that described in this study for Pekin ducks. The largest recovery exhibited by both rats and dogs consisted of a 30-40% decrease in the initial ventilatory response, but only after 3-4 weeks of exposure to 5% CO_2 (Lai et al., 1981; Jennings & Davidson, 1984). In humans, neither Schaefer et al. (1963) nor Guillerm and Radziszewski (1979) could demonstrate any adaptive changes in $\dot{V}_E$ after 30-40 days of 2% CO_2 inhalation. Clark et al. (1969), however, found a 20% recovery in $\dot{V}_E$ after 10 days of 4% CO_2 exposure suggesting that the degree of adaptation shown by humans may be related to the magnitude of the CO_2 stimulus. While most of these studies have demonstrated that the ventilatory response to chronically inspired CO_2 consisted of two phases (acute and chronic), two studies conducted upon dogs have shown a third phase to the chronic response. After $\dot{V}_E$ had completely adapted from its initial increase, it secondarily increased again during the last four days of a fourteen-day 5% CO_2 exposure (Jennings & Chen, 1976; Jennings, 1979). Thus, the final levels of $\dot{V}_E$ were somewhat greater than those seen after the initial recovery. Even more interesting, perhaps, was that a later study from the same laboratory, conducted under identical conditions and also upon dogs, showed the more classical, two phase respiratory response to chronically

inspired CO_2 (Jennings & Davidson, 1984).

The data from the present study, when compared with these other studies, show that Pekin ducks chronically exposed to hypercapnic conditions demonstrated a relatively large and rapid ventilatory adaptation.

B. The Effect of Chronically Inspired CO_2 Upon Acid-Base Homeostasis

Accompanying the ventilatory changes just described for male ducks during the latter 280 minutes of these experiments were significant changes in both arterial CO_2 tensions and arterial pH. Despite the large increase in $PaCO_2$ during the first 20 minutes of CO_2 exposure, the arterial P_{CO_2} of these birds continued to rise throughout the period of chronic CO_2 exposure (Fig. 3a) as a consequence of the decrease in ventilation. During the first twenty minutes of CO_2 exposure, an acidotic shift in pH accompanied the rise in $PaCO_2$. During the latter 280 minutes, however, the continuing rise in $PaCO_2$ was accompanied by an alkalotic shift in pH. In other words, pHa also demonstrated a partial recovery, of approximately 50% (range: 33-110%), towards its control level during chronic exposure to CO_2. These paradoxical changes in $PaCO_2$ and pHa suggest that the buffering ability of the blood must have changed considerably. Accompanying the recovery of arterial pH was a significant increase in the calculated arterial bicarbonate ion concentration ([HCO_3^-]) (Fig.3c), a rise that indicated the functioning of metabolic compensatory mechanisms. Changes in the relationships between $PaCO_2$, pHa and calculated [HCO_3^-] during the periods of chronic exposure are best illustrated in the pH/[HCO_3^-] diagram shown in Figure 6. The slope of the line connecting the control and 20 minute values represents the _in vivo_ buffering capacity of whole blood (extracelluar fluid and

erythrocytes). After twenty minutes, the slope of the line changed dramatically, reflecting the compensatory changes that must have occurred in the plasma to produce the paradoxical changes in pHa and $PaCO_2$. Such compensatory changes were presumably due to ion exchange processes between the plasma and other body fluid compartments.

For comparison with studies conducted on other species, the changes in pHa which occurred during the chronic exposure to CO_2 were expressed according to the method first developed by Siesjo (1971) and later refined by Lai et al. (1973). Termed "pH compensation", this method expresses the recovery measured in pHa (with metabolic compensation) as a function of the changes in pHa that would have occurred if $[HCO_3^-]$ had remained constant (without metabolic compensation). The equation used to convert the pH changes observed in this study to the more standard form of pH compensation was taken from Lai et al. (1973):

$$\% \text{ pH Compensation} = 1 - \frac{pH_{actual}}{pH_{[HCO3-] \text{ constant}}} \times 100 \quad (3)$$

Values obtained with this method are compared with the percentage recovery values in Figure 14 using the data collected from adult male ducks. Because $PaCO_2$ continued to rise after the initial twenty minutes, pHa would have continued to fall if the level of $[HCO_3^-]$ had not increased. Thus, the degree of pH compensation that was exhibited by male ducks after 300 minutes was actually higher (75%) then the percent recovery data would imply. The "pH compensation" values, therefore, give a better estimation of the extent of the metabolic compensation for the respiratory acidosis than the "percent recovery" values.

