THE INITIATION OF AND RECOVERY FROM DIVING BRADYCARDIA IN THE MUSKRAT

by

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B.Sc., University of British Columbia, 1967

M.Sc., University of British Columbia, 1970

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

ZOOLOGY

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1980

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ABSTRACT

Heart rate was found to be significantly lower in unrestrained diving muskrats than in those which were forced to dive. The response in the unrestrained animal represents a heart rate of about 9% of the resting rate and is similar to the cardiac responses recorded in freely diving pinnipeds. Apnea and bradycardia were initiated by water lapping the nares of the conscious animal. Anaesthesia abolished this narial reflex to submersion. In anaesthetized muskrats water was drawn into the nasal cavity causing transient apnea and prominent bradycardia by stimulating receptors located principally in the glottal and pharyngeal areas. Nerve blockade by reversible cooling and section demonstrated that these nasal receptors are innervated by the maxillary and inferior laryngeal nerves. In the conscious animal trigeminal neurotomy failed to affect the course of the response confirming that the muskrat has a number of external sensory mechanisms capable of initiating the diving reflexes.

Respiratory activity was shown to have a marked effect on heart rate when the muskrat was at rest and when water was passed through the nares. Cardioacceleration during nasal stimulation resulted from a central component and from neural input originating in fast adapting pulmonary receptors. Artificial ventilation not only increased heart rate but often tended to restore normal respiratory activity. Pulmonary deafferentation by steaming eliminated the Hering-Breuer reflex to maintained lung inflation as well as the cardioacceleration seen in response to artificial ventilation during nasal stimulation. The loss of the Hering-Breuer reflex occurred first suggesting that different receptors are involved. Lung deflation *per se* caused a reflex bradycardia but it appears that this does not potentiate the

narial reflex since nasal bradycardia was not reduced when lung inflation was maintained. Central and peripheral components arising from respiratory activity have their greatest effect during the recovery period.

Elimination of the carotid bodies delayed but did not abolish chemoreceptor driven bradycardia demonstrating that these are the most chemosensitive units but not the only ones responding to changes in blood gas tensions. No role however, has been found for the arterial baroreceptors. The barostatic reflex brought on by drug induced hypertension was triggered at a lower pressure than that found in the seal but it appears that this pressure would not be exceeded in the muskrat if heart rate remained low during a dive.

It is concluded that the cardiac response to submersion in the muskrat results from at least three reflex arcs. These reflexes originate from the nares, the lungs and from peripheral chemoreceptors. Although the chemoreceptors act to maintain the prevailing diving responses, it is likely that the external narial reflex accounts for almost all of the cardiovascular adjustment brought about in normal foraging dives since these are usually of short duration. The chemoreflex could play a significant role in dives exceeding one minute by prompting the animal to resurface when oxygen stores are depleted.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. David R. Jones for his advice and generous assistance throughout this study. I also wish to thank Drs. William K. Milsom and Richard Brill for their helpful suggestions in the preparation of the text and especially to my wife Judith who has borne much of the burden for far too long.

Financial support was provided by Teaching Assistantships from the Department of Zoology and the University of British Columbia Graduate Fellowships.

General Introduction

The respiratory and cardiovascular events which occur during submersion of diving animals have attracted considerable attention for over a century. These adjustments are well known to play a major role in the ability of the animal to tolerate the prolonged periods of asphyxia which can accompany submersion. This defense against asphyxia is most prominent in large aquatic mammals and is manifested by apnea, a dramatic decrease in cardiac function and an extensive redistribution of blood flow.

Burne (1909) was the first to suggest that selective redistribution of blood flow to the heart and brain occurred during periods of asphyxia. He proposed that during asphyxia blood flow favoured tissues which are vulnerable to hypoxia and was reduced to those areas which can be supported by anaerobic metabolism. Later it was found that many divers repay only a portion of the oxygen debt which would be incurred if resting metabolism was maintained during diving (Scholander et al., 1940; Andersen, 1961). In fact, metabolic rate declines during diving in birds (Pickwell and Douglas, 1968), reptiles (Andersen, 1961) and even in fish exposed to air (Leivestad et al., 1957).

Scholander et al. (1942a, b) were the first to establish that anaerobic glycolysis takes place in tissues which are deprived of circulation during diving. More recent work has revealed that energy in prolonged dives may be derived from carbohydrate and amino acid stores as well as glycolytic pathways (Hochachka et al., 1973). While there is little doubt that these biochemical processes play a role in the defense against asphyxia, it is difficult to assess their contributions in many animals.

However, in at least one species, the sea turtle which has been described as a "facultative anaerobe", the biochemical adaptations seem to be the most important in the defense against asphyxia (White and Ross, 1966; Hochachka and Storey, 1975).

While the mechanisms of the diving reflexes have not been revealed until recent years, it is now clear that their initiation, at least in mammals, is primarily of nasal origin (Andersen, 1966; Angell James and Daly, 1972a; Daly and Angell James, 1975). This recent work in fact confirms in principle the work of Beau (1860) who first suggested that the responses were, in part, reflexly initiated by impulses carried in the trigeminal nerve. His view was later disputed by Bert (1870) who believed that the cardiovascular adjustments resulted from voluntary breath holding. At the same time Kratchmer (1870) found electrical stimulation of the trigeminal root to be ineffective in evoking cardio-respiratory changes in both cats and dogs. Later however, his observations were questioned by François-Franck (1889) who was able to generate apneic and bradycardic responses from stimulation of the same nerve; results which were ultimately confirmed by Richet (1899) and Brodie and Russell (1900). Richet was also able to show that the efferent pathway of the cardio-depressant response in the duck was carried in the vagus nerve. His work in particular established the importance of nasal water contact in the initiation of the cardio-respiratory adjustments which are now known as the diving reflexes.

A number of important elements of the diving reflexes were explored in 1913. In that year Huxley (1913) demonstrated the involvement of a postural component thought to be associated with the diving responses in the duck. She was able to show in decerebrated animals that both apneic and cardiac

responses could be evoked by ventriflexing the head; a reflex that is able to function independently of higher centre control. In the same year this postural reflex was described as part of labyrinthine function and the afferent pathway shown to be carried by the cervical nerves (Patton, 1913). This was followed by a controversial piece of work in the duck concerning the effect of carbon dioxide on respiration (Orr and Watson, 1913). The paper was particularly significant because in spite of their erroneous conclusion that respiration is inhibited by carbon dioxide, recognition was given to both the roles of external and humoral drives during periods of asphyxia. This was later to have a profound influence on studies related to the diving condition. The role of the trigeminal reflex in diving was also studied in the same year. Experimenting with ducks, Lombroso (1913) demonstrated the presence of bradycardia when lung inflation was maintained and confirmed the earlier view held by Beau and Richet.

Studies on the initiation of the diving reflex were not resumed until Vincent and Cameron (1920) and later Koppanyi and Dooley (1928) concluded that submersion apnea in the duck was caused by wetting the beak, nares and mucous membranes of the nasal cavity. In a subsequent paper, Koppanyi and Dooley (1929) suggested that a postural component was also involved in the diving reflex of the muskrat. Much later, Andersen (1963a,c) and Feigl and Folkow (1963) confirmed that the reflex in the duck was initiated by specific receptors in the nares and that the subsequent bradycardia was augmented by hypoxia and hypercapnia. Andersen found that bradycardia and apnea were unaffected by decerebration but were abolished by division of the ophthalmic and mandibular branches of the trigeminal nerve. He concluded that the former branch was the major afferent nervous pathway of the

cardiac response and was triggered by water immersion per se.

In recent years, attention has been focused on a variety of factors which contribute to underwater endurance, particularly in aquatic mammals. Behavioural adaptations to diving have a marked influence on the responses in many diving animals. Birds and mammals which are accustomed to aquatic environments tend to relax when they are forcibly submerged (Irving, 1939; Andersen, 1966) yet still exhibit striking responses whether they struggle or not. Irving et al. (1941b) frequently observed that the diving ability of the seal was not fixed but in fact was labile and depended on a "steady disposition". Conditioning also seems to play a role in the expression of cardiovascular responses to diving. In experiments on human subjects, training has been shown to improve the diving reflexes and the duration of breath holding during face immersion (Whayne and Killip, 1967; Corriol and Rohmer, 1968); a consequence probably related to the state of apprehension of the untrained group. In dolphins trained to dive on command, diving bradycardia is prominent, and stable but quite unlike the wildly fluctuating heart rate which characterizes spontaneous diving (Elsner et al., 1966b). Irving et al. (1941a) also noted that the dolphin responds poorly to forced submersion and is easily asphyxiated in short dives of this nature.

While modification by conditioning tends to improve the tolerance to asphyxia, the diving reflex seems to be fully developed at birth and in some cases has been shown to decrease with age. In the elephant seal, bradycardia resulting from forced submersions is more pronounced in the newborn calf than in the adult (Hammond et al., 1969). In spite of this, the newborn calf appears incapable of dives exceeding two minutes. This, however, may be due to the lower blood oxygen capacity of the young animal

and a far greater brain to body weight ratio. In contrast to the elephant seal, the smaller pinnipeds tend to increase the intensity of the reflex to submersion as they mature (Harrison and Tomlinson, 1960) suggesting that experience improves diving performance in some species.

Fetal and hibernating animals respond in much the same manner as the diving mammals to hypoxic stress (Scholander, 1960). Bradycardia often occurs during delivery and is invariably accompanied by a post natal rise in blood lactate (James, 1962) similar to the changes which occur in non-circulated vascular beds following submersion in the diving animal (Scholander, 1940; Scholander et al., 1942a; Clausen and Ersland, 1970/71). Selective redistribution of blood which favours the heart and brain has also been observed in the fetal lamb during maternal hypoxia (Parker and Purves, 1967) although flow to the fetus is maintained (Elsner et al., 1969). In other studies on seals, it has been found that the onset of and recovery from asphyxia induced bradycardia in the fetus lags well behind that of the mother (Elsner et al., 1969).

The maintenance of apnea in utero has raised the question about whether the fetal and diving reflexes are similar. Tchobroutsky et al. (1969) noted that fetal apnea was related to an "inhibitory factor" (Barcroft, 1946) and the absence of external stimuli (Reynolds, 1962) and suggested that initiation of respiration at birth may result from their withdrawal. Respiratory airway fluid, known to be present in fetal development, is thought to be the inhibitory factor causing apnea. The onset of hibernation in certain animals is of particular interest because of the appearance of bradycardia before a decrease in body temperature sets in, indicating that its cause might be a primary vasoconstriction (Scholander, 1963). Arousal from the

hibernating state also seems to be related to a primary vasomotor response resulting in an increase in blood flow to the limbs and an elevation in metabolic rate (Eliassen, 1960b).

From an evolutionary sense, these reflexes which characterize such a broad range of physiological behaviour have given rise to speculation that they form part of a "general adaptation syndrome" common to all animals (Selye, 1949). Thus as in the neonate and hibernator, the adjustments to diving in the aquatic mammal do not imply a unique reflex but rather suggest them to be a refinement of a stereotypic reflex. Poikilotherms such as snakes (Johansen, 1959), turtles (White and Ross, 1966) and alligators (Andersen, 1961) display marked responses to submersion even though they appear much more slowly than in mammals. The reflexes have also been observed in anurans (Jones, 1966; West and Jones, 1976) in spite of their ability to provide metabolic needs through cutaneous respiration, and in fish exposed to hypoxia (Satchell, 1961) or removed from water (Leivestad et al., 1957).

Homeotherms are vulnerable to even short periods of asphyxia and not surprisingly possess reflexes which are far more dramatic than those found in the cold blooded species. In the harbour seal, for example, cardiac responses occur before submersion and underwater endurance is 4 to 5 times greater than that predicted from normal body oxygen stores if surface levels of metabolism are maintained (Irving et al., 1941b) and some of the larger pinnipeds are able to remain submerged for periods up to one hour (Kooyman and Andersen, 1969). The ability of the animals to withstand lengthy periods of asphyxia yet remain active during submersion has been shown to depend largely on anatomical and physiological features (Scholander, 1963; Elsner et al., 1966a; Elsner, 1969; Yonce and Folkow, 1970; Daly, 1972).

Anatomical adaptations for diving, especially those in aquatic mammals, have been recognized for over a century (Hunter, 1787; Burow, 1838). Modification of the respiratory organs in these divers however, is often misleading since some features are related to relief from the effects of pressure rather than to conservation of oxygen (Harrison and Kooyman, 1971; Hempleman and Lockwood, 1978). As a rule large tidal volumes relative to body weight and a slow breathing frequency are associated with the better divers (Kooyman, 1973). While this allows a higher degree of oxygen utilization during normal respiration, evidently none of the shallow diving animals takes advantage of this potential store. Shallow divers have been reported to exhale on submersion (Kooyman and Andersen, 1969) but this may not always be the case. Ducks (Andersen, 1966), muskrats (Errington, 1963) and seals (Kooyman et al., 1971) have been observed to release air during shallow dives. In contrast to pinnipeds, the deep diving cetaceans have relatively small lung volumes and diving often takes place with the lungs fully inflated (Kooyman and Andersen, 1969).

Because of the reliance of aquatic birds and mammals on available oxygen stores during diving, blood characteristics of these divers have received special attention. Richet (1899) was the first to note the significance of large blood volumes in ducks accustomed to prolonged dives. In some, such as the diving guillemot, the blood volume is nearly double that in similar terrestrial species (Irving, 1939). Cetaceans and pinnipeds in particular, possess large venous reservoirs which are highly oxygenated and provide sizeable oxygen reserves which can be drawn upon in asphyxic periods. Oxygen binding capacity and the Bohr factor have been considered

as likely adaptations to hypoxic environments since both ensure efficient utilization of hemoglobin bound oxygen. The oxygen binding capacity of the blood in many of these animals is unusually high and similar to that found in burrowing and high altitude mammals (Bartels, 1964; Hall et al., 1936) but oddly there seems to be little relationship between this adaptation and diving ability. While the oxygen capacity of seal blood is approximately 45% higher than in man, the bloods of the beaver, sea lion and some whales have been found to be no different from man (Irving, 1939). Similarly, the blood of many aquatic mammals does not have a large Bohr factor (Clausen and Ersland, 1968). Muscle tissue may also be a valuable source of oxygen during times of hypoxic stress. Robinson (1939) has estimated that oxygen in the form of oxymyoglobin accounts for 47% of the store available to the harbor seal during diving.

It has been claimed for many years that chemoreceptors in diving animals are less sensitive to asphyxia than those in terrestrial species. Apneic Weddell seals for example, have been shown to tolerate a PaO_2 of 8 mm Hg simultaneously with a $PaCO_2$ of 80 mm Hg without starting breathing (Elsner et al., 1970). In the hooded seal, voluntary breath holding can be maintained until PaO_2 is 14-16 mm Hg before rebreathing begins. Respiratory insensitivity has also been noted in the duck (Orr and Watson, 1913), the beaver (Irving, 1938) and a number of reptiles (Dill and Edwards, 1931; Johansen, 1959) suggesting that at least in aquatic animals, the withdrawal of chemoreceptor drive tends to promote diving responses. Andersen (1966) however, stated that on the basis of two studies in the seal (Robin et al., 1963) and duck (Andersen and Løvø, 1964) that respiratory drive was likely to be increased over the range of carbon dioxide tensions experienced in

diving and indirectly raised questions concerning the mechanisms of chemoreceptor integration with the primary responses occurring early in the dive. Like hypercapnia, hypoxia in the absence of other factors stimulates respiration (Andersen, 1959; Cherniak et al., 1970/71). Both Scholander (1940) and Elsner et al. (1970) have suggested that the tolerance of seals to hypoxia may be less than to carbon dioxide since the animals show signs of distress after breathing nitrogen for only a short time. Evidently the train of oxygen sparing adjustments is not initiated by ventilation hypoxia.

It is now generally recognized that at least two inputs contribute to the cardiovascular and respiratory reflexes associated with diving (Angell James and Daly, 1975) but only recently have their interactions been examined. A trigeminal reflex, identified by Andersen (1963c) is considered to initiate the necessary adjustments to submersion before any marked changes occur in blood gas tensions. A chemoreflex on the other hand, resulting largely from hypoxic and hypercapnic stimulation of the carotid bodies, is thought to intensify the primary reflexes in prolonged dives (Angell James and Daly, 1975). Thus it is clear that a finely set central mechanism is required to integrate the afferent inputs to evoke the appropriate cardiovascular and respiratory adjustments during diving.

In view of the interdependence of the cardiovascular and respiratory centres it is not surprising that a good deal of controversy still surrounds their respective roles in the control of the diving reflexes. In fact, it is the complexity of these central mechanisms which has often led to the confusion of cause and effect (Anrep et al., 1936a). Thus through the work of Bainbridge (1920) and Hering (1933) there persisted a belief that respiratory movement accompanied cardiac arrhythmia but was not its cause.

Historically, many workers believed that the respiratory influence on heart rate was a purely central phenomenon independent of peripheral factors (Traube, 1865; Snyder, 1915; Heymans, 1929). This uncertainty however, led Anrep et al. (1936a, b) to a series of classic experiments which clearly demonstrated that in dogs, sinus arrhythmia has both central and peripheral origins.

The conclusions of Anrep et al. proved to be significant in the light of the work of Daly and Scott (1958, 1962) in which they showed that primary cardiovascular reflexes evoked by carotid body stimulation could be greatly altered by lung ventilation. Increases in ventilation of spontaneously breathing animals are known to overrule bradycardia and vasoconstriction caused by apneic asphyxia: both responses being regarded as primary reflexes in the non-ventilated animal (Bernthal, 1938; Bernthal et al., 1951; Daly and Daly, 1959; Daly and Hazeldine, 1963). Thus it is clear that mechanical stimulation of lungs may evoke concomitant changes in central respiratory activity which tend to reverse the primary consequences of chemoreceptor stimulation. It is apparent from these findings that the state of respiratory activity determines both the nature and the degree of cardiovascular responses to asphyxia.

Recently considerable attention has been devoted to the interaction of the reflexes during conditions of acute stress such as hemorrhage, ventilation hypoxia and apneic asphyxia (Andersen, 1966; Daly, 1972; Daly and Angell James, 1975): the latter condition occurring most commonly during submersion. Much of the attention however, has been focused on the influence of chemoreceptor drive (Feigl and Folkow, 1963; Kawakami et al., 1967; Corriol and Rohmer, 1968; Daly and Angell James, 1975) and little credit has been given to the immediate effects of reflex apnea. It is now

clear that apnea *per se* initiated by water contact not only serves as a protective reflex by preventing the inhalation of water, but also allows the expression of the essential cardiovascular adjustments. In prolonged diving however, the primary chemoreceptor influence on respiration has been questioned since it fails to produce respiratory breakthrough as predicted from preliminary studies (Daly and Ungar, 1966). Attempting to resolve this paradox, Angell James and Daly (1972b) and Daly *et al*. (1977) have shown that the chemoreceptors respond differently during face submersion than during purely asphyxic conditions. When trigeminal and carotid body stimulation were applied together apnea was provoked in freely breathing dogs which persisted much longer than when nasal stimulation was given alone. The bradycardia and vasomotor responses were also found to increase in intensity. These results indicate that the trigeminal reflex not only facilitates the cardiovascular responses occurring during asphyxia but also tends to reverse the primary stimulating effect of chemoreceptors on respiration and delays premature breakthrough. Thus if this conclusion is extended to diving birds and mammals, it appears to resolve the paradox noted previously by Andersen (1966) that respiratory drive is increased by asphyxia in the duck and seal but not during asphyxia combined with nasal stimulation.

Lung collapse during submersion has led to speculation that it might be a requisite for optimum cardiovascular adjustment during diving (Andersen, 1966; Yonce and Folkow, 1970; Angell James and Daly, 1969b). Some avian divers when failing to exhale before submersion, develop bradycardia and possibly other responses only slowly (Andersen, 1963a; Eliassen *et al*., 1960a) which suggests that pulmonary receptors initiate increases in heart rate and tend to normalize blood flow following submersion. It has been

postulated for some time that the cardio-inhibitory centre is under the influence of two inputs from the lungs, one causing tachycardia during inspiration and the other bradycardia on expiration by way of the respiratory centre (Cordier and Heymans, 1935). Thus aside from static conditions, lung receptors responding to expansion and collapse of the lung are seen to have a dual capacity in stimulating and inhibiting the cardiovascular centre. Many animals display rhythmic bursts of cardiac activity in the first few breaths on emersion (Irving et al., 1941b; Johansen, 1959; Andersen, 1963a, b; Tchobroutsky et al., 1969); a reflex too rapid to be associated with a change in chemoreceptor activity. The afferent arm of the cardiac response is considered to originate in the nervous plexus of the bronchioles since the reflex is abolished by steam inhalation (Hainsworth et al., 1972). It would seem that lung inflation has its greatest effect at the onset of breathing after a dive not only because of the pronounced respiratory activity but also because of the depressed cardiovascular activity.

In summary, it appears that while the trigeminal and chemoreceptor reflexes have been well documented, the involvement of static and phasic lung inflations in the diving reflexes has been largely speculative. It has been claimed that the nasal and chemoreceptor reflexes interact to enhance the cardiovascular adjustments to diving (Angell James and Daly, 1973; Strømme and Blix, 1976; Elsner et al., 1977), the modulation of which rests with the occurrence of respiratory activity (Angell James and Daly, 1978). The role of arterial baroreceptors in the diving responses remains controversial. In birds, selective denervation of aortic baroreceptors has no significant effect on diving bradycardia (Jones, 1973; Jones and West, 1978), yet others have claimed that diving bradycardia results from the barostatic reflex in response to chemoreceptor induced peripheral vasoconstriction (Andersen and

Blix, 1974; Blix et al., 1974; Blix, 1975; Blix et al., 1975). Baroreceptors appear to contribute to the bradycardia caused by nasal stimulation in the rabbit (White and McRitchie, 1973); in other mammals their role has not been fully elucidated (Angell James et al., 1978).

The purpose of the present investigation was to examine the cardiodepressant and cardiac stimulating pathways in an accomplished diver, the muskrat (Ondatra zibethica osoyoosensis), to establish which are important in the initiation and maintenance of submersion bradycardia. Muskrats are easily obtained and managed in the laboratory and diving bradycardia appears to be unaffected by anaesthesia. The study was also undertaken in the hope that it would prove useful in elucidating central neural integration of the reflexes bearing on cardiac function.

Methods and Materials

Experiments in this study were carried out on 162 adult and subadult muskrats of both sexes varying in weight from 0.61 to 1.35 kg. The animals were trapped either in the sloughs and ditches of the Fraser River delta or in the marshlands in the vicinity of Pitt Meadows, B. C. Although experimentation was carried on throughout the year, virtually all the animals were trapped in the winter months (November through February) as trapping was not usually successful in the spring and summer. The muskrats were held in pens, usually in pairs, for up to 8 months and were sustained on a diet of high protein rabbit "chow" (Purina) supplemented with lettuce and carrots. The rabbit chow was initially medicated with 0.03% sulfaquinoxaline to prevent suspected hepatic coccidiosis but the drug was found to be detrimental since a number of muskrats succumbed to Tyzzer's disease (Chalmers and McNeill, 1977), usually between the first and second week of captivity. Each holding pen (3 x 1 x 1.5 m) was constructed of steel and contained an enclosed platform and a wooden nesting box. Water under the platform was kept at a depth of 0.5 m with a continuous flow.

Operative Procedures and Recording Techniques

All operative procedures were performed under urethane (950-1350 mg/kg) or nembutal (60 mg/kg) anaesthesia administered by intraperitoneal injection. Prior to injection the muskrats were sedated with ethyl ether vaporized in a closed jar; the procedure taking approximately 3 minutes. A number of experiments were carried out on animals paralyzed by the injection of curare (Tubocurarine, 2 mg/kg i.p.) and in some cases the areas of incision were infiltrated with local anaesthetic (Xylocaine, 2%). During all surgical procedures body temperature was maintained at 37.5±0.5^{o}C with an overhead lamp.

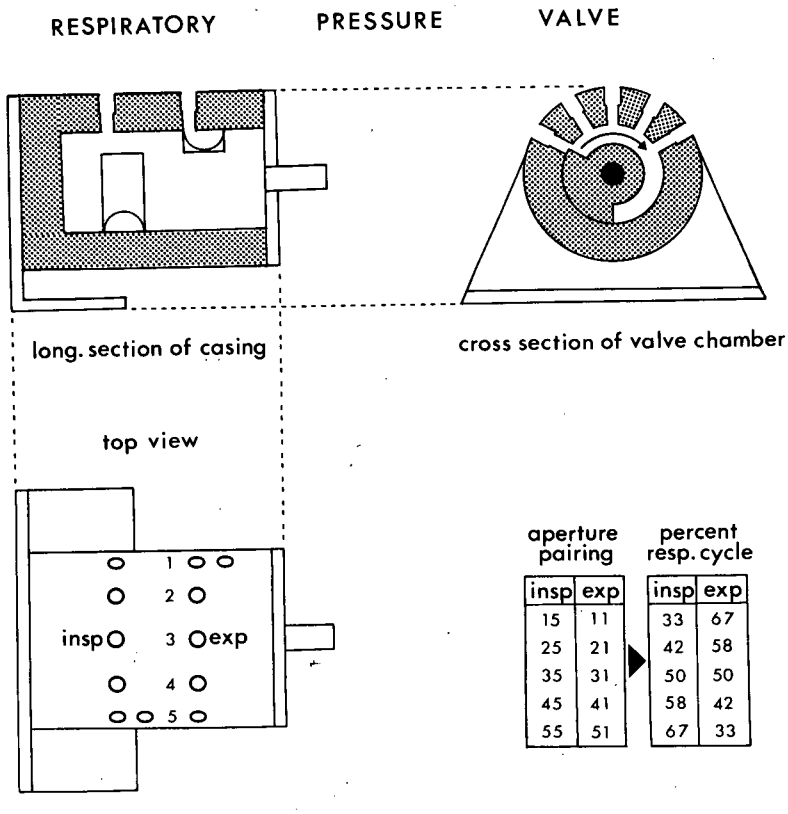
In preparation for recording electrocardiograms in unrestrained diving, the muskrats were first anaesthetized with nembutal and a siliconed baseplate (1 x 1.5 cm) bearing female microconnector plugs was placed subcutaneously between the clavicles. Bipolar electrocardiographic electrodes (formex coated copper wire, 0.5 mm diameter) were led from the microconnector plugs, beneath the skin and sewn in place at the chest and the base of the tail. The animals were allowed to recover overnight before experimentation. For restrained diving, the muskrats were sedated with ether and then fitted with surface chest and thigh copper wire electrodes. Submersion of the animal was done after recovery from anaesthesia and was made in an open topped perspex box (8 x 10 x 28 cm) which was perforated to allow rapid entry of water. The holding box was mounted above a larger perspex box containing water which could be raised quickly and locked at the desired level. Anaesthetized muskrats were also dived under the same conditions was described for restrained diving above.

Breathing was monitored by one of three techniques: pneumotachograph recordings from an attachment to a tracheal cannula, impedance changes across the chest or by thermistor recordings of expired and inspired air. A tygon cannula (2.5 mm o.d.) was inserted through a tracheal incision 1.0-1.5 cm above the bronchial bifurcation. The pneumotachograph output connections were made to a differential transducer (Hewlett Packard, type 268 B) and the signal amplified by a LVDT coupler (Beckman). In some cases the signal obtained from the coupler was passed through an integrating preamplifier (Hewlett Packard 3700A) to give tidal volume. The pneumotachograph was of a minimum size so that it added little to the volume of the dead space. Impedance changes were taken from a Harvard impedance pneumograph (Model 391). For thermistor recordings, a bead thermistor was

mounted either in the tracheal cannula or in the nasal cavity through a small hole bored in the nasal bone. The leads from the thermistor were connected to a standard bridge circuit. All signals were amplified by conventional means and displayed on a storage oscilloscope (Tektronix 564 B) and either on a Harvard 4 channel (Model 486) or a Beckman 2 channel (RS type) pen recorder.

Artificial ventilation was regulated using a small positive pressure valve (Fig. 1). The valve consisted of a two chambered teflon barrel (2.5 x 5.0 cm) and a brass casing (4.4 x 6.0 cm) which allowed the passage of air to and from a Y piece fixed to the tracheal cannula. Five equally spaced holes (30^{o} apart) and two offset holes were drilled in the casing and occupied 120^{o} of the perimeter so that the inspiratory and expiratory duration times could be varied between 33 and 67% of the respiratory cycle. The barrel was grooved to correspond with the two sets of holes in the casing and each groove was milled through 240^{o} of the barrel perimeter so that the two were offset by 120^{o} in the cross-sectional axis. External connections were made with polyvinyl tubing (4 mm i.d.) to the two valve chambers (expiration and inspiration) and the remaining holes were stoppered. Expiration was either passive (normal) or induced (aspiration). The valve and the shaft of the barrel were held in place by a brass cover fixed to the casing and were rotated by a variable speed motor attached by a flexible hose. Gas flow supplied to the inspiratory chamber was regulated by having the gas overflow from a side arm of the inlet tube. The side arm was placed so that the opening was at various depths in the water filled cylinder and a balloon was attached on the inlet side of the valve to lessen pressure fluctuations caused by valve closure.

Figure 1. Diagram of the respiratory valve. Refer to text for description.



aperture pairing		percent resp. cycle	
insp	exp	insp	exp
15	11	33	67
25	21	42	58
35	31	50	50
45	41	58	42
55	51	67	33

The ventilation valve possessed a number of advantages in that it could be placed some distance from the animal as well as allowing quick and easy adjustment of the ventilation rate, tidal volume and inspiratory-expiratory ratio. In many experiments inflation and deflation of the lungs was achieved by stopping the valve in the inspiratory or expiratory position respectively. A more suitable technique however, was to simply clamp either the inspiratory or expiratory tubes leading to the valve. In experiments involving the determination of the Hering-Breuer reflex to constant inflation, the latter was the only method used.

Prior to experiments involving cranial nerve section, the animals were anaesthetized with urethane and a dental burr was used to remove a portion (1 x 1.5 cm) of the parietal bone. Normally the saggital sinus was ligated, the dura reflected and the cerebral hemispheres aspirated with a curved pipet. Occasionally the frontal bone was pared to the floor of the cranial cavity to expose the maxillary nerve trunk and its branches. Bleeding was controlled by cauterization. The olfactory nerve, facial and petrosal nerves, mandibular and maxillary division of the trigeminal nerve were exposed intracranially after decerebration. The inferior and superior laryngeal, glossopharyngeal, sinus, vagus and phrenic nerves were approached ventrally through an incision running from the lower jaw to the thorax. Nerve blockade was accomplished either by section or by cooling in a specially designed thermode similar to that described by Douglas and Malcolm (1955). The thermode consisted of an insulated silver probe (7 x 4 x 1-3 cm) slotted to accommodate a nerve, and a supporting handle through which cooling fluid could be circulated. The device was cooled by drawing either ice water or dry ice cooled acetone through to an evacuated flask, and the temperature

controlled by varying the flow rate. Thermode temperature was normally held at 5-6^{o}C until nerve blockade was complete as judged by the response to water flow stimulation of the nasal passages.

Nerve recordings were made with conventional silver wire electrodes. The signals were amplified (Tektronix amplifier, type FM 122) and displayed on a storage oscilloscope (Tektronix, 564 B). The output was, in turn, connected in parallel with a two track tape recorder (Tandberg, model 64) and a window discriminator (F. Haer, No. 40-75-1). The signals from the latter were relayed to an audio amplifier and speaker. Taped nerve discharge was passed through a ratemeter (EKEG, model RT 682) and recorded on a Brush 220 pen writer (Gould). Electrodes similar to that described for recording were also used for nerve stimulation. The electrical stimulus was supplied by a Grass S 6 stimulator coupled with an isolation unit (Grass model S1U5). Dry glottal stimulation was achieved by inflation of a small balloon attached to a polyethylene tube inserted through a tracheal incision.

Diving conditions were simulated by situating a second tracheal cannula (oral facing cannula) just anterior to the first and allowing water or saline to flow from a reservoir usually set one meter above the animal. The mouth was not occluded and permitted some fluid to escape during nasal stimulation.

Pulmonary deafferentation was carried out by forcing steam from a boiling flask through an insulated tube (1 cm o.d., 3 mm i.d.) and into a small nylon tube (1.5 mm i.d.) inserted through a hole midway along the tracheal cannula. A rigid plastic sleeve with a side opening was placed over the junction to prevent leakage during steaming. The sleeve was rotated to seal the opening when the steaming cannula was withdrawn. A three-way tap was inserted between the large and small steam lines to ensure that maximum

temperature of steam at the exit was reached before its application to the lungs. Steam temperature normally exceeded 98°C a few millimeters from the tip if this procedure was followed. Deafferentation was most successful when the steam was delivered by artificial ventilation at a pressure at 12 cm H_2O since it precluded the possibility of animals becoming apneic.

Arterial blood pressure and oxygen tension in the muskrats were measured by means of a cannula forming an arterial-arterial or arterial-venous loop, usually on the left side. The carotid artery was exposed by a mid-line incision in the neck and was cannulated with polyethylene tubing (PE 90) to make a connection with a pressure transducer (Harvard Apparatus, type 377) and a flow-through cuvette containing an oxygen electrode (Beckman, No. 315752). When the cuvette was in use the pressure transducer was connected to a side arm of the carotid loop. In some experiments, a carotid artery-jugular vein loop was used to improve the loop circuit time and when this was used the response time of the system depended on the nature of the membrane covering the electrode. Using a teflon membrane reduced the 90% response time of the electrode to 1.7 seconds. The electrode was calibrated with air or nitrogen equilibrated saline applied to the side arm of a T-piece on the upstream end of the cannula and removed through a second T-piece on the downstream loop. Calibration salines were flowed past the electrode from a pressure head which was adjusted to be close to that of the animal's blood pressure. The cuvette holding the electrode was enclosed in a water jacket which was maintained at 37°C by the flow of water from a reservoir containing a heater-stirrer unit.

Experimental Protocol

Muskrats instrumented to give EKGs in unrestrained dives were transferred the day after the operation to a 3 meter diameter wooden stave tank filled with water to a depth of one meter. Approximately 2/3 of the tank area was covered with wire mesh a few centimeters below the water line to encourage long diving periods. A nesting platform (0.5 x 0.5 m) was floated on the uncovered water and the animals were connected to a preamplifier with a thin insulated wire which was maneuvered by a light rod during diving. As a rule, two muskrats were held in the tank at the same time for the diving trials. Diving was prompted by a scare stimulus from the operator and the point of submersion was recorded by an operator activated event marker on the chart recorder. Successful recordings of the EKG were obtained only up to the sixth post-operative day.

For muskrats dived in the restraining box, submersion was carried out by quickly raising the lower water filled box until the animal was fully submerged. In these experiments the dive was generally terminated at 40 seconds although some animals were held under water for periods of up to 5 minutes without drowning. To ensure complete recovery, a period of at least 15 minutes was allowed between successive dives. A group of anaesthetized animals were also dived as described for restrained diving. This group however, was not able to tolerate submersions as well as when they were conscious and as a result only a few of these dives exceeded one minute.

Nasal stimulation was effected by passing water or saline through the oral facing cannula with the animal in the supine position. Normally a flow of 32 ml/min was generated when the reservoir was kept at a height of 1 m.

When required, the flow rate was reduced by lowering the pressure head. Prior to nasal stimulation, water was run to the end of the cannula and the tube was clamped so that maximum flow rates were achieved in less than a second when the clamp was removed. Following a period of stimulation (usually 20 seconds or less) air was forced through the cannula to remove residual fluid from the nasal passages. Failure to remove the water resulted in decreased responsiveness to subsequent stimulation. Water stimulation of the external narial region alone was carried out in curarized artificially ventilated muskrats. In each case the animal was held in the prone position and the nose drawn through a hole in a sheet of dental dam which formed one side of a small box (10 x 10 x 8 cm). Air was continuously blown through the oral facing cannula and exited through the nares to prevent the entry of water. The water level in the box was raised by pouring water from a beaker into the open top or by raising the water level from a reservoir and drained by removing a plug from the bottom.

The effect of decerebration and of sectioning or cooling the cranial nerves on the respiratory and cardiovascular responses to nasal stimulation was studied. The delimitation of the neural pathways for the responses was confirmed by recording from nerves innervating the areas which gave apneic and bradycardic responses to punctate stimulation and by electrically stimulating the central ends of these nerves. Nerve recordings of discharge in the phrenic, nasociliary and inferior laryngeal nerves were taken from the active cut ends of the nerves. The nerve ends were prepared for recording by removing the sheath and dividing the nerve body into small bundles. In all preparations the indifferent electrode was grounded to an underlying baseplate with a portion of extraneous nerve of the same diameter as that of

the active bundle. The baseplate was earthed, the recording area immersed in a pool of mineral oil and a ring of silicone sealant was applied to the area of incision to prevent the seepage of oil into the fur.

The effect of lung receptor input on the responses to nasal stimulation was studied in anaesthetized muskrats which were artificially ventilated over a range of pressures from 4 to 22 cm H_2O. Ventilation was maintained throughout periods of nasal stimulation with water and saline, before and after lung deafferentation. The effect of maintained inflation and deflation of the lungs on heart rate, blood pressure and PaO_2 were investigated in animals paralyzed with curare. Steam deafferentation was carried out in steps. Normally, two inhalations of steam were given in succession and the Hering-Breuer reflex and the cardiac response to ventilation during nasal stimulation were checked before further steaming. Generally 3-6 breaths of steam was sufficient for pulmonary deafferentation but in a few cases further steaming was necessary.

Baroreceptor stimulation was produced by the rise in blood pressure following intra-arterial injection of 5 µg/kg of adrenaline, while carotid body stimulation was achieved by injection of 80-200 µg/kg of potassium cyanide. The carotid sinus baroreceptors were denervated by section of the sinus nerve and was judged to be successful when the blood pressure rise following adrenaline failed to cause bradycardia. Denervation of the carotid body chemoreceptors was also carried out by sinus nerve section and was considered complete if injection of cyanide produced no increase in respiration.

The effects of baroreceptor and chemoreceptor denervation on the responses to nasal stimulation and maintained lung inflation and deflation

were investigated. In some experiments chemoreceptors were stimulated by artificial ventilation at a controlled frequency and tidal volume, with a gas mixture of nitrogen and 5% carbon dioxide. Blood oxygen was continuously recorded during this procedure. The cardiovascular responses to normocapnic anoxia were recorded before and after sinus nerve section. Heart rate and arterial oxygen tension were also monitored during nasal stimulation with and without maintained ventilation during asphyxia.

In the text and figures, the numerical values used in referring to determinations of variables in a group of animals, are given as means ± the standard error of the means (±S.E.M.). The standard student t-test was applied in a few cases to determine the statistical significance of the difference between groups. In such cases, 95% was considered the fiducial limit of confidence. In trials demonstrating a decrease in heart rate, bradycardia was recognized when the cardiac interval lengthened by more than 10% from the value in the control period.