Figure 14. Schematic representation of pH recovery (dotted area) versus pH

compensation (dotted plus lined area) in chemoreceptor-intact adult male

ducks during chronic CO_2 exposure. Only mean values for pHa are given. For

calculation of pH compensation, see text.
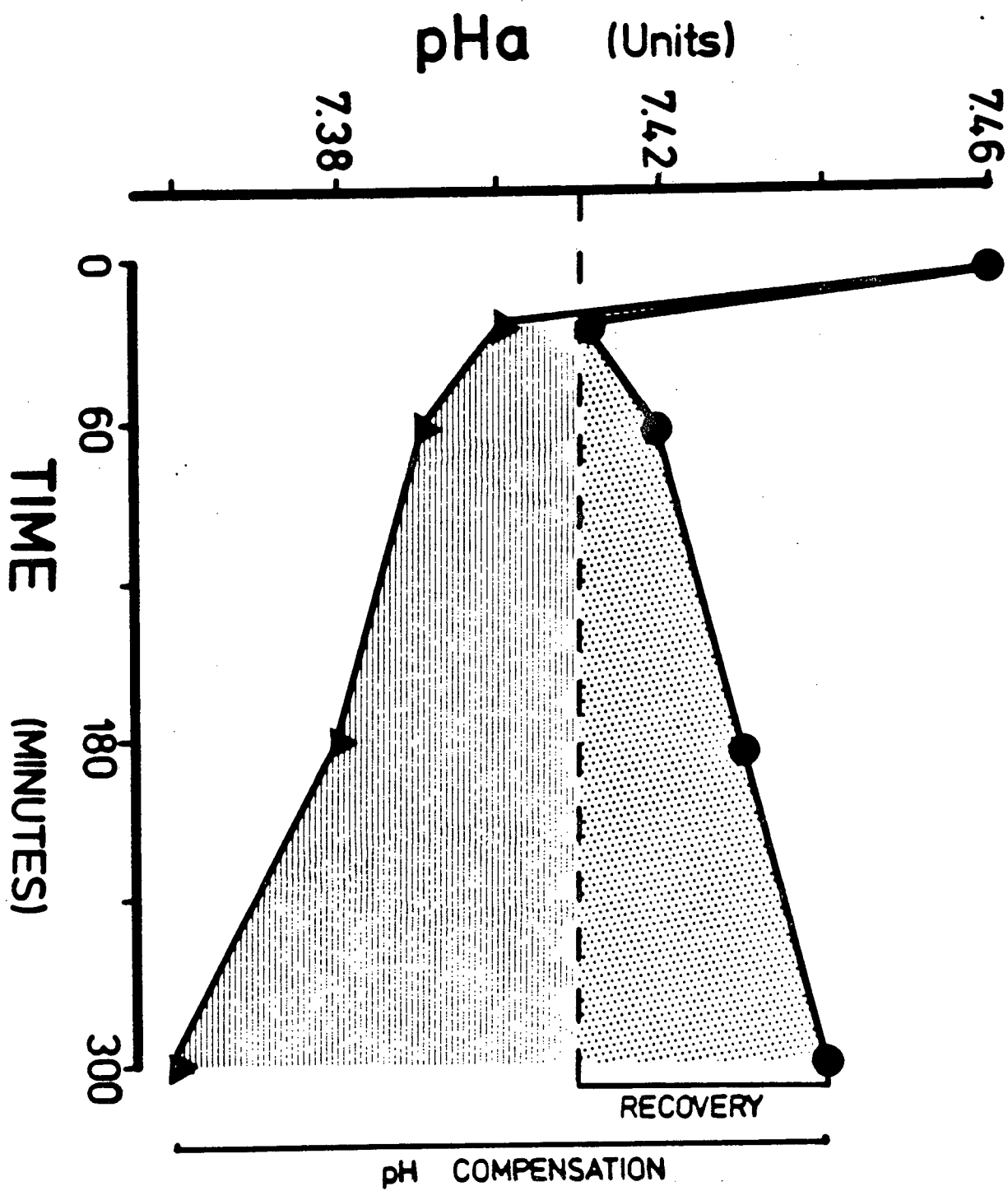
Amongst the other vertebrates for which pH compensation values during chronic hypercapnia have been reported, only fish exhibit a degree of compensation greater then the 75% exhibited by the adult male ducks of this study. While the inter-species variation has been reported to range from 0-100%, the average degree of pH compensation exhibited by fish exceeds 80% (Toews et al., 1983; Heisler, 1986 Cameron & Iwama, 1987). Terrestrial vertebrates, on the other hand exhibit a degree of pH compensation that is considerably lower. Amphibians exposed to 3-5% CO_2 for at least 24 hours demonstrate pH compensation ranging from 0% in the mudpuppy (*Necturus maculasa*) (Stiffler et al., 1983) to 30% in the toad (*Bufo marinus*) (Boutilier et al., 1979; Toews & Heisler, 1982; Stiffler et al., 1983; Boutilier & Heisler, 1988). In the only two species of reptile that have been examined, the western painted turtle (*Chrysemys picta bellii*) and the tegu lizard (*Tupinambis nigropunctatus*), the range of pH compensation varied from 30-40% (Silver & Jackson, 1985; Glass & Heisler, 1986). The degree of compensation exhibited by mammals, however, varied with the degree of arterial hypercapnia. In general, small changes in $PaCO_2$ (< 1.4 fold increase) produced a high degree of compensation (> 50%) while larger changes produced responses that were comparable to those displayed by both amphibians and reptiles (Heisler, 1986). Thus, as with the respiratory adaptation, the degree of pH compesation observed in birds was relatively high and rapid.

## C. CO_2 Versus pH ([H$^+$]) as the Unique Stimulus of Ventilation During Chronic Hypercapnia

Considerable controversy has always existed as to whether P_{CO_2} provides a unique stimulus to respiratory chemoreceptors independent of its effects on pH ([H$^+$]). Much of

this stems from the difficulty in separating these two potential stimuli. In the present study, however, the metabolic compensation which developed to offset the chronic respiratory acidosis made this possible. Thus, although the data collected from the acute portion of this study could not distinguish whether the rise in P_{CO_2} or the fall in pH was responsible for the increase in $\dot{V}_E$, when time was allowed for compensatory processes to occur, the effects of pH upon ventilation were uncoupled from those of P_{CO_2}.

The data collected from this study clearly demonstrate that ventilation decreases significantly while F_ICO_2 remains constant and arterial P_{CO_2} increases during chronic exposure to hypercapnia. Thus, neither the changes in inspired CO_2 nor arterial P_{CO_2} correlate well with the changes observed in ventilation. An excellent correlation does exist, however, between the changes observed in $\dot{V}_E$ and pHa arguing that at least under conditions of chronic hypercapnia, the changes observed in $\dot{V}_E$ are primarily a function of the changes observed in pH (Fig. 15). Minute ventilation, however, is the product of V_T and f_b which appear to be differentially affected by CO_2 and H^+ as specific stimuli. The fact that f_b decreases suggests that it is primarily influenced by pH. Tidal volume, on the other hand, remains constant while $PaCO_2$ increases and $[H^+]$ decreases, suggesting that the level of V_T is determined by a combination of the two stimuli. Thus, although levels of $\dot{V}_E$ are really a function of both pH and P_{CO_2} the adaptive changes observed in $\dot{V}_E$ during chronic CO_2 exposure are primarily a function of compensatory changes in pH.