Results

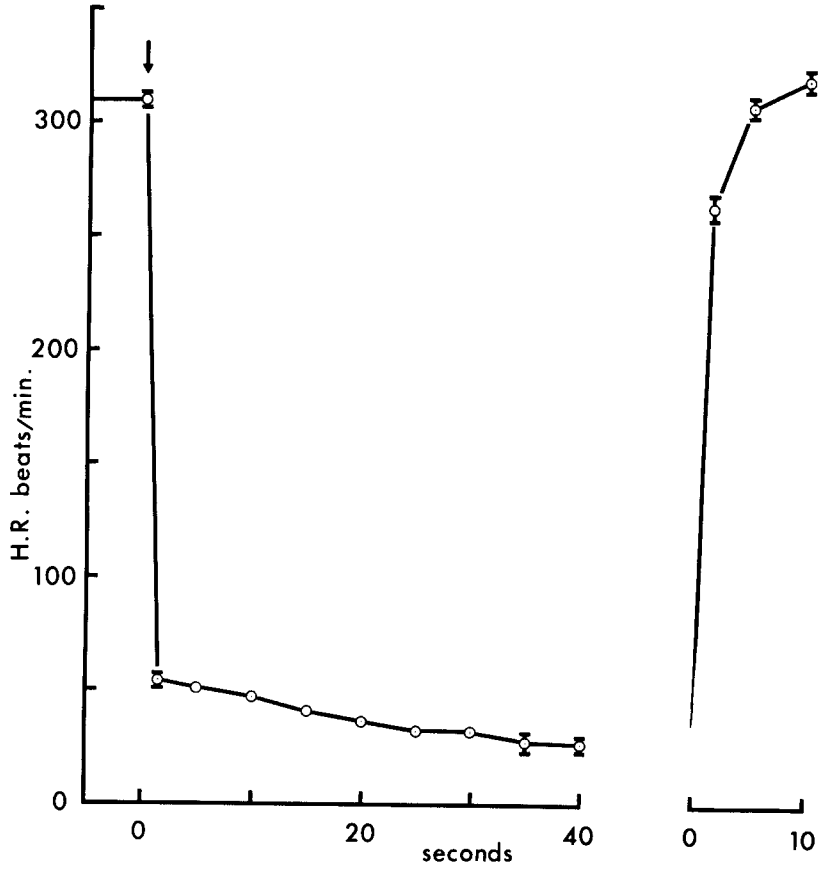
1. Respiratory and Cardiac Responses to Diving.

1.1. Cardiac Responses to Unrestrained Diving.

Under the conditions imposed by the dimensions of the diving tank and its covering wire set beneath the surface of the water, the muskrats displayed two distinct types of diving behaviour to a scare stimulus. In approximately one half the dives, the animals resurfaced immediately after crossing the "open" water which was approximately 1/3 the tank area. In such cases (n = 50) the dives never exceeded 5 seconds in length. The second type of dive was characterized by the muskrats swimming directly under the wire netting and resurfacing after a rest period which varied from 6 to 63 seconds. While it was difficult to determine if the animals dived after inspiration, it was obvious that they continued to expel air during the course of the dive. This was also noted in muskrats which dived voluntarily for periods of up to 10.5 minutes without recording attachments. In all cases the animals were allowed to resume normal behaviour (grooming, feeding, etc.) before a second scare stimulus was given.

The EKG of the muskrats during resting periods and moderate activity was regular and displayed no sinus arrhythmia. On average, heart rate fell from 310±3 to 54±3 beats/min (n = 102) in the first measurement following submersion (1-2 seconds) and declined to 27±3 beats/min (n = 3) at 40 seconds (Fig. 2). Due to the unrestricted nature of the trials the mean duration of dives exceeding 5 seconds (long dives) was 17.5±4.1 seconds and thus the time course of heart rate represents a decreasing number of dives. Recovery of

Figure 2. Mean heart rate during unrestrained dives exceeding 5 seconds. Each point represents the mean rate for all dives (n = 102 at time 0, n = 3 at 40 seconds) in five muskrats. Standard error of the mean is given for S.E.M. greater than 4.


H.R. beats/min.
300
200
100
0
0
20
40
seconds
0
10

heart rate on resurfacing was marked by a short period sinus arrhythmia and normal rates were reached after 5 to 10 seconds. Post-diving tachycardia was not observed in any of the trials.

Evidence was obtained from one muskrat that not only did the cardiac response precede submersion but also that the degree of response was related to dive duration but because of electrical interference caused by swimming these observations could not be confirmed in the other muskrats. In transitory dives of 4 seconds or less the first cardiac interval after submersion was the longest, averaging 0.82±0.03 seconds (n = 50) while in those dives 5 seconds or longer the first cardiac interval was nearly doubled to 1.56±0.08 seconds (n = 37, Fig. 3). The same relationship was evident for the remaining cardiac intervals up to 4 seconds for the shorter dives and in each case the groups were significantly different at the 99% confidence limit. In dives which were 5 seconds or longer there was no correlation between the initial cardiac response and the time underwater.

The mean time periods by which bradycardia preceded submersion were 0.30±0.02 and 0.43±0.02 seconds for the short and long dives respectively. These intervals were measured from the time of the first appearance of bradycardia to the event marker activated at the point when the head touched the water. The animals however, did not show a reliable anticipatory response to resurfacing and when this response was present, it was not pronounced (Fig. 4).

When the muskrats were stimulated to rapid successive dives, the intervening recovery of heart rate was slight and the initial cardiac interval of the second dive was increased. Although heart rate in the resting animal was usually stable, in many instances the muskrats displayed

Figure 3. Duration of the first four cardiac intervals of transitory (stippled) and long dives (unstippled) ±S.E.M. Unlike the long dives (6-63 seconds), the muskrat did not swim under the covering wire in the transitory dives (2-4 seconds). Trials were on one muskrat.

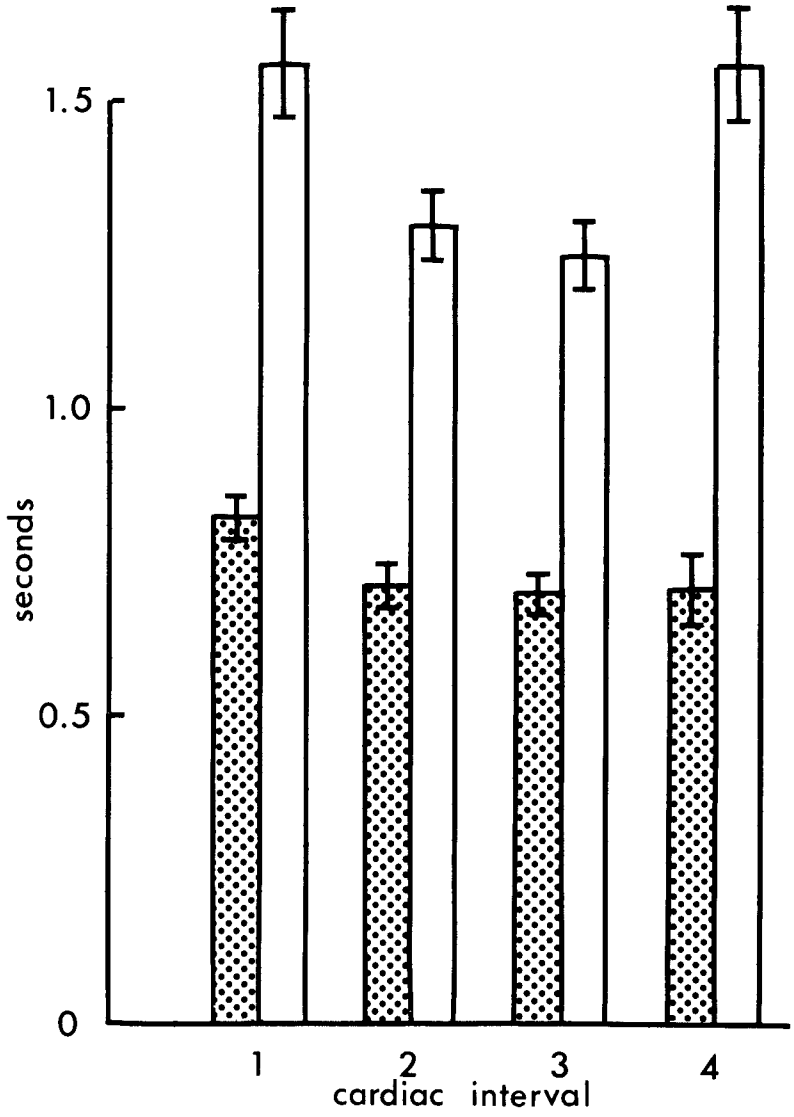
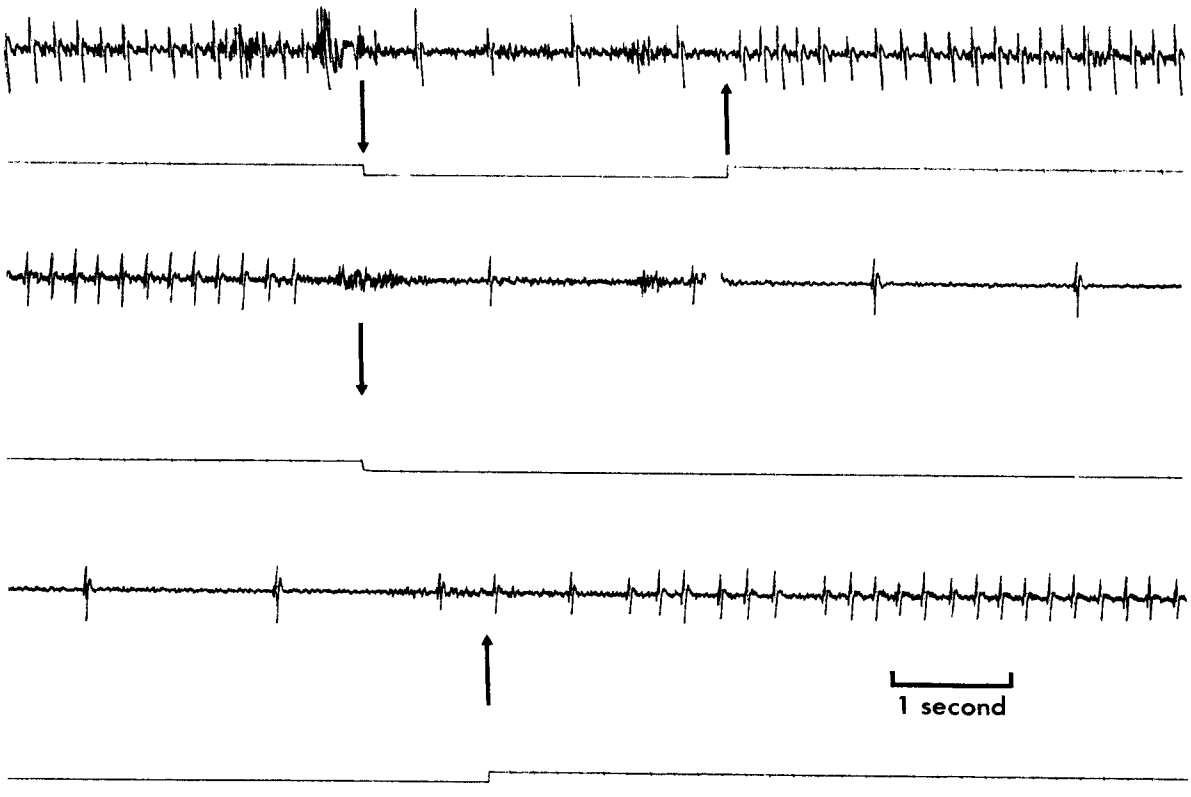

1.5
1.0
0.5
0
seconds
1
2
3
4
cardiac interval

Figure 4. Electrocardiogram during unrestrained diving. Top trace, during a transitory dive (duration 2.5 seconds); bottom traces, continuous recording during an 11 second dive. In the latter dive the muskrat swam without pause beneath the covering wire (see text). Note that the electromyocardial artifact on both traces signifies the movement preceding the point of submersion at downward pointed arrows. Resurfacing is indicated by the upward pointing arrows. Time marker, 1 second.


1 second

transient bradycardia when disturbed, most notably in response to tactile, noise and visual stimuli (Fig. 9).

1.2 Cardiac Response to Restrained Diving

The mean resting heart rate of 11 restrained muskrats was significantly lower (266±3 beat/min, n = 66) than those in the "natural" environment (310±3 beat/min, n = 102). During restrained dives, nostril closure and apnea occurred immediately on submersion and bradycardia followed after a latent period of 300±10 msec (n = 60); with heart rate falling to 78±4 beat/min within 1 to 2 seconds (Fig. 5). A rate of 51±2 beat/min was reached after 5 seconds and thereafter heart rate remained relatively stable until the animals were resurfaced.

Heart rate increased rapidly on emersion, reaching the pre-dive rate after 10 seconds. The increases were always associated with the recovery of normal respiration; heart rate rose markedly during the inspiratory phase. As in unrestrained diving, heart rate seemed unaffected by movement or struggling (Fig. 6, top) and no tachycardia was evident in the recovery period. Neither the latent period to the onset of bradycardia nor the time course of the response were changed when the unanaesthetized animals were forced dived into saline (0.9%) or when the water temperature was varied between 4 and 38^{o}C.

1.3 Respiratory and Cardiac Responses to Diving During Anaesthesia

Both the resting heart rate and the cardiac response to diving while the muskrat was anaesthetized (urethane, 1250 mg/kg, i.p.) were similar to those trials on the restrained animals (Fig. 7). In 9 experimental animals, mean

Figure 5. Mean heart rate during restrained diving. Each point represents the mean response in 11 muskrats (n = 60) for dives terminated at 40 seconds. The arrows indicate the points of submersion and resurfacing. Standard error of the mean is given for S.E.M. greater than 4.

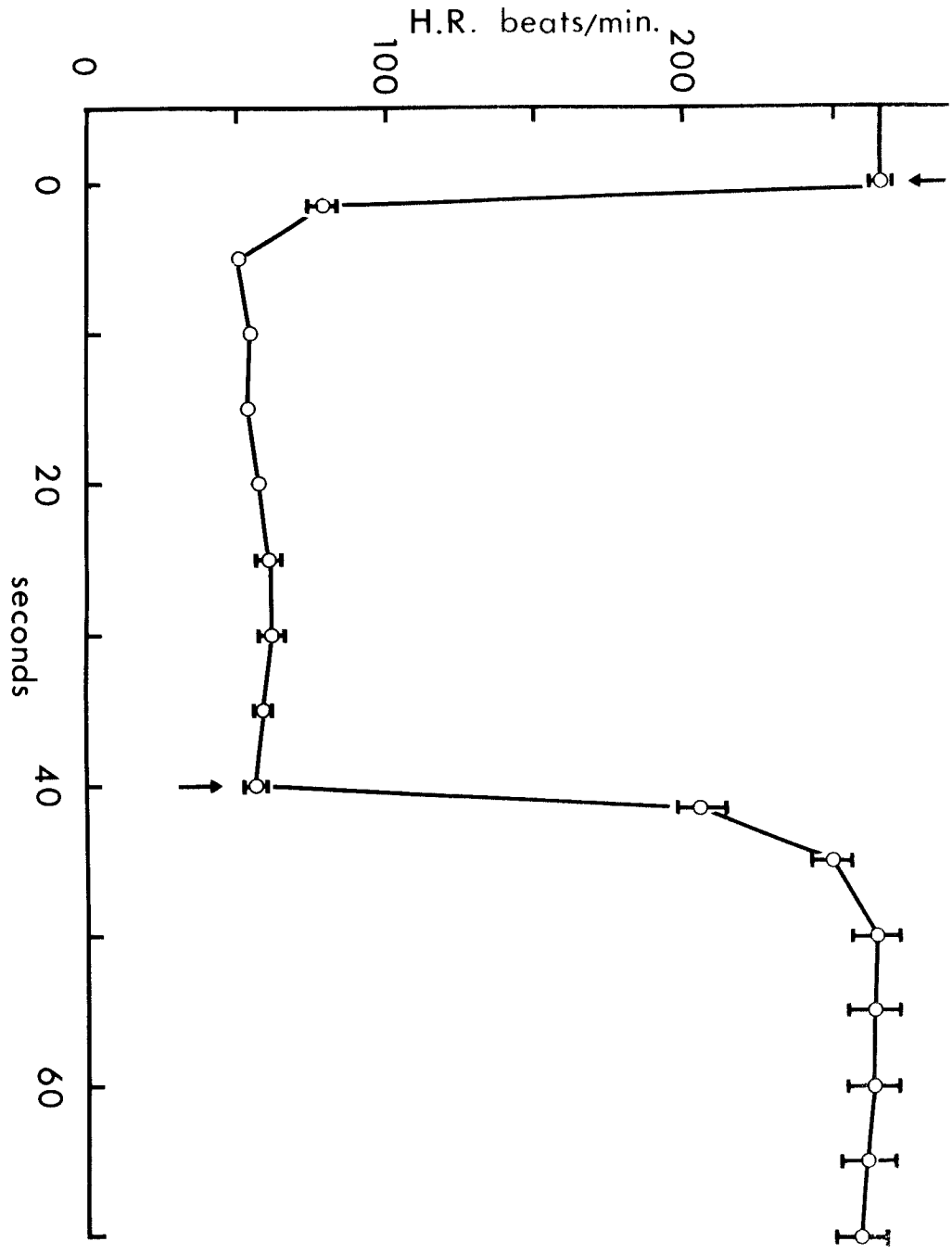

H.R. beats/min.
0
100
200
0
20
40
60
seconds

Figure 6. Electrocardiogram during diving in the restrained (above) and anaesthetized muskrat (below). The points of submersion and resurfacing are indicated by the arrows. Sustained apnea did not occur in the anaesthetized animals. Time marker is 1 second.

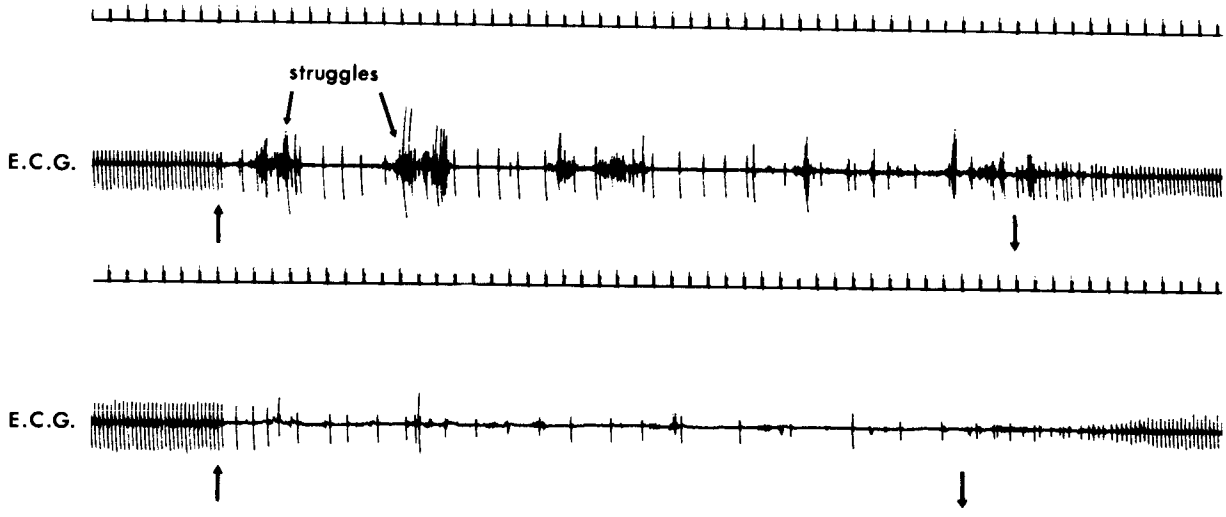

struggles
E.C.G.
E.C.G.

Figure 7. Mean heart rate during diving in the anaesthetized muskrat. Each point represents the mean value of a minimum of 39 trials in 9 muskrats in which all dives were terminated at 40 seconds. The arrows indicate the points of submersion and resurfacing. Standard error of the mean is given for S.E.M. greater than 4.

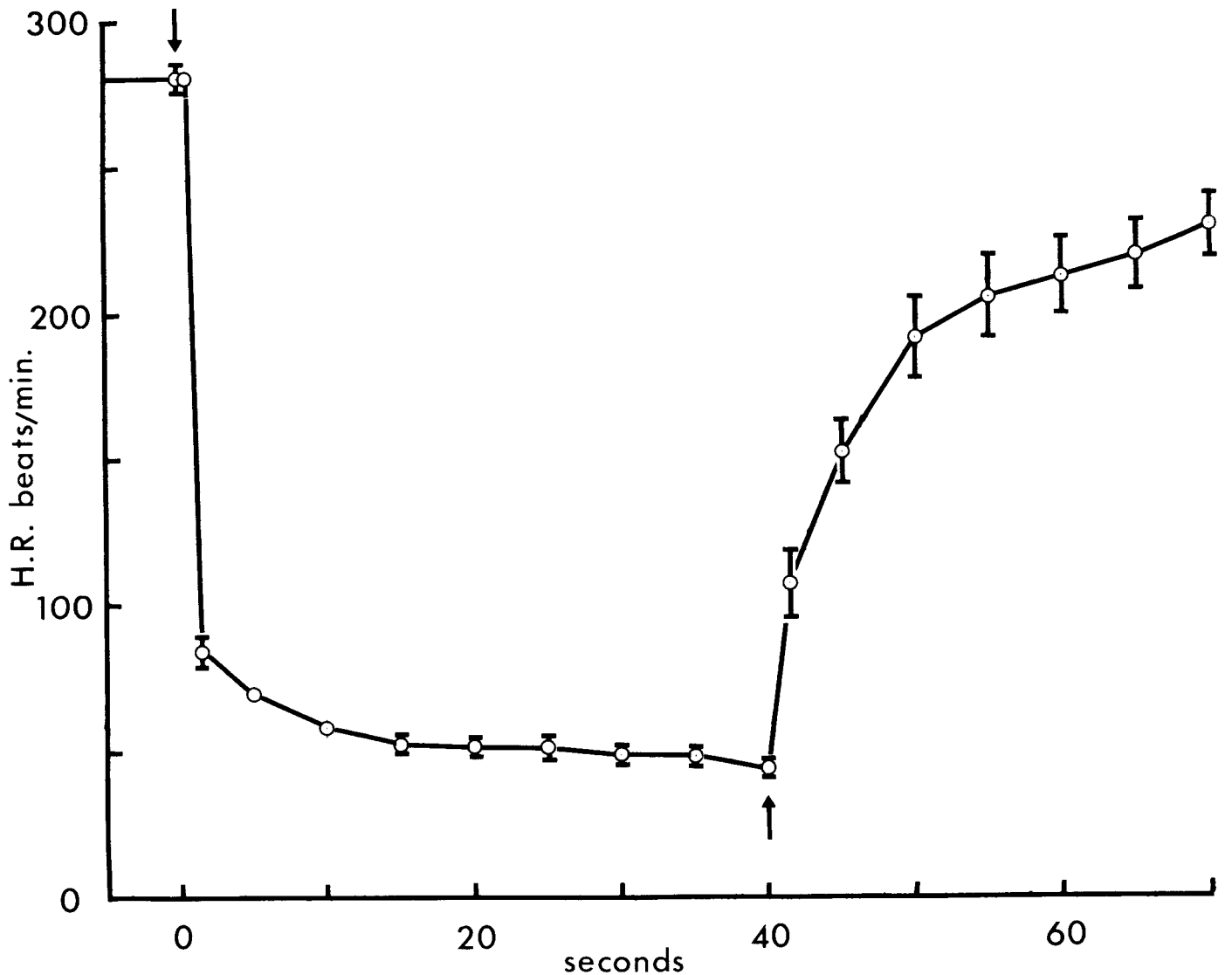
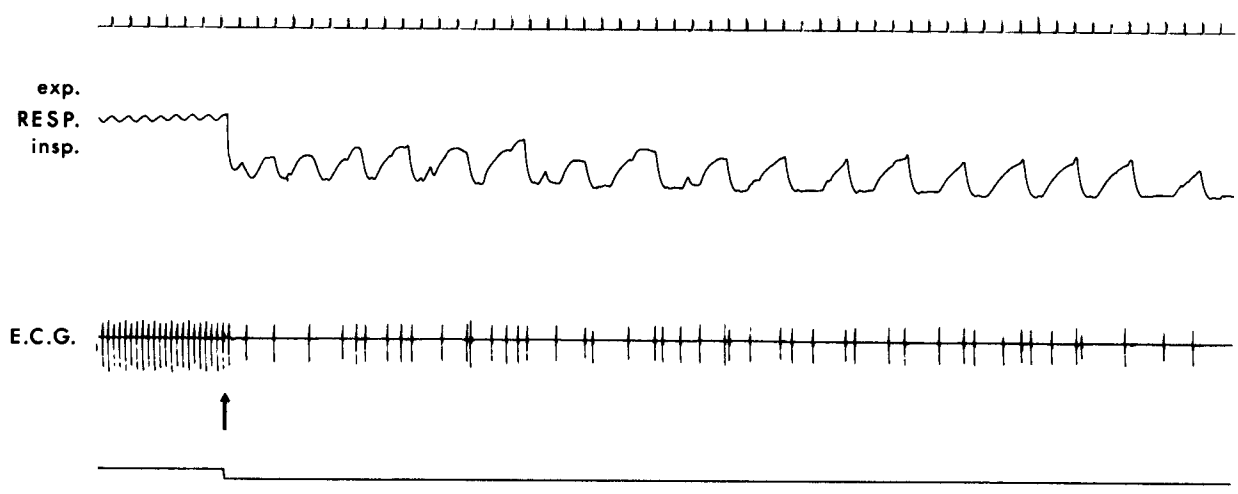

300
200
100
0
H.R. beats/min.
0
20
40
60
seconds

Figure 8. Respiratory and cardiac responses to submersion in the anaesthetized muskrat. Traces top to bottom, time (1 second marker), tracheal air flow (inspiration downwards), EKG and event trace. Respiration recorded from a thermistor set in the nasal passage indicated the directional flow of air in the pre-dive state, and inhalation of water during submersion. Note that a significant bradycardia occurs despite continued respiratory efforts. The arrow marks the point of submersion.


exp.
RESP.
insp.
E.C.G.

heart rate fell from 281±5 to 84±4 beats/min (n = 56) after an initial latent period of 591±40 msec and continued to decline to a rate of 39±2 beats/min (n = 39) after 40 seconds of submersion. As a rule however, respiratory movements continued during submersion and these tended to increase with dive duration.

That water was taken into the nasal cavity in dives in which the animals were anaesthetized was confirmed by recordings taken from a thermistor set in the nasal cavity (Fig. 8). A second thermistor placed in the trachea and larynx established that inhaled water was not drawn past the glottis but was expelled during the expiratory phase of the cycle. Heart rate on resurfacing was slow to recover and generally required 60 seconds to reach the pre-dive rate. Interestingly, the anaesthetized muskrats could not tolerate submersion in isotonic saline (0.9%) for more than a short time. On three occasions muskrats were drowned after saline submersions of less than 40 seconds and were found to have taken the fluid into the lungs.

2. Responses to Water Stimulation of the Nasal Area.

2.1. External Narial Stimulation.

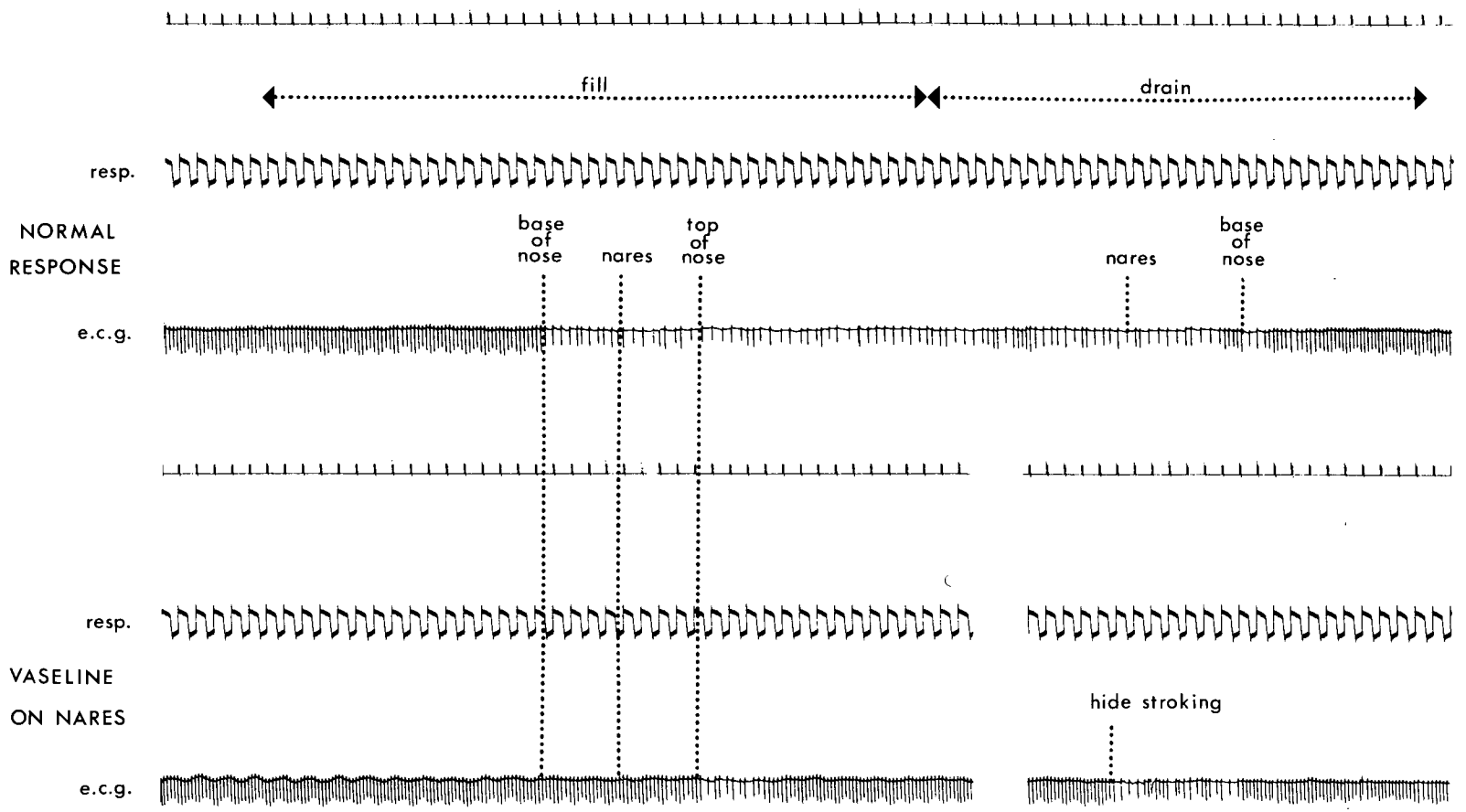
Visual observations on the normal muskrat at rest on the surface of the water indicated that apnea was triggered by water contact with the nares. The narial flaps adjacent to the nares seemed to function in both a sensory and motor capacity. Not only could the diving responses be evoked from this area but it was also apparent that the flaps prevented water from entering the nasal passages. In the unanaesthetized animal, water was never observed to enter the nares. Following anaesthesia however, the narial reflex was lost since water was taken into the nasal passages. Anaesthesia

also abolished the respiratory and cardiac responses to narial water stimulation when the animals were allowed to breathe through an exposed cannula.

In unanaesthetized curarized muskrats which were artificially ventilated (8-12 cm H_2O), pouring water over the external nares or raising the water level over the nose resulted in a moderate bradycardia which was not sustained (Fig. 9). In these experiments water was excluded from the nasal passages by a continuous but gentle flow of air through the oral facing cannula. Heart rate fell from a mean of 292±6 to 76±12 beats/min (n = 6) on submersion. As a rule, the response was better sustained when the nose was kept underwater than when water was poured on the nares. Stopping ventilation in expiration just before water stimulation of the nares caused heart rate to fall further than when ventilation was maintained. Virtual elimination of the response was obtained if the nares was covered with a fine layer of vaseline (Fig. 9) providing that water was excluded from the nasal passages. It is also noteworthy that curarization preserved the cardiac responses to various stimuli; sudden stimuli such as noise, light flashes and hide stroking could also evoke transient responses even when artificial ventilation was continued.

Postural changes had little effect on the heart rate when the muskrats were subjected to sham (dry) dives. Passive head ducking in either the normal or anaesthetized animal did not evoke bradycardia when this was carried out in a gentle manner similar to that observed in unrestrained dives. Furthermore, in animals which were force dived in a vertical head-down position, cardiac responses were no different from those which were dived in the prone position and suggest that the postural responses noted by Koppányi

Figure 9. Response of heart rate to narial water stimulation in a curarized artificially ventilated muskrat. Top to bottom, time marker (1 second signal), ventilation (inspiration downwards) and electrocardiogram. Ventilation was given at an inspiratory pressure of 8 cm H_2O and a frequency of 1 hz. A gentle flow of air passing from the oral facing cannula out the nares was maintained throughout to prevent water from entering the nasal passages. In the top and bottom (left) recordings, water level was slowly raised above the head and then drained resulting in a moderate but maintained bradycardia. Vaseline applied to the nares abolished the response until water was raised near eye level. The traces at the bottom right indicate that the response to tactile stimuli remains in spite of curarization and forced ventilation.


fill
drain
resp.
NORMAL
RESPONSE
base
of
nose
nares
top
of
nose
nares
base
of
nose
e.c.g.
resp.
VASELINE
ON NARES
hide stroking
e.c.g.

and Dooley (1929) have little significance in the diving ability in the muskrat.

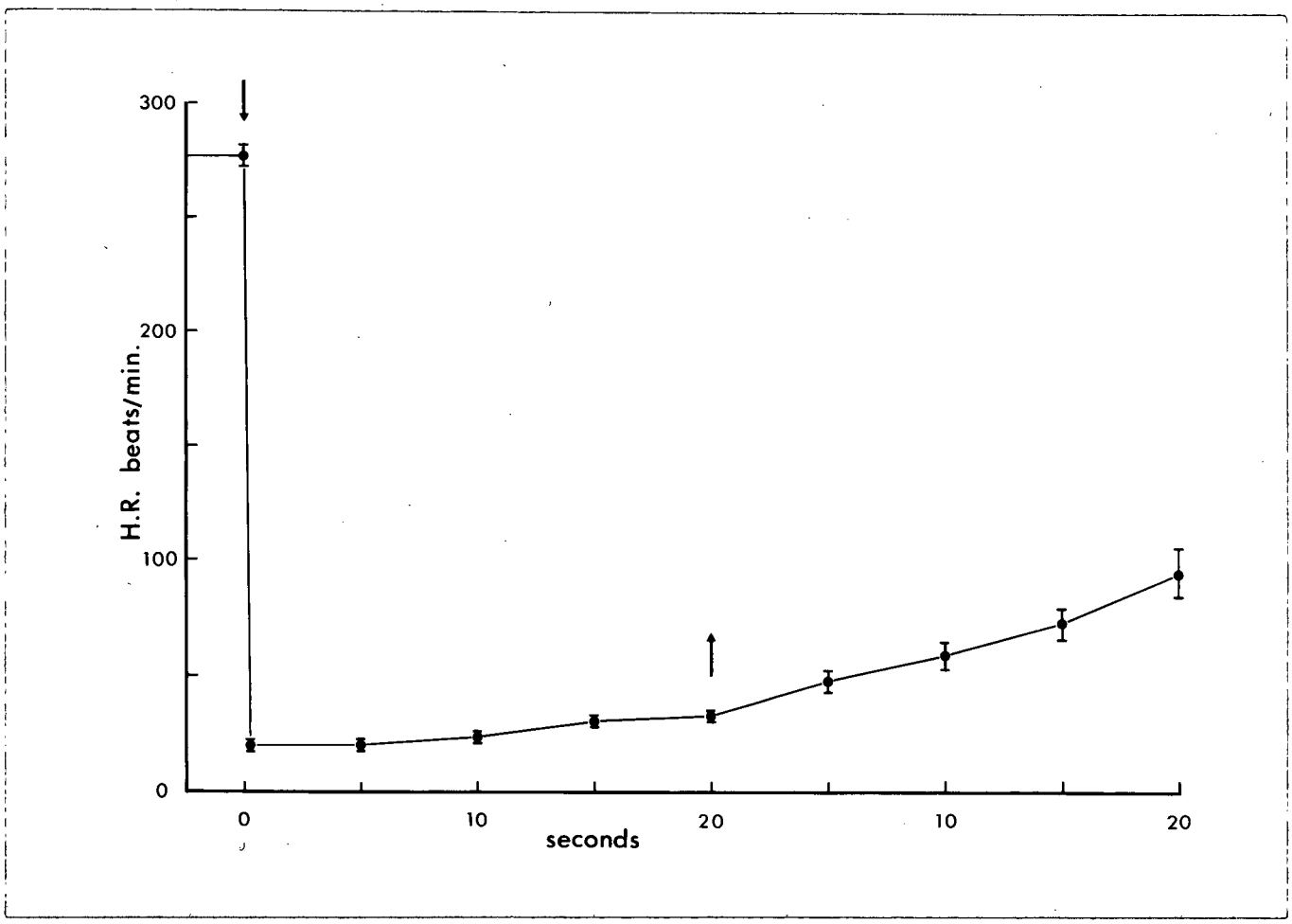
2.2 Internal Nasal Stimulation.

Passing water or saline through the nasal passages to exit at the nares in curarized or anaesthetized muskrats caused varying degrees of apnea and bradycardia depending on the flow rate and the ionic composition of the fluid. Small jets of air (50 µl) directed into the nares could also produce a bradycardic response but this tended to be slight and short lived. Nasal water flow driven by a 100 cm head provided a flow rate of 32 ml/min and gave a pronounced bradycardia and an apnea which lasted from 15 to 40 seconds with maintained stimulation in the anaesthetized animal.

In 12 anaesthetized animals which remained apneic over the experimental trial period (20 seconds), heart rate fell from a mean pre-stimulation rate of 277±5 to 20±2 beats/min (n = 34) when water flow began (Fig. 10). Bradycardia occurred at a mean of 320±27 msec after the onset of water flow but heart rate gradually rose during stimulation and reached a rate of 33±2 beats/min after 20 seconds. When water flow was stopped, the recovery to pre-stimulation heart rate although much slower than in the conscious animals, was always simultaneous with the re-establishment of breathing; usually beginning 5 to 10 seconds after water flow.

The water driven responses were preserved by clearing the nasal passages of water between trials and these could normally be maintained over a five or six hour experimental period. If during extended periods (6 to 10 hours) the responses to water flow stimulation decreased, they were soon lost altogether. In such cases, respiratory and cardiac responses were lost

Figure 10. Mean heart rate in the muskrat during apneic nasal water flow stimulation. Each point represents the mean response in 34 trials in 12 muskrats ±S.E.M. The onset and termination of water flow (32 ml/min) are indicated by the arrows. Water remained in the nasal passages approximately 30 seconds after water flow was stopped.