These data corroborate the study of Dodd and Milsom (1987) which also suggested that $\dot{V}_E$ was a single function of pH in birds under conditions of chronic hypercapnia. Similar results have also been described for several other vertebrate species. The complete ventilatory recovery exhibited by the dogfish exposed to chronic hypercapnia was also

Figure 15. Changes in $\dot{V}_E$ and pH that were observed between 20 and 300 minutes, expressed as percentages of the changes initially observed between 0 and 20 minutes for chemoreceptor-intact adult male ducks. It should be pointed out that although the percent-changes were identical, the direction in which the two variables were changing were opposite as $\dot{V}_E$ was decreasing as pHa was increasing.
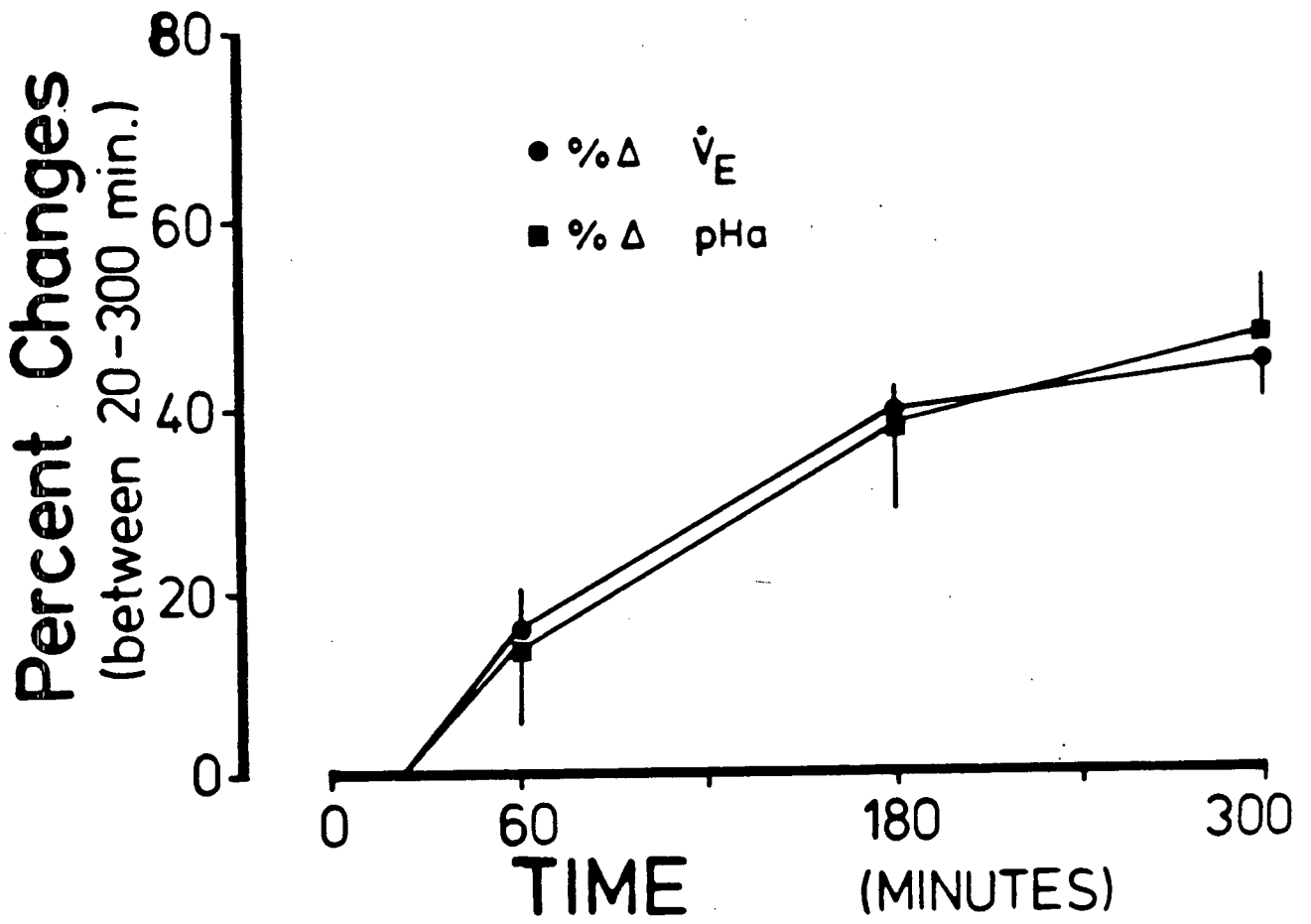
accompanied by a large increase in plasma [HCO_3^-] and a 70-80% recovery in arterial pH after only four hours (Randall et al., 1976). Likewise, the rainbow trout also exhibited a 6-8 fold increase in arterial [HCO_3^-] and complete recoveries in both ventilation and pH after 3-5 days of chronic CO_2 exposure (Janssen & Randall, 1975; Eddy et al., 1977).

Data from mammalian studies also reveal a tight correlation between changing levels of ventilation and pH during chronic hypercapnia. The temporal changes recorded in the arterial pH and [HCO_3^-] of rats chronically exposed to 5% CO_2 closely paralleled the 35% reduction recorded in ventilatory drive (Lai et al., 1981). Similarly, the 40% recovery in ventilation recorded in dogs over 26 days of chronic hypercapnia was paralleled by an approximate recovery of 40% in pHa and a 4-5 mM increase in arterial [HCO_3^-] (Jennings & Davidson, 1984). In the studies conducted on both humans (Guillerm & Radziszewski, 1979) and turtles (Silver & Jackson, 1985), where no ventilatory adaptation occurred, there was also no pH recovery.

Although most of this study was conducted upon male birds, some female ducks were included in the study. The respiratory responses of the adult female ducks to both acute and chronic exposure to elevated levels of CO_2, however, were significantly different from those shown by either adult or juvenile male ducks.

While acute inspiration of CO_2 stimulated respiration in female ducks, the maximum levels of $\dot{V}_E$ obtained were significantly lower than those observed in male birds (Fig. 4; Table 2). This was the result of smaller increases in both V_T and f_b in the female ducks (Fig.4) despite similar changes in $PaCO_2$ and pHa suggesting that the sensitivity of the respiratory system to CO_2 was less in female ducks then in male ducks.

After the first twenty minutes of chronic hypercapnic exposure, female ducks failed

to demonstrate the progressive fall in f_b that was always observed in males. As a result, ventilatory adaptation did not occur in the female ducks over the period of chronic CO_2 exposure (Fig. 4). Female birds also did not exhibit any metabolic compensation and thus pH did not return towards control levels (Fig. 5). The fact that neither $\dot{V}_E$ nor pHa showed any recovery further supports the hypothesis that the adaptive changes in ventilation seen during chronic CO_2 exposure are a singular function of compensatory changes in pH.

D. Possible Mechanisms Responsible for the Changes Observed in

Acid-Base Homeostasis During Chronic Hypercapnia

These data suggest that male birds are capable of rapidly correcting, at least partially (50% pH recovery) the acid-base disturbance imposed by the chronic inhalation of CO_2. Thus, it is clear that birds must have the ability to both rapidly mobilize and rapidly exchange ion stores between various body compartments.

Conventional acid-base analysis has traditionally revolved around the Henderson-Hasselbach equation and the measurements of pH, P_{CO_2} and HCO_3^- (calculated). Recently, however, Stewart (1981; 1983) has rejuvenated the physicochemical theory of acid-base regulation clinically referred to as the "anion gap" (Cameron, 1989). This latter theory of Stewart's requires us to recognize the existence of both dependent ($[H^+]$ (pH), $[HCO_3^-]$) and independent (P_{CO_2}, strong ion difference (SID), total weak acid (A_{TOT})) variables in acid-base regulation. Thus, all changes that were measured in pHa in this study must have been the result of changes in at least one of the independent variables listed above.

These data show that the changes measured in arterial P_{CO_2} and pH, during chronic hypercapnia, occur paradoxically. Thus, the changes measured in pH could not be

accounted for by the changes measured in P_{CO2}. Changes in A_{TOT} also did not appear to have been responsible for the changes measured in pH. In plasma, the only significant weak acids that exist are proteins, and their concentrations are regulated primarily by the liver (Stewart, 1983), a process that requires considerable time (days to weeks). Thus, the changes that were observed in pH when birds chronically inhaled elevated levels of CO_2, must have necessarily been due to changes in SID. Plasma collected from adult male ducks in this study, however, failed to show any significant changes in any of the plasma strong ions (Na^+, K^+, Ca^{++}, Cl^-, lactate$^-$) between conditions of rest and chronic hypercapnia, despite the significant changes that were observed in pH (Table 1). It is quite probable, therefore, that the resolution of the analysis used during this study was not sufficient to detect the change that must have occurred in SID. Differences in analytical resolution may also explain why some studies of chronic hypercapnia have reported changes in SID (Eddy et al., 1977; Stiffler et al., 1983) and others, including this one, have not (Silver & Jackson, 1985; Cameron & Iwama, 1987). Because of the inability to detect changes in SID, the Henderson-Hasselbach equation was employed to help explain the changes that were observed during this study. However, it must not be overlooked that while the Henderson-Hasselbach equation gives a quantitative measure of the magnitude of the metabolic compensation that was required to produce the observed changes in pHa, it does not explain the specific ion movements involved.

Unfortunately, while the contribution of renal mechanisms to acid-base regulation has been well described in mammals (Lai et al., 1973; Cogan, 1984; Tannen & Hamid, 1985), their involvement in the acid-base homeostasis of birds remains unknown. In addition to the kidneys, extra-renal structures downstream from the kidneys may also

contribute to acid-base homeostasis in birds. Following the delivery of ureteral urine to the cloaca, the urine transverses to the colon, perhaps as far as the caeca (Long, 1982). Long and Skadhauge (1983) have shown that in resting chickens, cloacal fluid pH is significantly more alkaline (~1 pH unit) than ureteral urine. This observation suggests the presence of ion exchange sites in the lower intestinal tract of birds. Extra-renal modification of urine pH, and thus extracelluar pH, has certainly been shown to occur in both amphibians and reptiles (Schlib & Brodsky, 1966; Frazier & Vanatta, 1972; Ludens & Fanestil, 1972; Tufts & Toews, 1985). The net result of such ion exchange is the acidification of the mucosal fluid (urine) and the alkalization of the serosal fluid (extracellular fluid). Thus, while purely speculative, it is tempting to suggest that the avian hindgut and the amphibian/reptilian urinary bladder function analogously and contribute towards acid-base homeostasis.