300
200
100
0
H.R. beats/min.
0
10
20
10
20
seconds

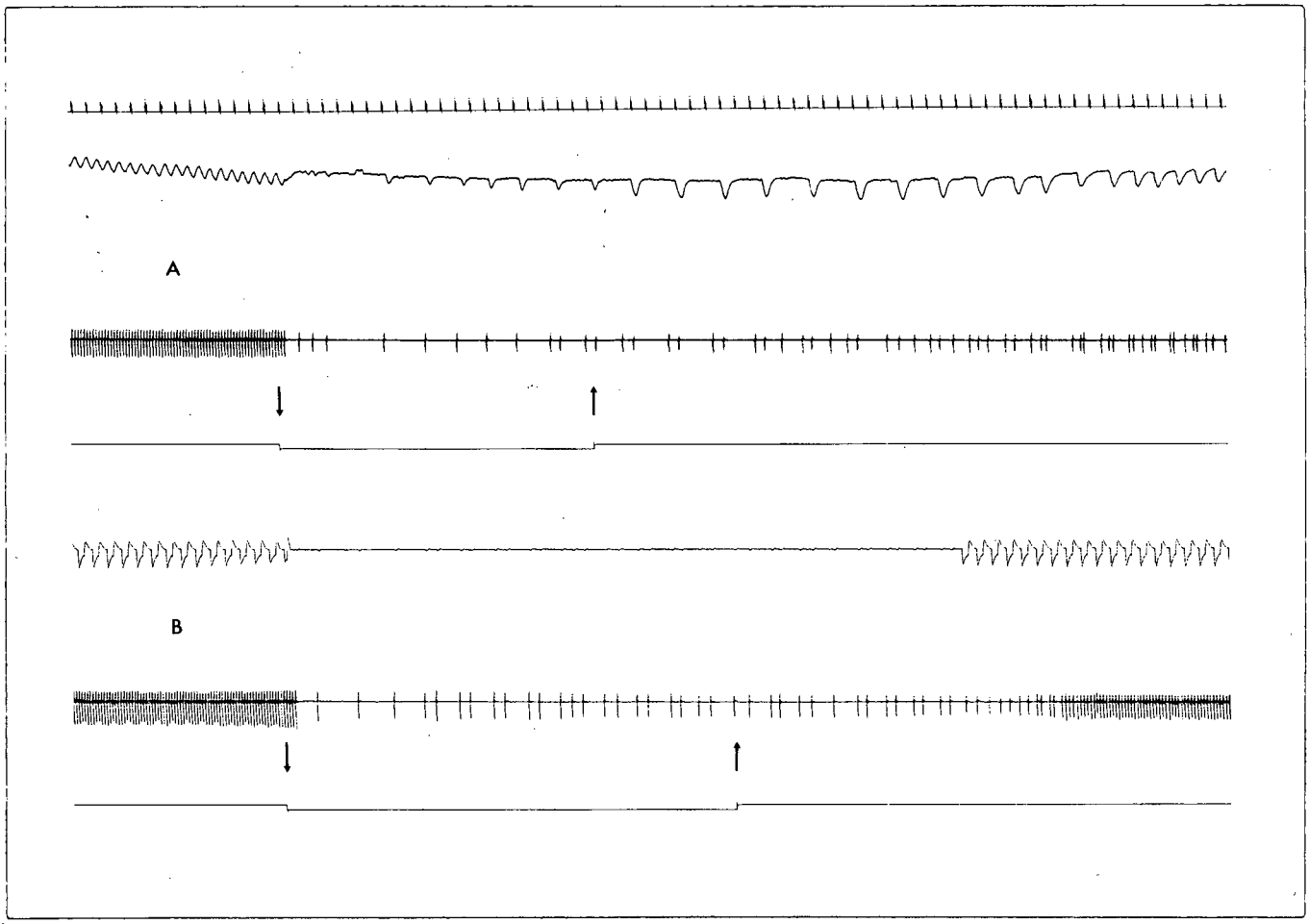
simultaneously in spite of surface respiration and heart rate remaining normal. The loss of the reflexes appeared to be related to central impairment of the bradycardia reflex arc since asphyxiation also failed to produce reflex slowing of the heart. This phenomenon has also been noted in anaesthetized dogs in which the nasal responses are prone to disappear spontaneously (Angell James and Daly, 1972a).

The substitution of 0.9% saline for water or lowering the water flow rate always caused a less intense and more unpredictable bradycardia than that caused by 32 ml/min water flow. Apnea at the onset of stimulation was neither immediate nor lasting (Fig. 11) and respiratory breakthrough occurred throughout, which in turn increased heart rate with each breath.

During non-apneic stimulation, a well defined relationship existed between the inspiratory phase and the appearance of EKG spikes. In the initial period of stimulation an EKG spike always occurred simultaneously with inspiration (Fig. 11, top), but as respiratory drive increased a second (and occasionally a third) spike appeared near the peak of inspiration. This relationship continued until sinus arrhythmia was no longer present. The pattern of activity revealed that the single spikes preceded the respiratory deflection by 200-300 msec but when paired spikes were present, the first advanced closer to the beginning of lung inflation while a second appeared near to but not at maximal inflation. As the stimulus continued, a slight phase shift occurred in which the second of the two advanced on the first to give a double spike that was associated with early inspiration.

The pattern of sinus arrhythmia in the artificially ventilated animal suggested that the influence of respiration on heart rate combined both a central and a peripheral component as noted by Anrep et al. (1936b).

Figure 11. Cardiac responses to non-apneic water flow stimulation in the anaesthetized and curarized anaesthetized muskrat. Top to bottom in each recording from time trace (1 second marker); pneumotachogram (inspiration downwards), electrocardiogram and event trace. The upper recording shows the response to low water flow stimulation (10 ml/min) in which the integrated tracheal air flow trace is punctuated by breakthrough inspirations (A). The bottom recording shows the cardiac response of a curarized muskrat in which ventilation was stopped during and after water flow. Forced ventilation was delivered at 10 cm H_2O and 1 hz. Water flow is indicated by arrows.


A
B

Observations in the curarized animal during asphyxia however, tend to show that the typical paired sequence may arise exclusive of lung inflation (Fig. 11, bottom trace).

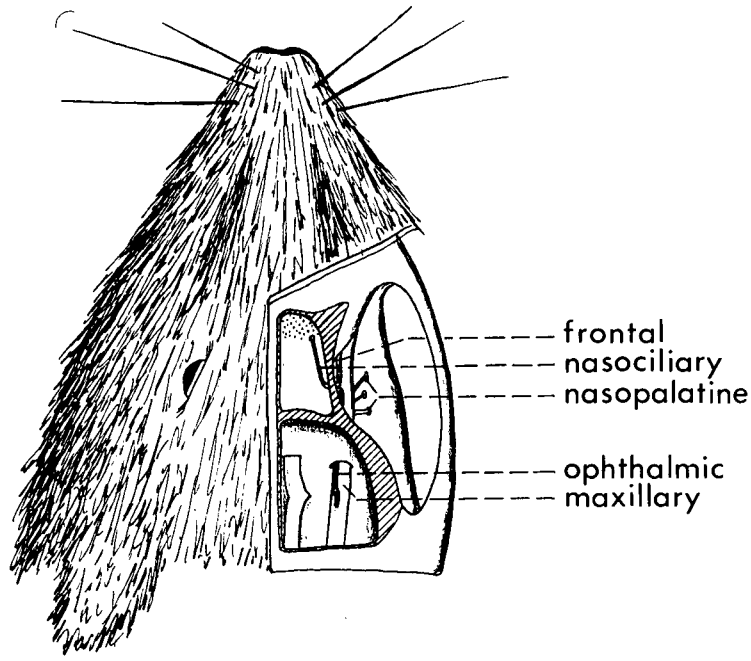
3. Afferent and Efferent Pathways of the Diving Reflexes.

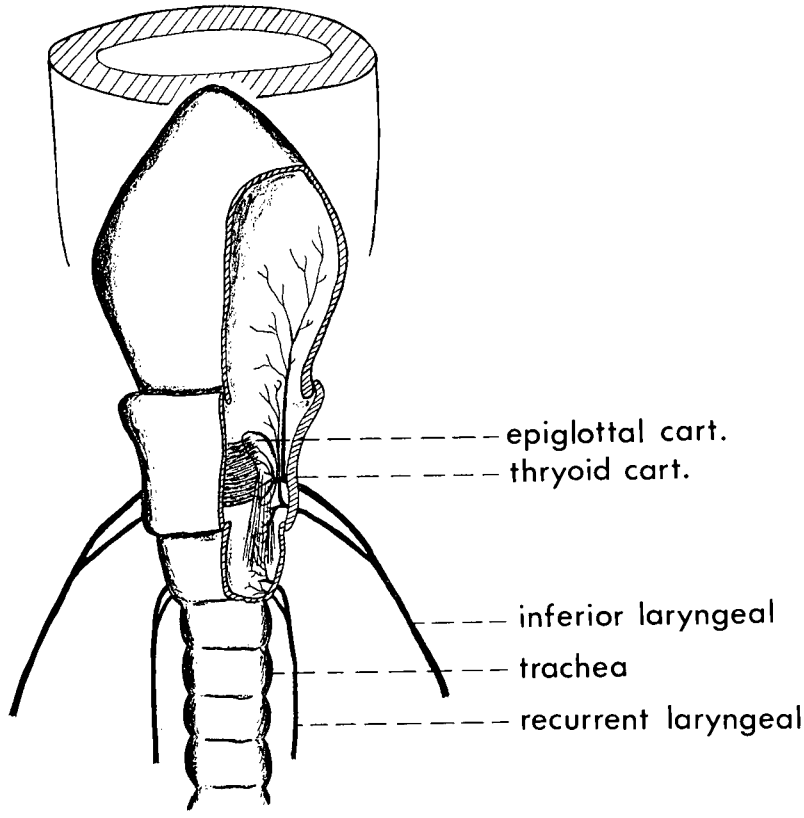
3.1. Morphology of the Nasal Receptor Areas.

While the inferior laryngeal division of the vagus was readily accessible, blockade of the maxillary branch of the trigeminal was hampered by its location deep within the basisphenoid bone and the variability of the ancillary branches near the anterior lacerated foramen and within the orbit. Cardiac and respiratory responses to water flow were not affected when the maxillary division was sectioned or cooled rostral to the exit of the nasopalatine and sphenopalatine branches. Abolition of the maxillary contribution to the nasal responses however, occurred when blockade was attempted at a level just caudal to the foramen where the maxillary and ophthalmic divisions of the trigeminal unite to form the main trigeminal trunk (Fig. 12).

Microscopic examination of the nasopalatine branches revealed that these nerves are given off from one or two sub-maxillary bundles which pass ventrally and medially through the basisphenoid, presphenoid and maxilla bones. The branches emerge within the nasal cavity to innervate the nasal mucosa and the base of the molar teeth and reappear beneath the epithelial tissues of the hard palate. From their exit on the medial side of the third molar, two branches were distinct, one of which passed forward to innervate the anterior portion of the hard palate while the second ran posteriorly along the medial side of the maxillary ridge to give off finer branches to the soft palate. In two operations in which the pharynx and palate were

Figure 12. Major nerves supplying the nares and the nasal and glottal mucosa. Top, cutaway diagram of the frontal portion of the skull in which the parietal bone, frontal lobe, olfactory lobe, right eye and lacrimal gland have been removed to show some branches of the maxillary and ophthalmic divisions of the trigeminal nerve. Bottom, ventral exposure of the glottal area.


frontal
nasociliary
nasopalatine
ophthalmic
maxillary


epiglottal cart.
thryoid cart.
inferior laryngeal
trachea
recurrent laryngeal

exposed by mid-ventral jaw section, the dorsal anterior surface of the soft palate appeared to the the most sensitive to probe, suction or electrical stimulation of the epithelial tissues as judged by respiratory and cardiac responses. As a rule stimulation of these areas evoked transient apnea and slight bradycardia. Similar but less intense responses could be elicited from the pharyngeal and rostral nasal cavity areas.

The innervation of the larynx and posterior pharyngeal mucosa by the inferior and recurrent laryngeal nerves is shown in the lower half of Fig. 12. The muscles of the epiglottal cartilage appeared to contain fibers from both divisions but only the anteriorly directed branches of the inferior laryngeal nerve were found to extend to the pharyngeal musculature where they terminate on the ventral and lateral sides of the epithelium. The area most sensitive to probe and electrical stimulation was found to be near the anterior end of the glottis but the more anterior epithelium, innervated by the superior laryngeal division failed to cause any cardiac or respiratory responses on stimulation.

3.2 Afferent Pathway of the External Narial Reflexes.

Blockade of the afferent pathway of the external narial reflexes was attempted in three unanaesthetized muskrats. In each case the animals were denervated under general anaesthesia (nembutal) and were allowed to recover fully before experimentation. Maxillary nerve section at the level of the zygomatic arch and infiltration of the narial region with 2% xylocaine failed to affect the respiratory and cardiac responses to forced dives. As in the normal animals, apnea occurred when the water lapped the nares and none appeared to enter the nasal passages.

In two animals visual input was eliminated by sewing the eyes closed in addition to frontal maxillary section but both responded in a manner similar to that in normal forced submersions. It was concluded that while these results do not rule out frontal maxillary nerve involvement in the initiation of the primary nasal reflexes, the afferent limb likely includes many of the small branches which reside in the nasal passages and unite with the ophthalmic division and possibly the maxillary division within the orbit. Because of the inaccessible location of these nerves within the nasal cavity, no reliable results were obtained to confirm their involvement in the reflexes.

3.3 Afferent Pathway of the Internal Nasal Reflexes.

3.3.1 Nerve Blockade of the Afferent Pathway.

The afferent pathway of the nasal water flow reflexes was investigated by nerve section, reversible nerve cooling, irreversible freeze blockade and by electrical stimulation of the central ends of the cut nerves. Of the 17 muskrats examined, 10 were decerebrated. In animals which were decerebrated by aspiration to the level of the thalamus, normal respiration rate, tidal volume and heart rate were unaffected although the cardiac response to nasal water flow was significantly reduced. When water flow of 32 ml/min was allowed to pass through the nares heart rate rose to 31±5.8% of the normal rate from the pre-decerebrate response of 8.3±1.0%.

Section or cooling blockade of the following nerves had no effect on either the respiratory or cardiac responses to the water flow stimulus: olfactory (n = 10), ophthalmic (n = 10), mandibular (n = 10), facial (main branch, n = 2), superior petrosal (n = 3), glossopharyngeal (main branch

distal to sinus nerve, n = 3), sinus (n = 5), superior laryngeal (n = 6), recurrent laryngeal (n = 3). In 4 of 6 muskrats in which both the maxillary division of the trigeminal nerve and the inferior laryngeal branches of the vagus were sectioned or cooled, the respiratory and cardiac responses were fully abolished. In the remaining two animals section of the two divisions resulted in only slight decreases (less than 5%) in normal heart and respiration rates during the water flow stimulus. Table I summarizes the mean results of maxillary and inferior laryngeal nerve blockade. It is evident from these data that while the maxillary nerve dominates cardiac inhibitory input, it has less effect than the inferior laryngeal nerve on the respiratory response (Table I).

It was clear from the results that response loss was dependent on the order of blockade of the two nerves. Table II compares the mean data of the cardiac and respiratory responses when the sequence of nerve blockade was reversed in one half the trials. Typically, when nerve blockade was carried out after the loss of the first nerve, the loss of the response was greater than if nerve blockade had been done alone. The table includes data from denervations by section and cold blockade since there was no difference in response loss between the two techniques. In two of eight animals in which inferior laryngeal blockade was carried out first, there was no noticeable decline in the cardiac response to water flow even though there was an obvious decrease in the respiratory response. In the remaining animals the loss of the cardiac response due to blockading the inferior laryngeal nerve first never exceeded that when the maxillary nerve was blockaded first.

Figure 13 shows the results from one muskrat which demonstrate that the sequential elimination of the water driven responses was achieved by bilateral

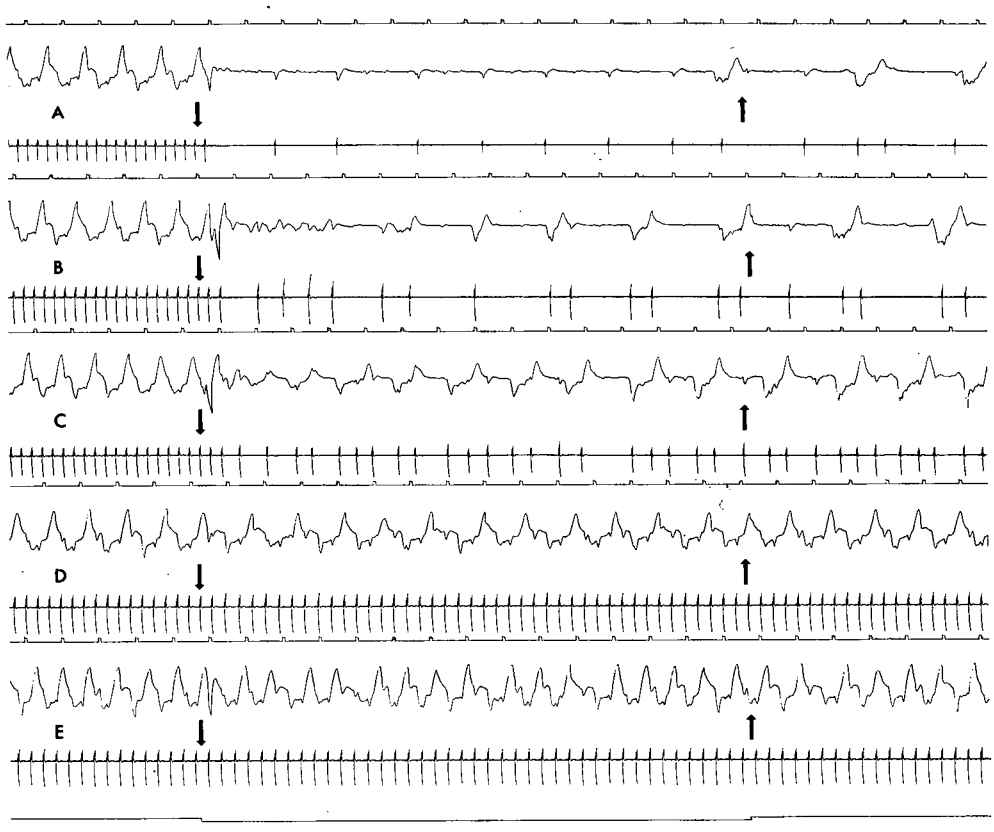
Table I. Effect of nerve blockade and electrical stimulation on heart rate and respiration. Blockade was tested by determining response loss to nasal water flow.

Number of animals	Cranial nerve	Branch	Average loss of response to blockade (%)		Effect of low voltage stimulation on respiration and heart rate
			Resp.	H.R.	
10	I	olfactory	0	0	0
10	V	ophthalmic	0	0	transient decrease
10	V	maxillary	44.5	68.5	sustained decrease
10	V	mandibular	0	0	0
2	VII	main	0	0	0
3	VII	superior petrosal	0	0	0
3	IX	main (distal)	0	0	0
5	IX	sinus	0	0	transient decrease
3	X	cervical (main)	0	100	sustained decrease
4	X	superior laryngeal	0	0	0
11	X	inferior laryngeal	55.5	31.5	sustained decrease
3	X	recurrent laryngeal	0	0	0

Table II. Effect of nasal water flow on heart rate and respiratory minute volume in 14 muskrats before and after bilateral section or cold blockade of the inferior laryngeal and maxillary nerves ±S.E.M. Each water flow period was 20 sec and heart rates and minute volumes are the averages for this period. On average each test was done 3 times on each animal.