In addition to the renal and extra-renal mechanisms suggested above, the mobilization of bicarbonate from bone may also contribute towards the increase detected in extracelluar $[HCO_3^-]$. Several mammalian studies have confirmed that bicarbonate exchange between blood and bone does occur under conditions of elevated arterial P_{CO_2} or decreased pH (Bettice & Gamble, 1975). Thus, the reduced degree of pH compensation shown by the juvenile birds of this study, whose bones were still undergoing ossification, could have been due to the reduced exchange of bicarbonate between extracelluar fluid and bone. Furthermore, studies conducted upon laying hens have shown that the calcium carbonate ($CaCO_3$) found in eggshells was originally derived from the stores of calcium and bicarbonate found in the blood (extracelluar fluid) (Hunt & Simkiss, 1967; Mongin, 1968). During shell formation, the removal of HCO_3^- from the blood at the shell gland would result in the development of a temporary metabolic acidosis (Mongin, 1968; Hodges, 1970).

Compensation of this acidosis took the form of increased H^+ secretion and HCO_3^- reabsorption at the level of the kidneys (Mongin, 1968; Prashad & Edwards, 1973). When the shell formation had been completed and the removal of HCO_3^- from the blood had ceased, the blood became alkalotic until renal compensation could once again re-establish normal acid-base balance. Under resting conditions, therefore, the blood of female birds cycled from a state of acidosis to alkalosis depending upon the stage of eggshell formation. Pekin ducks are not seasonally laying birds but instead lay eggs year round. Because the relationship between shell formation and acid-base homeostasis was overlooked in this study, no attempt was made to establish egg-laying cycles for the birds used in these experiments. In hindsight, it is very possible that the respiratory acidosis from the chronic inspiration of CO_2 was superimposed upon an already altered state of acid-base balance. Therefore, if at the time of the experiment, some HCO_3^- had already been directed towards the formation of eggshells, the amount of HCO_3^- available to buffer acid-base derangements would have been reduced and the capacity of the bird to compensate for further acid-base derangements would have been considerably reduced.

It is possible that any, or all, of the above mechanisms were responsible, at least partially, for the increases calculated in plasma $[HCO_3^-]$ and thus, the metabolic pH compensation shown by the ducks of this study when chronically exposed to CO_2. Although these changes were small when compared with those recorded in some other vertebrates, they occurred at rates that were much faster. Would extracellular $[HCO_3^-]$, and thus metabolic compensation, have continued to increase if the period of CO_2 exposure were longer than five hours, or would an upper limit to such compensation have been reached (Heisler, 1986)? While this study can not answer this question, it does suggest

that the mechanisms responsible for the pH compensation occur at a rate that is unsurpassed by other air-breathing vertebrates.

E. The Relative Contribution of Peripheral Chemoreceptors to the Ventilatory Response

The data collected from chronically hypercapnic male and female ducks provide substantial support for the hypothesis that ventilatory adaptation and metabolic pH compensation are causally related phenomena. Given the increasing number of studies which suggest that central chemoreceptors are the dominant site at which respiration is chemically controlled (Berger et al., 1977; Hitzig & Jackson, 1978; Milsom et al., 1981; Loeschcke, 1982; O'Regan & Majcherczyk, 1982; Hitzig et al., 1985; Jennings & Szlyk, 1988), it would be naive to conclude from this study that ventilation is necessarily following changes in arterial pH. Previous studies have shown that the changes in pH in both cerebrospinal fluid (CSF) and cerebral extracelluar fluid (ECF$_c$) are either equal to or greater than those changes measured in arterial blood pH during chronic respiratory acid-base derangements (Fencl, 1986; Kazemi & Johnson, 1986). Therefore, it is probable that ventilation was actually following changes in central pH rather than peripheral pH in the present study. Unfortunately, the existence of such a relationship between ventilation and central pH can not be demonstrated directly in this study although the data collected from the chronic denervation experiments indirectly suggests this.

During the acute phase of CO_2 inhalation, it was inferred previously that central chemoreceptors played the dominant role in the generation of the hypercapnic ventilatory response in birds and that carotid-body and pulmonary receptors collectively contributed less then twenty percent towards the overall respiratory response. Under chronic conditions

of inspired CO_2, the data also point towards a dominant role of the central chemoreceptors in the generation of the respiratory response.

Under chronic hypercapnic conditions, carotid-body chemoreceptors appeared to contribute little towards the process of ventilatory adaptation as both intact and denervated groups of animals exhibited similar recoveries in minute ventilation. In fact, other than a slight difference in the peak level of $\dot{V}_E$ reached after the first twenty minutes of CO_2 exposure, both groups of birds subsequently exhibited identical changes in ventilation (Fig. 8). The degree of pH compensation that was exhibited by denervated birds was slightly greater then that exhibited by intact birds but not significantly so (ie. 85% vs. 75%). Although both groups of birds exhibited identical changes in pH over the latter 280 minutes of each experiment, denervated birds exhibited larger changes in $PaCO_2$ (Fig 9a, b, c). The fact that both intact and carotid-body denervated birds exhibited recoveries in ventilation and pH that were nearly identical, indicates that carotid-body chemoreceptors contribute negligibly towards the control of breathing under conditions of chronic hypercapnia.

As previously discussed, pulmonary receptors obviously contribute towards the regulation of breathing pattern during acute exposure to elevated levels of inspired CO_2, but do not contribute significantly towards the increase in minute ventilation. Pulmonary denervation produced absolute levels of V_T and f_b that were significantly higher and lower, respectively, then those observed in chemoreceptor-intact ducks of similar age (Table 4). However, despite the changes in breathing pattern, pulmonary denervated birds exposed to chronic hypercapnia still maintained V_T while f_b progressively declined just as in their intact counterparts (Fig 11b,c). Therefore, the pulmonary denervated ducks, during chronic

hypercapnia, exhibited a recovery in $\overset{\bullet}{V}_E$ that was not dissimilar to that seen in chemoreceptor-intact ducks (Fig. 11a). These data suggest, therefore, that pulmonary receptors contribute little towards control of the overall level of ventilation during chronic hypercapnia.

Pulmonary denervated birds, while always acidotic in comparison to intact birds, also still exhibited a substantial pH recovery after the first twenty minutes of CO_2 exposure (Fig. 12b). In fact, the pH recovery exhibited by these birds (77%) was not significantly different than that exhibited by intact birds of similar age (86%) (Fig. 12b). Thus, although breathing pattern was significantly altered, the denervation of pulmonary receptors had little effect upon the magnitude of the initial ventilatory response or the degree of either ventilatory or pH recovery that was observed.

In summary, Figures 16 and 17 illustrate all the relative changes that were observed in respiratory and acid-base variables in male birds both with and without peripheral chemoreceptors. As all birds responded similarly to chronic CO_2 inhalation, it was clear that changes at the peripheral chemoreceptors contributed little towards the ventilatory adaptation seen during the chronic hypercapnia. Thus, these data suggest that central chemoreceptors play the dominant role in the control of ventilation during chronic hypercapnia in birds.

Unfortunately, because both groups of chemoreceptors (carotid body and pulmonary) could not be successfully removed together, arguments regarding the role of central chemoreceptors in the ventilatory response to chronic hypercapnia depend upon three assumptions. Firstly, it is assumed that both groups of peripheral chemoreceptors contribute towards the overall control of ventilation in an additive rather than an interactive fashion.

Figure 16. The changes observed in $\dot{V}_E$ in chemoreceptor-intact adult (●) and

juvenile (▲) male ducks as well as carotid body (■) and pulmonary

receptor (▼) denervate male ducks during 300 minutes following a step

increase in inspired CO_2 from 0 to 5%. Data are expressed as a percentage

of control (0% CO_2) values.
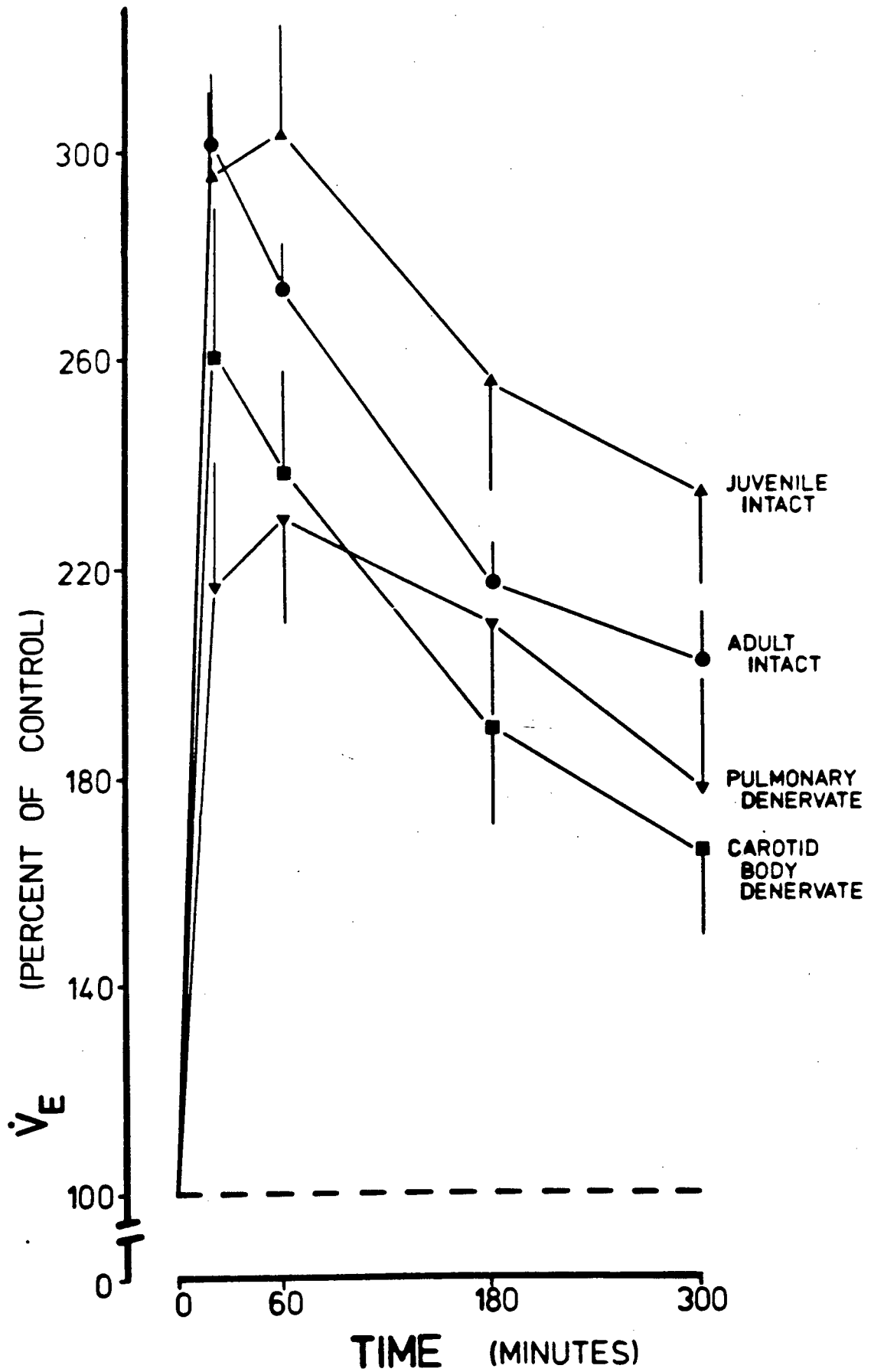
Figure 17.   Changes in $\dot{V}_E$, V_T, f_b, $PaCO_2$, pHa and arterial [HCO_3^-] that were