	Normal animals		Decerebrate		Bilateral IL blockade	Bilateral IL and max blockade	Bilateral max blockade	Bilateral max and IL blockade
	Control	Water flow	Control	Water flow				
Heart rate beats/min	277±13	25±4	286±13	53±10	93±18	270±18	177±14	275±8
Minute volume ml/min	214±8	0	189±12	22±10	101±15	171±9	89±18	181±17
Number of animals	14	14	11	11	8	3	7	3

Figure 13. The effect of unilateral and bilateral section of the maxillary (max) and inferior laryngeal (IL) nerves on the respiratory and cardiac responses to nasal water flow. Traces from top to bottom, time (1 second marker), pneumotachogram (inspiration downward) and electrocardiogram. A, decerebrate response to nasal water flow (32 ml/min); B, after left max section; C, after bilateral max section; D, after bilateral max and left IL section; E, after bilateral max and IL section. The slight downward deflections in the tracheal air flow trace during the control stimulation resulted from cardiac contractions and not from respiratory breakthrough.


A
B
C
D
E

section of the maxillary and inferior laryngeal divisions. In the pre-section control trace (A) apnea due to the water flow was maintained for 15 seconds following decerebration but heart rate had increased from 13 to 34 beats/min. When the right (B) and left (C) maxillary nerves were divided, apnea and a pronounced bradycardia did not occur as the recording was marked by breakthrough inspirations and sinus arrhythmia. Following the section of the right (D) and left (E) inferior laryngeal nerves, nasal stimulation caused only a slight irregularity of the respiratory pattern and no appreciable change in heart rate or minute volume. For trials of this nature afferent blockade was considered complete if minute volumes decreased by less than 5% during the 15 second period of water stimulation. In all cases, a short period of asphyxia by tracheal tube clamping was carried out to confirm that the chemoreceptor reflexes remained intact after the abolition of the water driven responses.

3.3.2. Electrical Stimulation of the Afferent Pathway.

Table I includes the effects of low voltage (1 V) stimulation of the central ends of 12 nerves considered as possible contributors to the afferent pathway. Although the responses to nerve stimulation were always less intense than those initiated by nasal water flow, apneic periods of 5 to 10 seconds could be evoked by stimulation of both the inferior laryngeal nerve and the main maxillary trunk of the trigeminal nerve. Electrical stimulation of the maxillary division anterior to the mid-point of the orbit had no effect on either respiration or heart rate but stimulation of branches located in the rear of the orbit usually gave transient responses. These were found to be the nasopalatine and sphenopalatine nerves which enter the

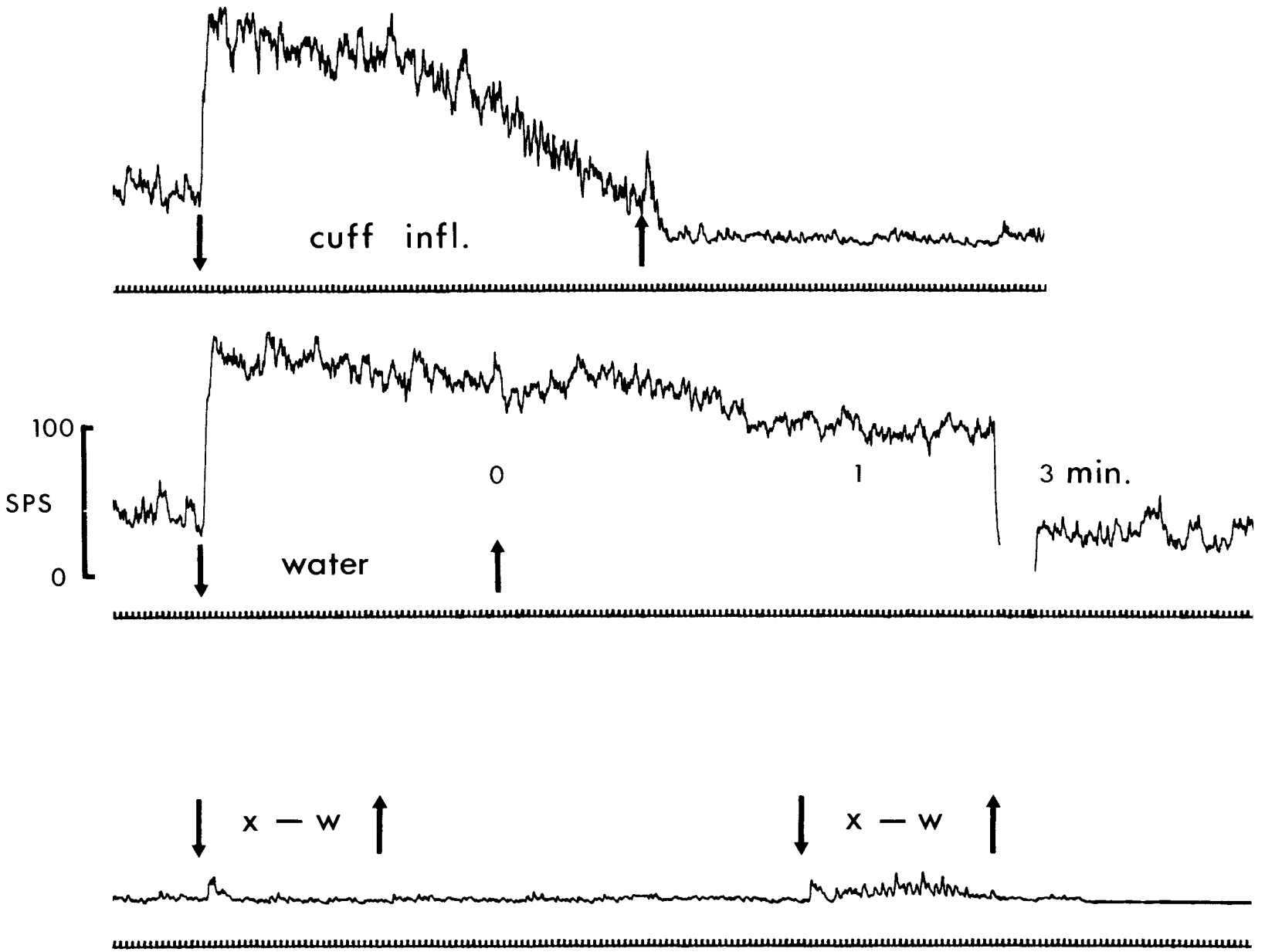
orbit from the nasal passages and join the maxillary bundle on the floor of the cranial cavity.

On the basis of electrical stimulation, the involvement of other small branch nerves could not be ruled out. Higher voltage stimulation (1 to 5 volts) of the nasociliary branch of the ophthalmic division of the trigeminal nerve evoked transient respiratory and cardiac responses in two of the five animals investigated. A response to 1 volt stimulation of the glossopharyngeal nerve resulted only if the electrode was placed central to the entry of, or in contact with the sinus nerve. As with water flow stimulation, when the stimulus strength was sufficient to evoke a response, some inhibition of breathing always accompanied the cardiac response. Respiratory responses however, could often be evoked without an obvious decrease in heart rate.

3.3.3. Recordings from the Inferior Laryngeal Nerve.

Nerve activity from one of the two afferent limbs was monitored to support its involvement in the internal nasal reflex. The response to stimulation by water flow, punctate stimulation and constant pressure stimulation of the glottal area was recorded from silver wire electrodes placed on the distal end of the divided inferior laryngeal nerve. Glottal stimulation by water flow was achieved by the technique previously described for nasal stimulation (via the oral facing cannula) while constant pressure was applied by a balloon in the nasal cavity inflated to 10 cm H_2O. The patterns of nervous discharge from a multi-fiber preparation to both stimuli were similar in onset and peak discharge but water flow always maintained activity much longer than did cuff inflation (Fig. 14). In fact, in water flow trials, nerve activity remained above the pre-stimulation level

Figure 14. Integrated discharge from the left inferior laryngeal nerve in response to pressure and water flow stimulation of the pharynx. Top, response to cuff inflation (10 cm H_2O) of the glottis; middle, response to a water flow of 32 ml/min; bottom, response to water flow after two minute topical anaesthesia (xylocaine, 1%) and during recovery from the anaesthetic (X-W). The vertical axis measures discharge rate of the multiple-fiber preparation in spikes per second (SPS). The periods of water flow are indicated between the arrows. Water was not flushed from the nasal passages in the middle and bottom traces. Note the break in the middle trace. Time, 1 second marker.


cuff infl.
100
SPS
0
water
0
1
3 min.
x — w
x — w

until the second minute after water flow was stopped. Cuff inflation caused a decline in spike activity over a one to two minute period and at this time discharge became less than in the unstimulated condition.

In each preparation (n = 3), the abolition of both the nervous activity and the respiratory and cardiac responses to water flow stimulation was complete following the two minute instillation of xylocaine (1%) into the glottal area (Fig. 14, bottom trace). The recovery of discharge activity from the anaesthetic was rapid, beginning about one minute after it was flushed out and reached pre-stimulation level after approximately 5 minutes.

3.4. Efferent Pathway of the Cardiac Reflex

Vagal blockade by section and cooling was attempted in 5 muskrats but only the former procedure resulted in complete abolition of reflex bradycardia. In urethane anaesthetized animals, division of the vagi at the level of the larynx increased heart rate by 15±4 beats/min from a normal rate of 286±6 (n = 3) and abolished the cardiac response to nasal stimulation and asphyxia. In both cases a test period of 30 seconds was used. Blockade of the intact vagal nerve trunks by thermode cooling while reversible, was less successful in abolishing reflex bradycardia. On cooling to 5-6^{o}C the initial response to nasal water flow was absent but periodic bradycardia occurred if water flow was continued. The normal response was re-established when the thermodes were returned to ambient temperatures. It was concluded that because of the relatively large diameter of the vagal trunk, core temperatures of less than 8^{o}C were not achieved over the nerve channel length in the thermode. The failure of complete blockade may have been caused by nerve "hurdling", noted by Douglas and Malcolm (1955) and would likely be prevented by decreasing thermode temperatures or increasing the length of the nerve channel.

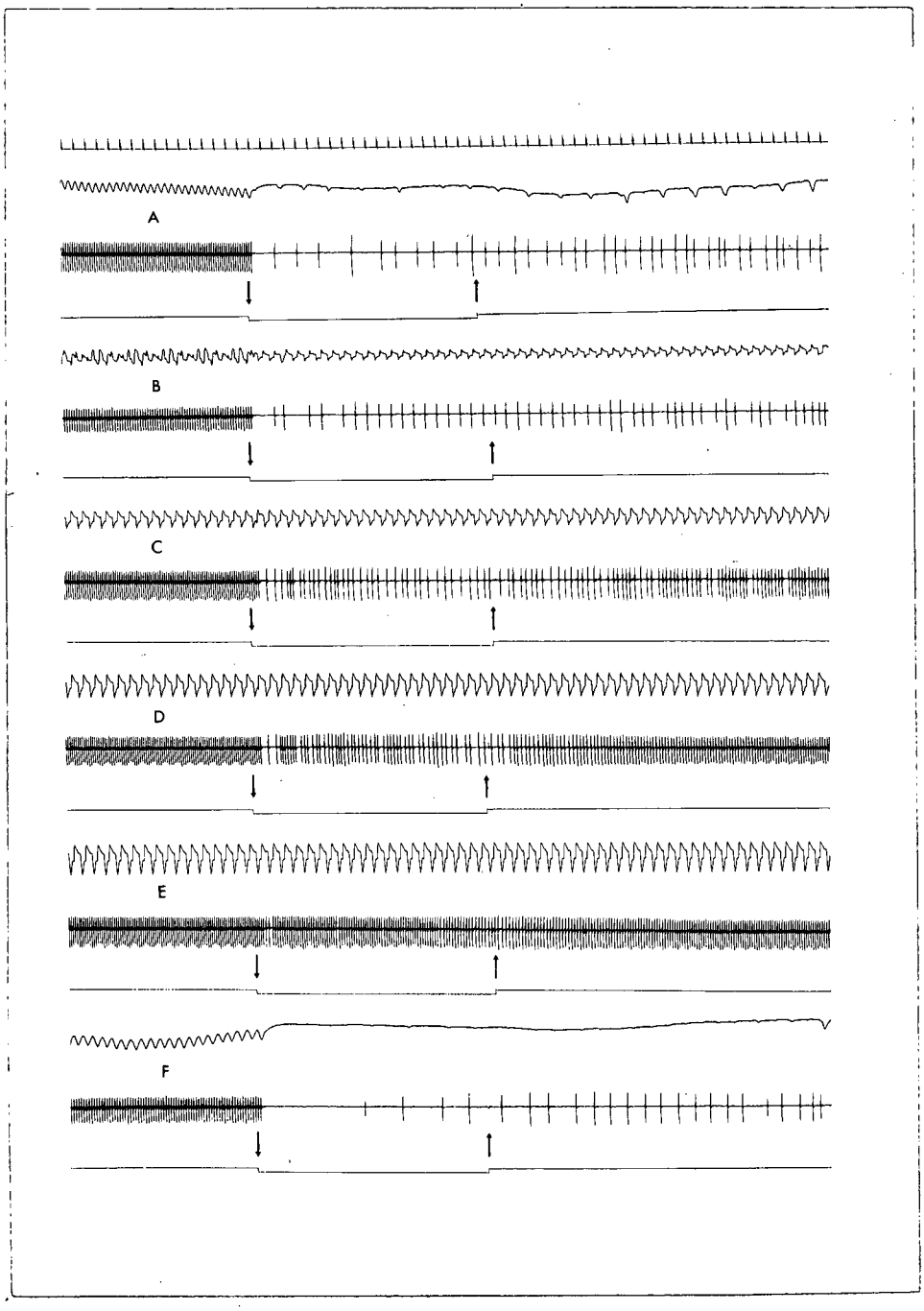
4. Effect of Lung Input on Respiration, Heart Rate and Blood Pressure.

4.1. Effect of Artificial Ventilation on Bradycardia Caused by Nasal Stimulation.

Artificial ventilation of seventeen anaesthetized muskrats caused slight cardioacceleration when minute volume exceeded that during unassisted ventilation. At four times the minute volume of 150±4 ml/min (n = 10) heart rate increased about 10 beats/min. This effect was independent of CO_2 washout as it occurred in animals ventilated with 95% O_2 and 5% CO_2. It was evident that artificial ventilation drove respiratory motor neurone output, monitored by phrenic nerve discharge, when the rate was close to the normal breathing rate (70±6 per min, n = 10). This response was evoked with tidal volumes (1.6 ml) less than that measured in the freely breathing animal (2.3±0.2 ml, n = 10) and motor discharge increased with increasing inspiratory pressure. Slowing artificial ventilation below the normal rate of free breathing caused phrenic discharge to occur synchronously with both spontaneous and forced inhalations.

Since the effect of artificial lung inflation on the cardiac response may also depend on the state of central respiratory neurone activity, a comparison was made between the responses during apneic and non-apneic conditions. Non-apneic responses were elicited by saline flow (32 ml/min) or by low water flow (15 to 25 ml/min). These stimuli resulted in highly variable cardiac responses. Maintaining a normal or elevated level of ventilation throughout the period of nasal water or saline flow always affected the level of bradycardia, although no level of ventilation was found which eliminated the bradycardia at the beginning of stimulation (Fig. 15). Individual variation of the cardiac response to weak nasal stimulation was

Figure 15. Effect of artificial ventilation on the cardiac response to non-apneic nasal stimulation. Top to bottom from time trace (1 second marker) pneumotachogram (inspiration downwards), electrocardiogram and event trace indicating water flow. A, nasal saline flow (32 ml/min) in the freely breathing animal; B, saline flow during 4 cm H_2O ventilation; C, saline flow during 8 cm H_2O ventilation; D, saline flow during 12 cm H_2O ventilation; E, saline flow during 16 cm H_2O ventilation; F, nasal water flow (32 ml/min) in the freely breathing animal. Arrows accentuate the period of nasal stimulation. Respiratory traces in A and F are given in the integrated form.


A
B
C
D
E
F

particularly large during artificial ventilation. Six of eight muskrats became apneic during a 20 second period of low water flow but only one was consistently apneic during saline flow (Fig. 16). In the latter animal, when apnea occurred at the onset of stimulation, the cardiac response was always less with saline flow than with water flow.

When spontaneous breathing occurred during nasal stimulation, the response of heart rate was much greater than when artificial ventilation was given during apneic stimulation in the same animals. Heart rate responses to low water flow rate or to saline flow at any given level of spontaneous (breakthrough) ventilation were achieved only when artificial minute volume was increased 4 to 5 times in the apneic trials (Fig. 17). With strong nasal stimulation (water flow 32 ml/min) artificial ventilation had no effect if tidal volume was kept below 4 ml. When tidal volume exceeded this, heart rate became "locked" to the respiratory frequency in the range of the normal rate of breathing (Fig. 18).

Substituting 5% carbon dioxide in oxygen for air during artificial ventilation had no effect on the relationship between the cardiac and ventilation frequencies during strong nasal stimulation (Fig. 19). Slowing the ventilation rate during stimulation caused the heart to beat irregularly, but at a faster rate than in non-ventilated preparations, while at higher rates and volumes of ventilation it was not unusual for heart rate to remain high in the initial period of stimulation, falling to the ventilation frequency after a period which varied from 3 to 10 seconds.

Figure 16. Respiratory and cardiac responses to 32 ml/min water and saline nasal flow in 8 muskrats. Average minute volumes (M.V.) and heart rates (H.R.) are expressed as percent inhibition of the control rates. The trial period was 20 seconds in length. An apneic response to water flow is shown in 6 of the 8 animals.

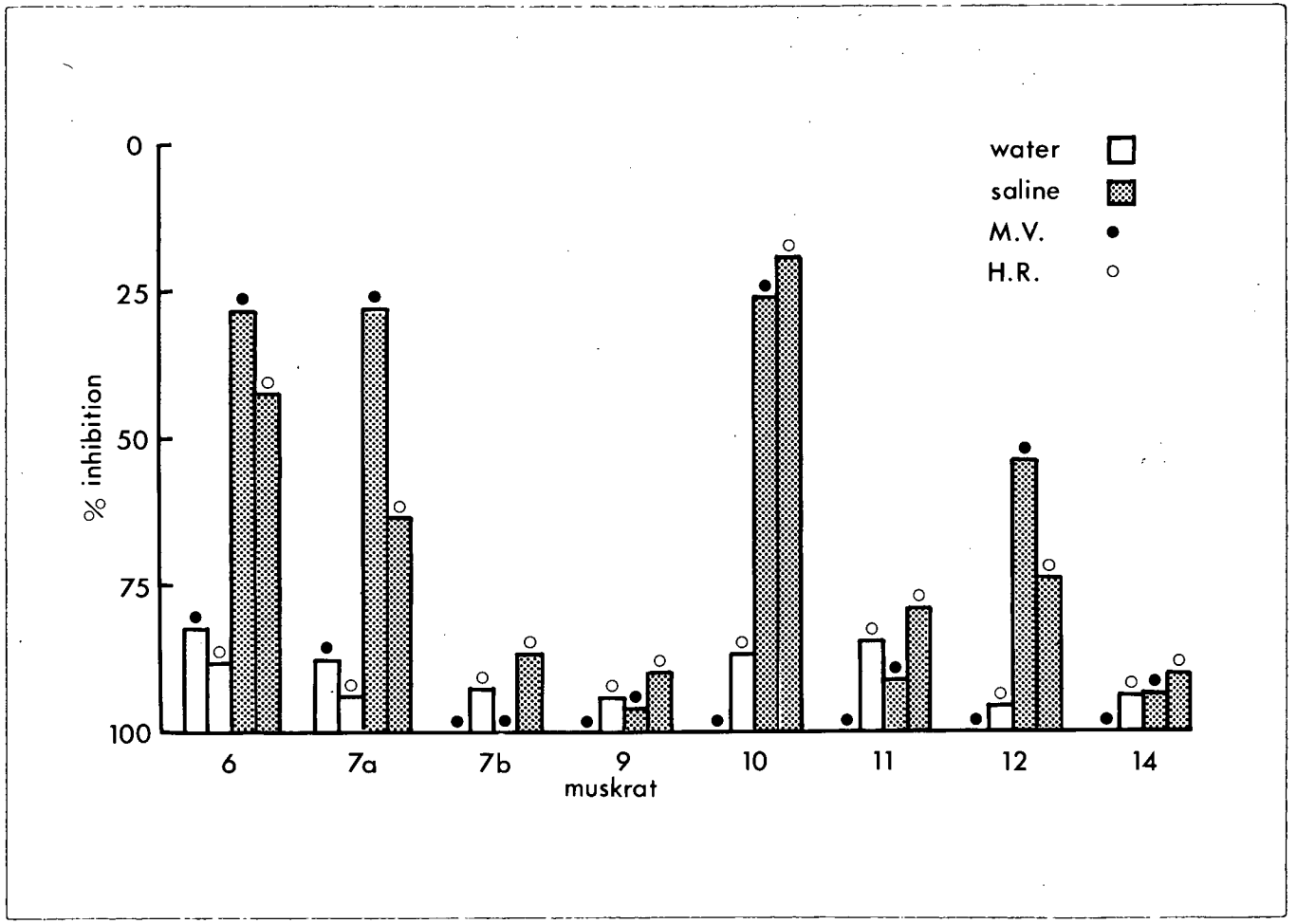

0
25
50
75
100
% inhibition
water
saline
M.V.
H.R.
6
7a
7b
9
10
11
12
14
muskrat

Figure 17. The effect of spontaneous ventilation and artificial ventilation on the cardiac response to weak nasal stimulation. Open (water) and closed (saline) circles describe the relationship between heart rate and minute volume during periods when nasal stimulation failed to cause apnea. The triangles describe the relationship between heart rate and artificial ventilation at the minute volume shown when the nasal stimulation caused apnea. Open squares indicate the control values in the non-stimulated spontaneously breathing animal. The lines were fitted to the points by eye.