observed between 20 and 300 minutes, expressed as percentages of the

changes initially observed between 0 and 20 minutes. A negative

value indicates the return of that variable towards its control level.
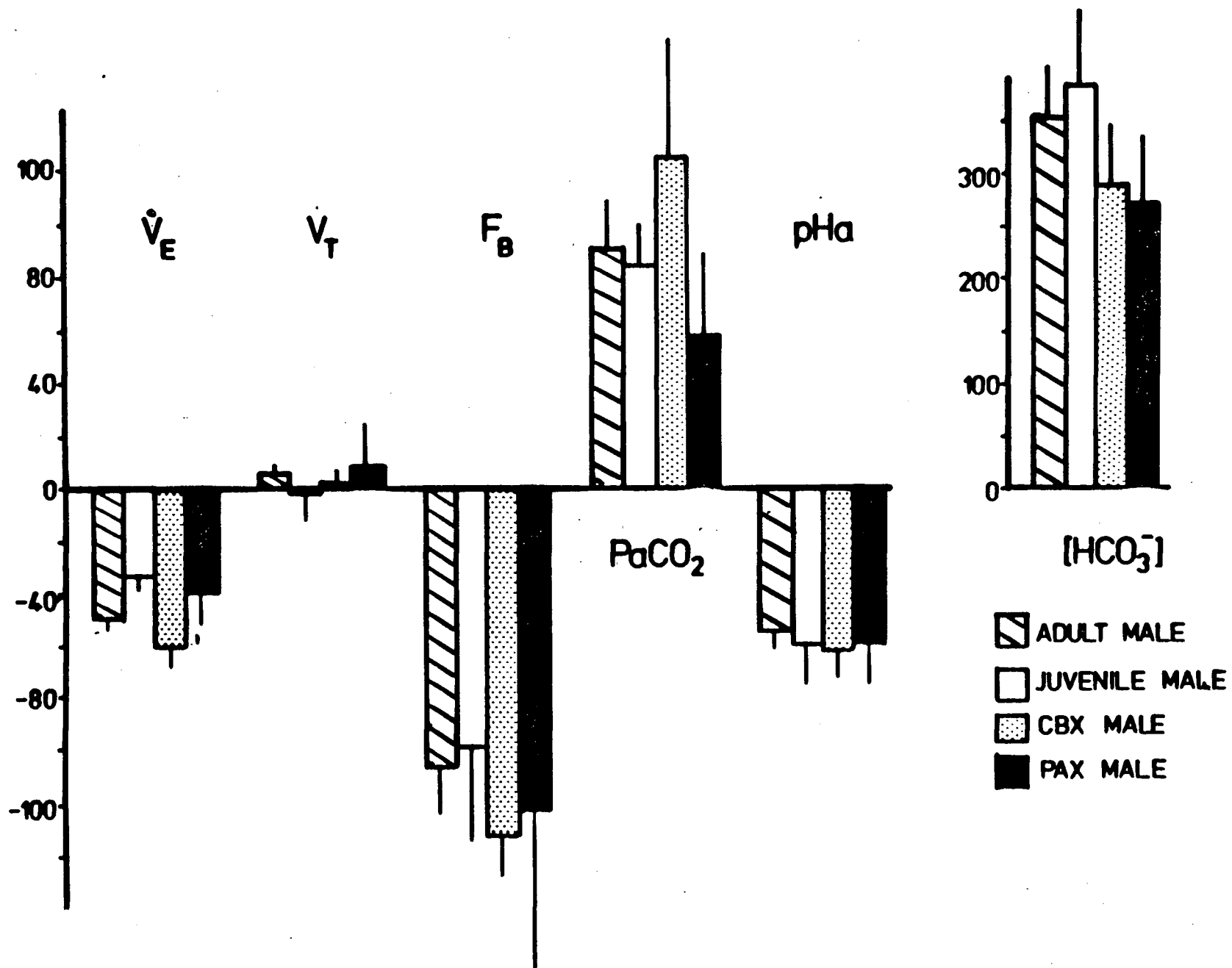
Thus, removal of one group of receptors would have little effect upon the relative contribution of the remaining group to the ventilatory response. Secondly, it is assumed that little or no redundancy exists between carotid-body and pulmonary receptors with regards to their effects upon ventilatory control. In other words, the contributions of the denervated chemoreceptor group were not simply shifted to the group of chemoreceptors that remained. Finally, and perhaps most importantly, it was assumed that no other groups of peripheral chemoreceptors existed in birds other then the carotid-body and pulmonary receptors. Based on these three assumptions, the data from this study strongly suggests that central chemoreceptors, responding to changes in pH, are responsible for the changes in ventilation that are observed during chronic CO_2 inhalation.

F. The Relative Contribution of the Central Chemoreceptors to the Ventilatory Response

The data from this study, and several other ventilatory reflex studies conducted upon birds (Sebert, 1978; Jones et al., 1979; Sebert, 1979; Milsom et al., 1981), suggest that central chemoreceptors are the predominant receptor-group involved in the chemical control of respiration. The exact location of these receptors, however, remains unknown in birds. Because relatively few studies have examined central chemoreception in birds, and because a large number of similarities exist between birds and mammals with regards to the chemical control of respiration (Bouverot, 1978), avian and mammalian respiratory control systems are believed to be the same. Thus, avian central chemoreceptors are thought to exist on or near the ventrolateral surface of the medulla, the same location in which they are found in mammals (Schlaefke et al., 1970; Bledsoe & Hornbein, 1981; Loeschcke, 1982; O'Regan & Majcherczyk, 1982).

The data from this study suggests that the ventilatory adaptation that was observed during the chronic inspiration of CO_2 reflected an adaptational change that had occurred at the level of the central chemoreceptors. While several plausible mechanisms for this central adaptation exist, the most obvious one suggested from these data was that the signal to these receptors had changed due to a changed ionic composition (ie. [H^+], [HCO_3^-]) in the region of the central chemoreceptors. However, for the sake of completeness, several other possible mechanisms should be briefly mentioned, including receptor adaptation, respiratory muscle fatigue and/or changes in the processes of central integration.

It was possible that the changes observed in ventilation were not due to changes in stimuli, but due to the adaptation of the central chemoreceptors themselves. The adaptation of neural receptors has been a phenomenon quite commonly described in the literature. In fact, adaptation[1] has been a feature quite commonly described for sensory receptors, such as the mechanoreceptors located in the skin (pacinian corpuscles), muscle (muscle spindles) and airways (airway stretch receptors) (Catton, 1970; Lahiri et al., 1982; Sant'Ambrogio et al., 1983). Adaptation has also been described for chemoreceptors exposed to CO_2, although these were carotid-body rather than central chemoreceptors (Dutton et al., 1967; Black et al., 1971; Lahiri et al., 1982). However, adaptation of these chemoreceptors was very rapid with the maximum response reached after only 20 seconds. If chemoreceptors were responsible for the adaptation to chronic CO_2 exposure, a decrease in their sensitivity to CO_2 should have also occurred. After chronic exposure to CO_2, however, the ventilatory

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[1] Adaptation is a process whereby the discharge of neural receptors diminishes after an appropriate stimulus has been introduced and maintained" (Sant'Ambrogio et al., 1983).

sensitivity of birds to CO_2 has been shown not to change (Dodd & Milsom, 1987). Therefore, both the time course of the adaptive response and the absence of a sensitivity change suggest that the changes observed in this study were not the result of central chemoreceptor adaptation.

Another possible way of explaining for the changes that were observed is that the respiratory muscles fatigued since the level of ventilation was at least doubled throughout the 5 hour period of hypercapnia. However, fatigue was highly unlikely. Birds subjected to high ambient temperatures maintain respiratory rates that not only far exceed those measured in this study, but that also last for much longer periods of time (Calder & Schmidt-Nielsen, 1968). Furthermore, Butler (1980) and Brackenbury et al. (1982) have also shown that during exercise, ventilation can increase to 5 to 7 times normal levels.

Finally, it is possible that the changes observed in ventilation resulted from temporal changes that took place as the signals were centrally integrated in the brain. Unfortunately, because many of the mechanisms of integration remain unresolved, no definite conclusions can be reached at this time from the data collected during this study.

While neither cerebral spinal fluid (CSF) nor cerebral extracelluar fluid (ECF_e) pH were measured during this study, it is assumed that changes in central pH parallel those changes measured in arterial pH during periods of chronic hypercapnia (Fencl, 1986; Kazemi & Johnson, 1986). Thus, data from this study suggest that a change in pH, acting upon central chemoreceptors, is the mechanism most likely responsible for the concomitant ventilatory adaptation that was observed.

It has been widely accepted in the literature that the [H^+] of the environment surrounding the central chemoreceptors is the major chemical stimulus to central

chemoreceptors (Mitchell, 1966; Berger et al., 1977; Berkenbosch et al., 1978; Loeschcke, 1982; O'Regan & Majcherczyk, 1982). During acute CO_2 exposure, therefore, the initial decrease observed in pH stimulates central chemoreceptors to stimulate ventilation, regardless of the integrity of the peripheral chemoreceptors. During chronic respiratory acidosis, the recovery of arterial pH, due to metabolic processes, must also have occurred centrally. Therefore, the $[HCO_3^-]$ of ECF_c should have increased in a manner that paralleled that calculated for plasma, as has been shown for mammals (Bleich et al., 1964; Leusen, 1972; Hasan & Kazemi, 1976; Nattie & Edwards, 1981; Loeschcke, 1982; Nattie, 1983). Current theories explaining the increases in CSF/ECF_c $[HCO_3^-]$ during chronic hypercapnia in mammals include both passive and active mechanisms. Leusen (1972) was amongst the first to firmly establish the exchange of HCO_3^- between the plasma and CSF at the blood-brain barrier. Loechcke (1982) further suggested that such exchange occurred rapidly (seconds to minutes) and involved a specific carrier protein and Cl^- as a counterion. At approximately the same time, Nattie and Edwards (1981) proposed that increases in CSF/ECF_c $[HCO_3^-]$ occurred by two general processes. Firstly, the ionic composition of the freshly formed CSF was altered by carbonic anhydrase-dependent P_{CO2} specific mechanisms. This process occurred relatively rapidly in mammals (minutes to hours) with the increase in $[HCO_3^-]$ provided by CO_2 hydration. The second process, which occurred much slower (hours), involved the exchange of ions (including HCO_3^-) across the blood brain barrier from the plasma to the CSF/ECF_c. Therefore, while it has been well accepted that under conditions of respiratory acidosis metabolic compensation occurs in the central compartments, the exact mechanisms by which this occurs are still debatable.

CONCLUSIONS

The results of this study indicate that acid-base compensation for respiratory acidosis occurs extremely rapidly in the male Pekin duck but not in the female duck. While this suggests that mobilization of bicarbonate ions may be important in alleviating acid-base disturbances of respiratory origin, the exact mechanism(s) involved remain unclear. Accompanying the recovery in pHa was a similar recovery in minute ventilation. The fact that the changes in $\dot{V}_E$ paralleled those occurring in pH and not P_{CO_2} suggests that during chronic hypercapnia, the changes observed in $\dot{V}_E$, in birds, are a function of metabolic acid-base compensation. Furthermore, the existence of similar changes following peripheral chemoreceptor denervation indicates that the hypercapnic ventilatory response originates at the level of the central chemoreceptors located in the brain.

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