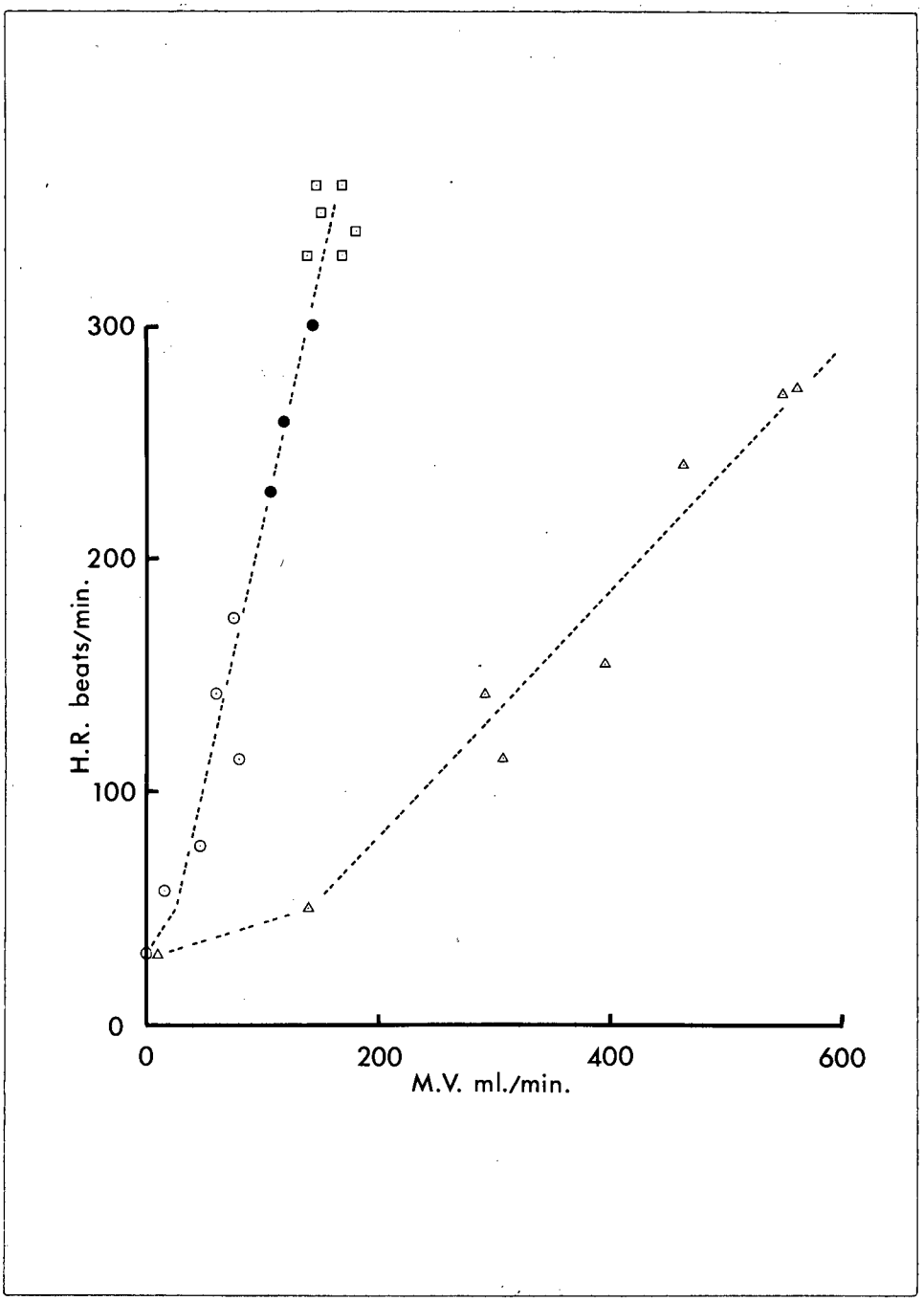

300
200
100
0
H.R. beats/min.
0
200
400
600
M.V. ml./min.

Figure 18. Effect of artificial ventilation on the cardiac response to strong nasal stimulation. Top to bottom from time trace (1 second marker), pneumotachogram (inspiration downwards), EKG and event trace indicating water flow (32 ml/min). A, artificial ventilation at 4 cm H_2O; B, artificial ventilation at 8 cm H_2O; C, artificial ventilation at 16 cm H_2O; D, maintained lung inflation at 16 cm H_2O. Ventilation was given at 1 hz.

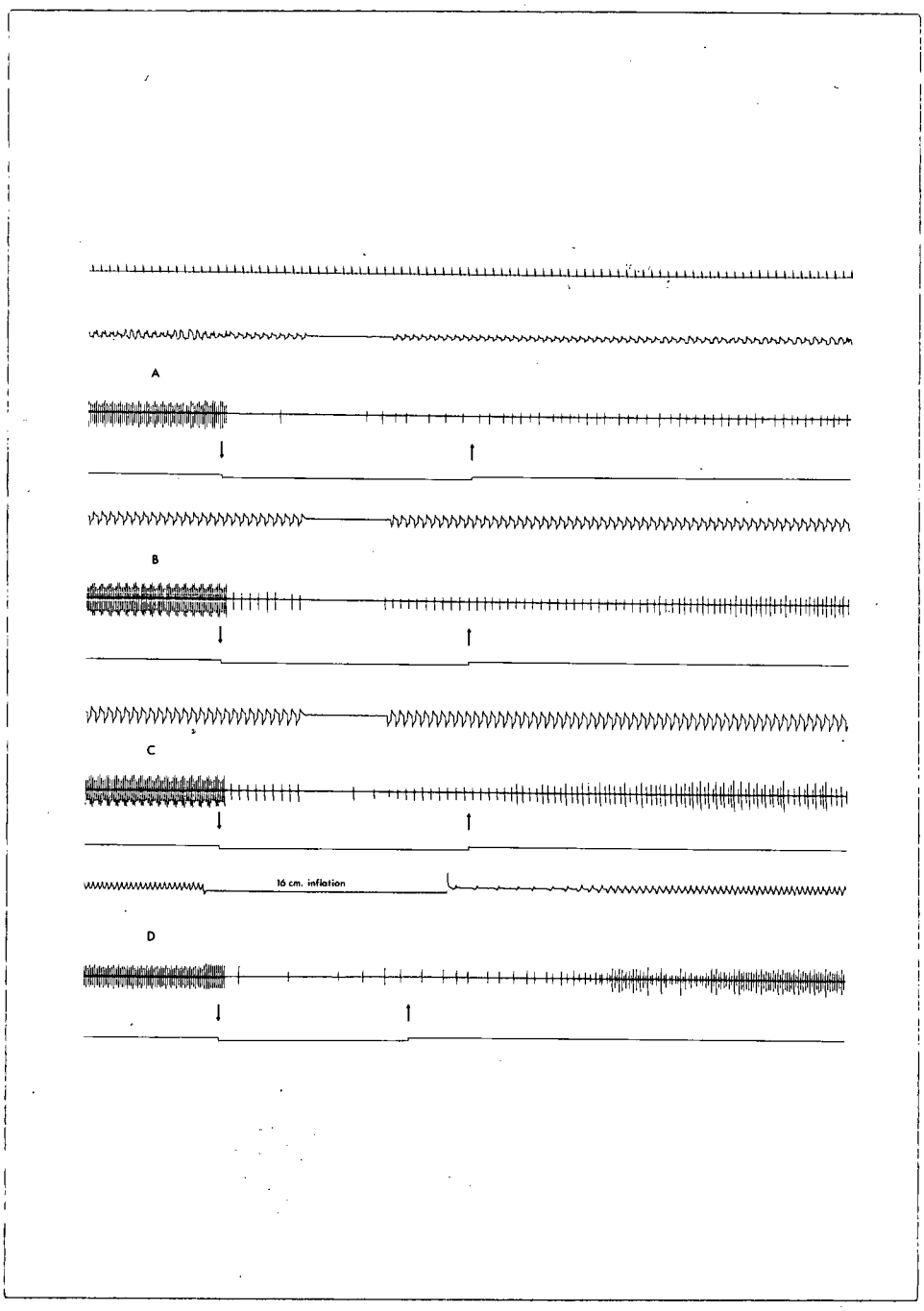
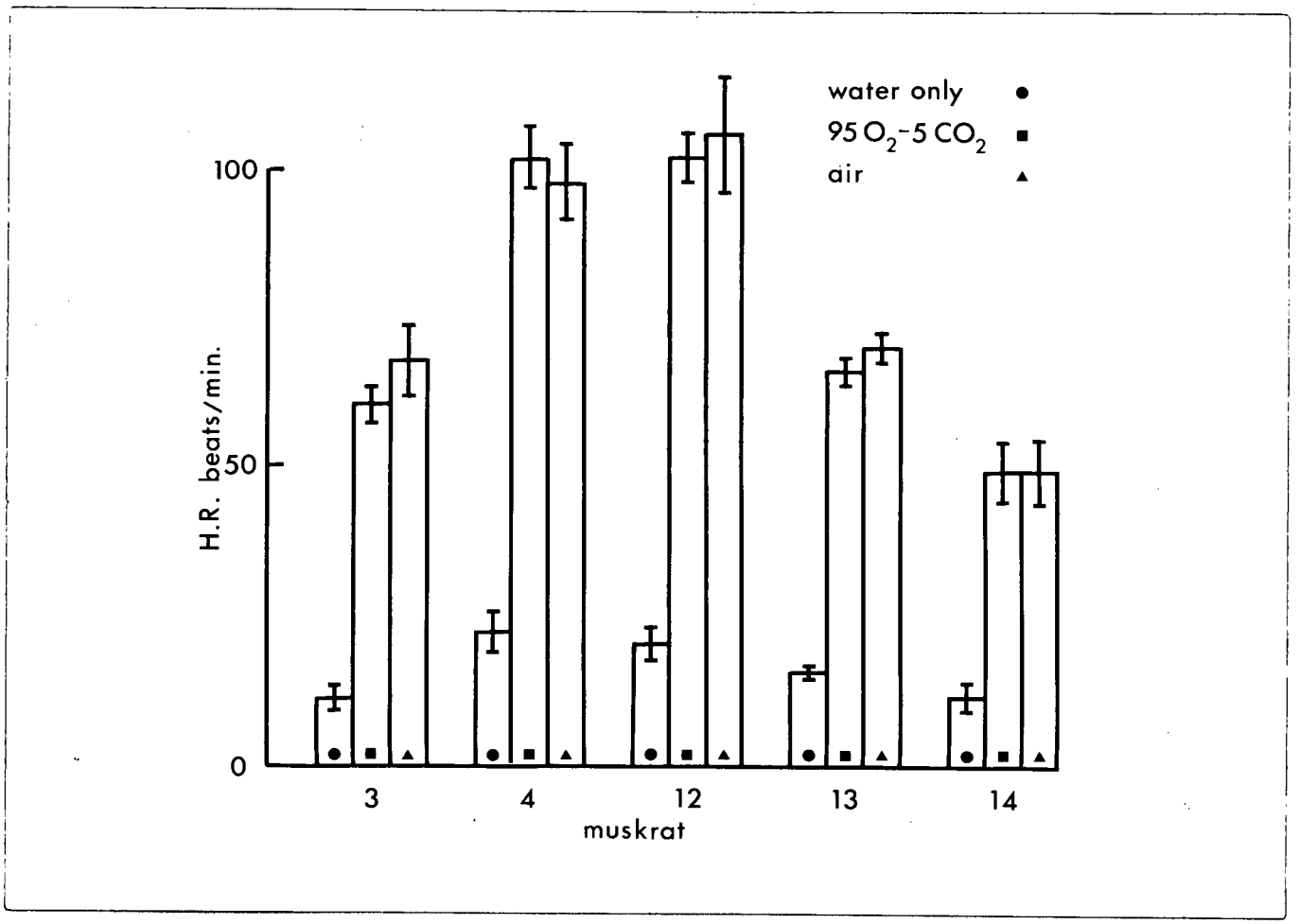

A
B
C
16 cm. inflation
D

Figure 19. Cardiac response during ventilation with 95% oxygen-5% carbon dioxide gas mixture and air. Vertical bars represent means of the control bradycardic response, 5% CO_2 ventilated and air ventilated trials ±S.E.M. Inspiratory pressures of 16 cm H_2O at 1 hz were given in all trials in which the response to ventilation was tested. Water flow of 32 ml/min caused apnea in each of the 15 second trial periods.


water only
95 O2-5 CO2
air
100
50
0
H.R. beats/min.
3
4
12
13
14
muskrat

4.2 Effect of Maintained Lung Inflation on the Cardiac Response to Nasal Stimulation.

The effect of maintained lung inflation on bradycardia caused by nasal water flow was investigated in trials on three muskrats by comparing heart rate during constant inflation with that in animals which were allowed to breathe normally before water flow began. Apneic responses to constant inflation (Hering-Breuer reflex) were normally achieved at 10-12 cm H_2O and at this pressure were maintained for approximately 15 seconds. When pressure was applied simultaneously with nasal stimulation the heart rate was no different from that when nasal stimulation was given alone (Fig. 20). All data were collected from animals which were apneic over the 15 second trial period.

4.3 Effect of Pulmonary Deafferentation on the Respiratory and Cardiac Responses to Artificial Ventilation.

Deafferentation of the lungs was achieved by allowing the animals to breathe steam. As a rule, two breaths of steam abolished the Hering-Breuer reflex to constant inflation yet heart rate was still affected by artificial ventilation frequency during strong nasal stimulation (Figs. 21 and 22). However, after 4 breaths of steam, the "rate locking" effect of ventilation frequency was abolished and lung inflation had no apparent effect on heart rate. Deafferentation, as judged by the loss of the cardiac response to ventilation, was followed by spontaneous breathing during artificial ventilation and the pacing of the normal breathing by lung inflation was no longer evident (Fig. 21E).

Lung deafferentation by steaming was attempted in 10 muskrats (Fig. 23). In freely breathing animals complete deafferentation had little effect on

Figure 20. Cardiac response to artificial ventilation and maintained inflation during strong nasal stimulation in three muskrats. The vertical bars represent the means of the control response ±S.E.M. (unassisted, left) and the response to lung inflation and ventilation at 16 cm H_2O. Animals were apneic in all trials.

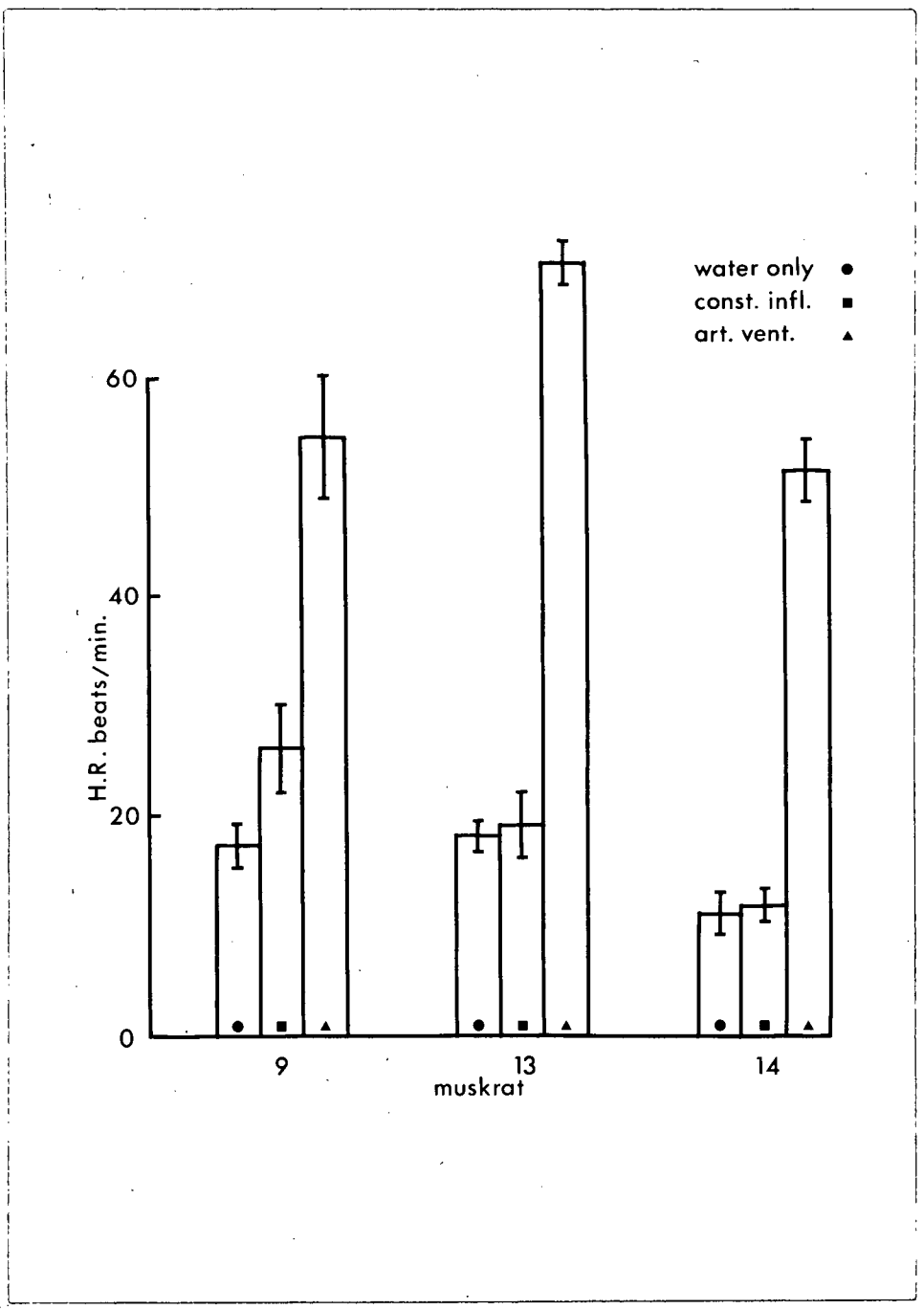

water only
const. infl.
art. vent.
60
40
20
0
H.R. beats/min.
9
13
14
muskrat

Figure 21. Separation of the responses to artificial ventilation and maintained inflation. Top to bottom from time trace (1 second marker), pneumotachogram (inspiration downwards), EKG and event trace (dots in B and C). A, control response to 12 cm H_2O, 1 hz ventilation during apneic water flow stimulation; B, Hering-Breuer reflex to 15 cm H_2O maintained inflation in the freely breathing animal; C, loss of the Hering-Breuer reflex after one breath of steam (15 cm H_2O); D, 12 cm H_2O artificial ventilation after one breath of steam; E, 12 cm H_2O artificial ventilation after three breaths of steam. Nasal water flow (32 ml/min) is indicated by the arrows. Note the irregularity of tracheal air flow before water flow and the loss of the pacing of heart rate during water flow in the latter recording.

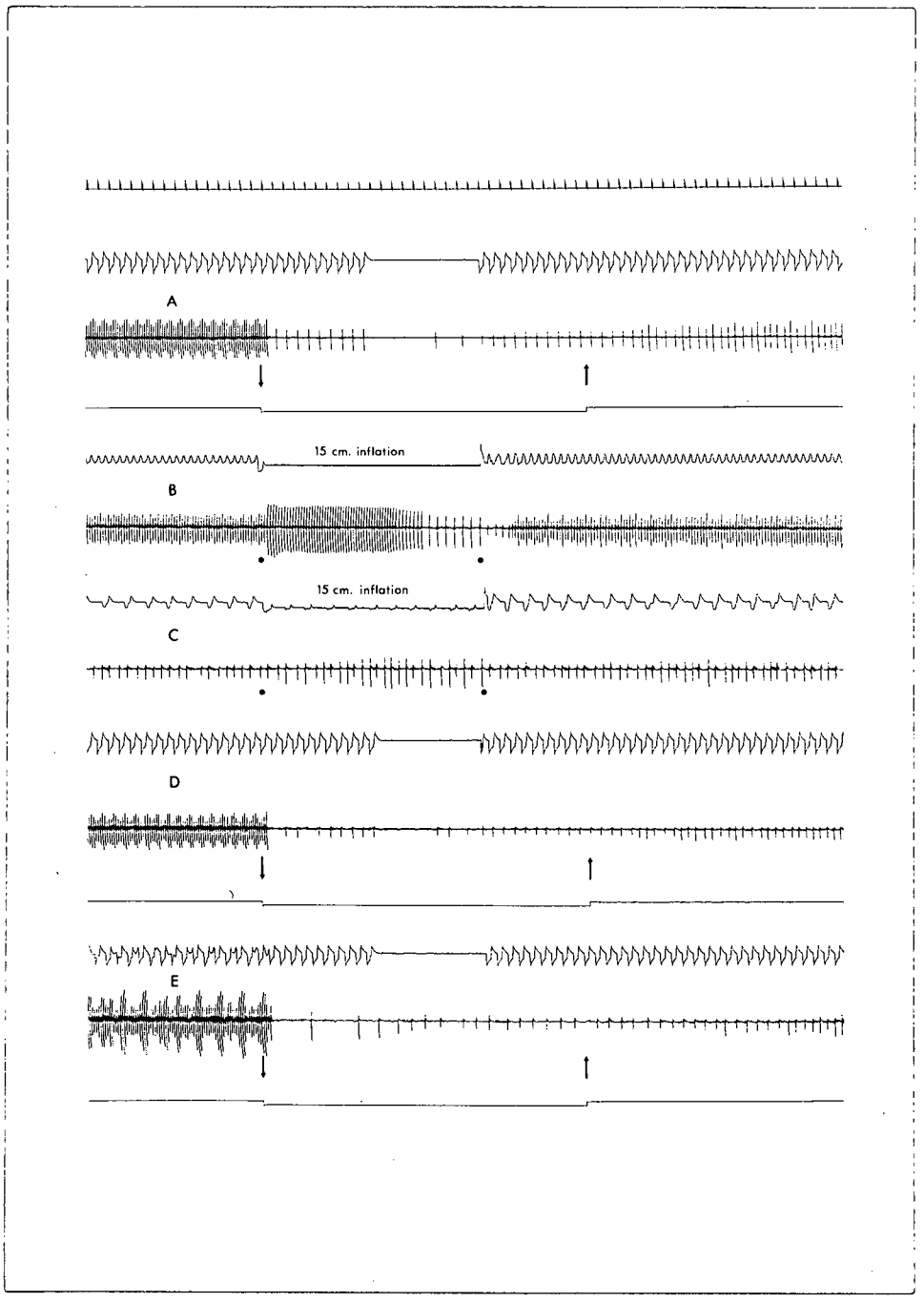

A
15 cm. inflation
B
15 cm. inflation
C
D
E

Figure 22. Loss of cardiac response to ventilation after sequential steaming in one muskrat. Histograms show cardiac response to 12 cm H_2O ventilation at 1 hz during water flow stimulation (32 ml/min) ±S.E.M. Top, control response with period of asphyxia indicated above in diagrammed artificial ventilation trace (A.V.); middle, after one breath of steam; bottom, after three breaths of steam. Water flow marker corresponds to a period of 30 seconds. The increase in heart rate after secondary steaming is the normal recovery pattern described earlier.

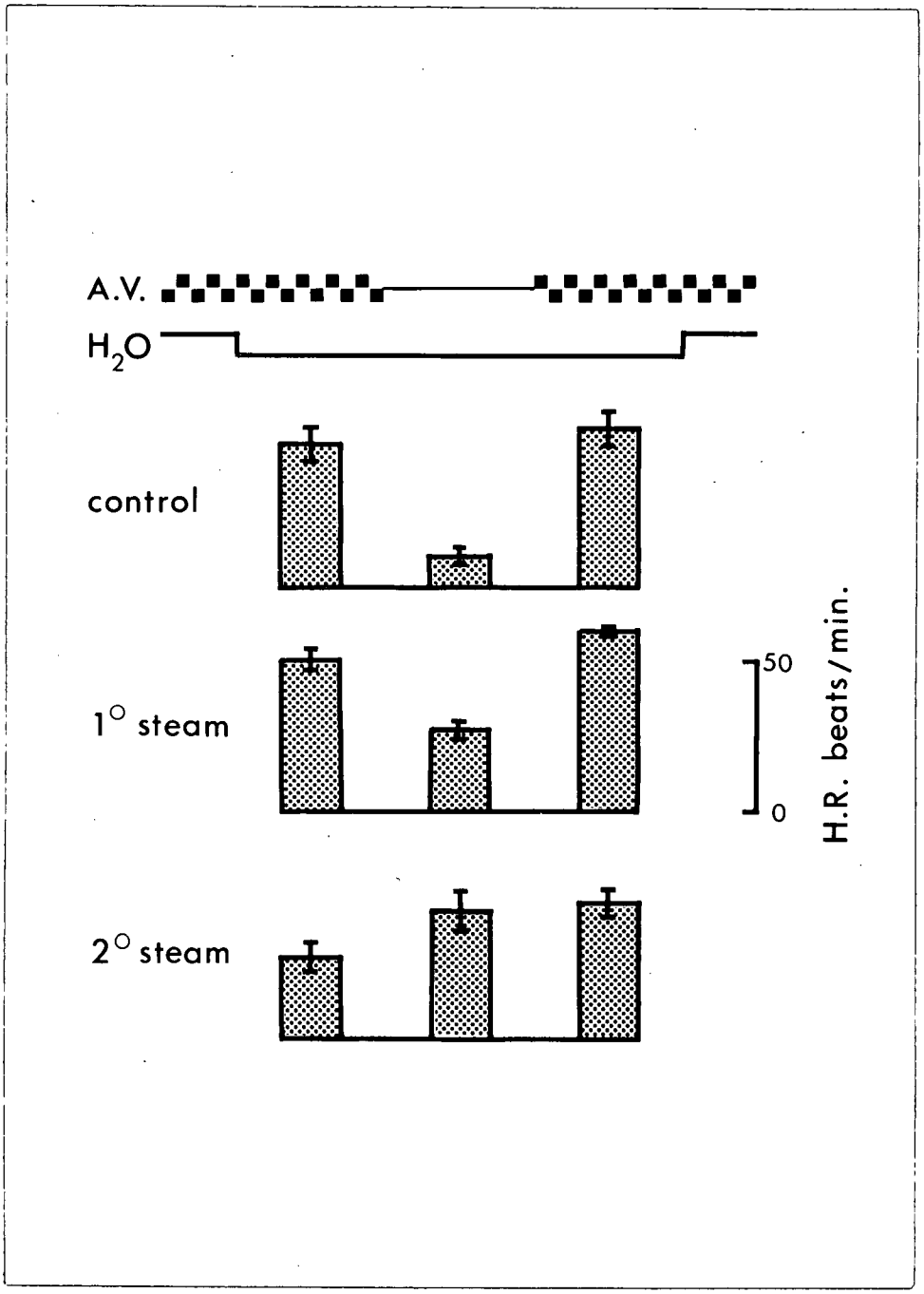
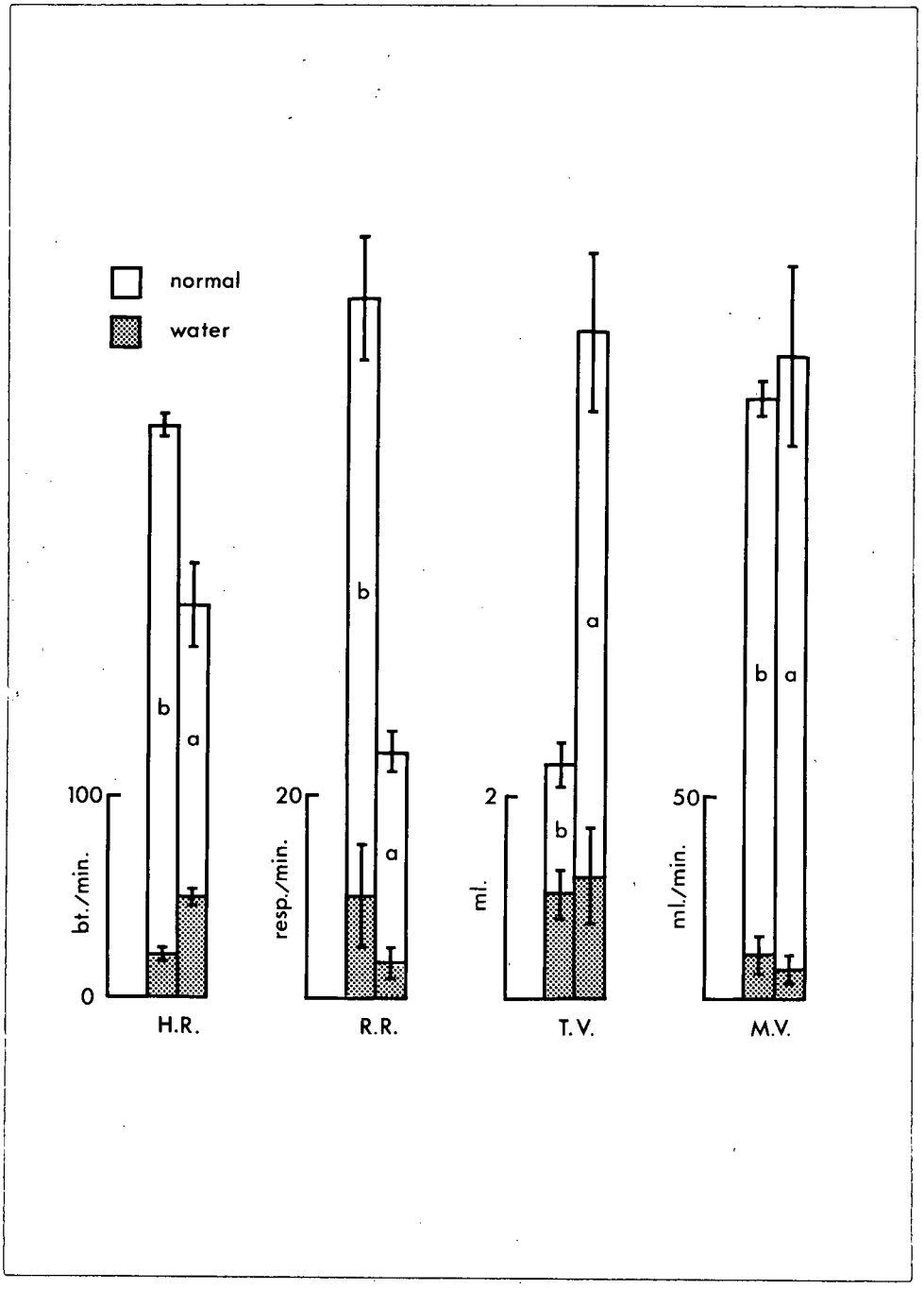

A.V.
H_2O
control
1° steam
2° steam
50
0
H.R. beats/min.

Figure 23. Effect of lung steaming on respiration and heart rate and their responses to strong nasal flow stimulation (32 ml/min). Vertical bars indicate the means of the heart rate (H.R.), respiratory rate (R.R.), tidal volume (T.V.) and minute volume (M.V.) before (b) and after (a) the loss of the Hering-Breuer reflex to constant inflation (12 cm H_2O) in 10 muskrats. Stippled bars show responses to water flow stimulation in a 20 second time period. Note that on average, the animals were non-apneic during nasal stimulation both before and after steaming. Values are the means ±S.E.M.


normal
water
100
0
bt./min.
b
a
H.R.
20
resp./min.
b
a
R.R.
2
ml.
b
a
T.V.
50
ml./min.
b
a
M.V.

minute volume (before 150±4 ml/min, n = 10; after 161±24 ml/min, n = 10) since although tidal volume increased by 3 times (before 2.3±0.2 ml/min, n = 10; after 6.7±0.3 ml/min, n = 10) respiratory frequency fell to one third the normal rate (before 70±6 per min, n = 10; after 24±2 per min, n = 10). Heart rate in the freely breathing controls was 286±6 (n = 10) and this fell to 197±22 (n = 10) in the lung denervates. Strong nasal stimulation caused heart rate to fall to 21.3±3 beats/min (n = 10) in intact freely breathing animals yet in the denervates heart rate only fell to 48.5±2 beats/min (n = 9). There was no apparent effect of deafferentation on the respiratory response to nasal water flow.

The loss of the cardiac response to artificial ventilation was demonstrated by plotting the heart rate response resulting from nasal stimulation combined with artificial ventilation ranging to a maximum of 324 (Fig. 24) to 875 ml/min. In each case ventilation frequency was one hz and deafferentation was considered complete when the heart failed to respond to increasing ventilation (i.e the heart rate-minute volume slope approached zero, Fig. 25). The failure to abolish the response in two animals apparently resulted from inhaling steam too rapidly (1 hz) since those steamings carried out at a slower rate (0.3 to 0.5 hz) were more effective.

Figure 25 also indicates the variability of the pre-treatment response to artificial ventilation. The higher slopes given for muskrats 5, 9, 13 and 14 were due to the fact that heart rate was not paced by ventilation frequency even at large ventilation pressures while in the other animals pacing of heart rate by ventilation was pronounced during strong nasal stimulation.

Figure 24. Abolition of the cardiac response to artificial ventilation by steaming. The response to 1 hz artificial ventilation is shown before (triangles) and after (circles) 4-6 breaths of steam. Ventilation pressures ranged up to 16 cm H_2O. The stippled blocks indicate the range of control heart rate-minute volume plots before (above) and after (below) the application of steam in one animal. Nasal stimulation was achieved with a water flow which caused apnea for 15 seconds. The lines were fitted by linear regression techniques and were based on a minimum of 6 points in each animal ($r = 0.99$, $m = 0.255$, and $r - 0.99$, $m = -0.007$ respectively for the pre- and post-treatment lines).

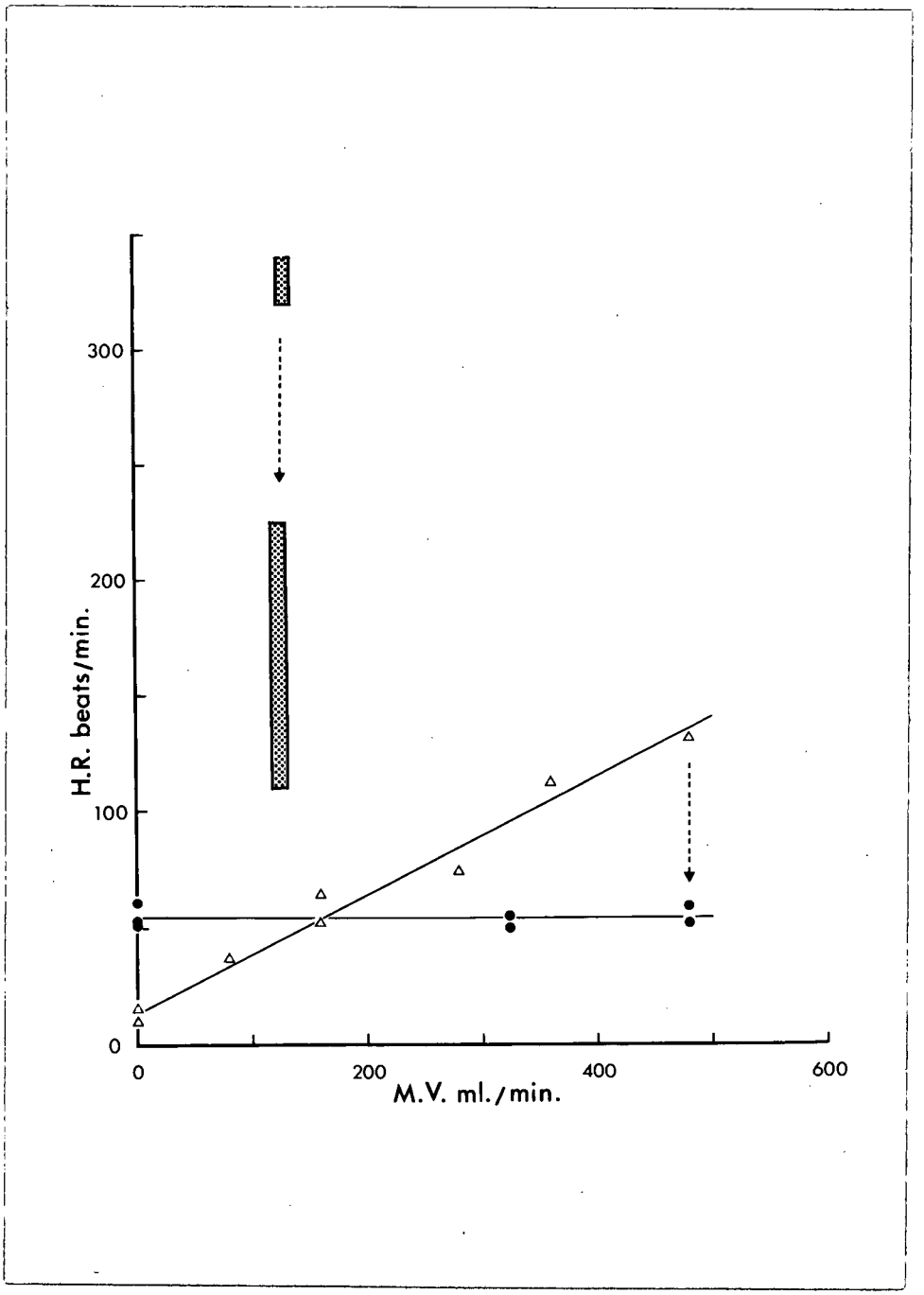
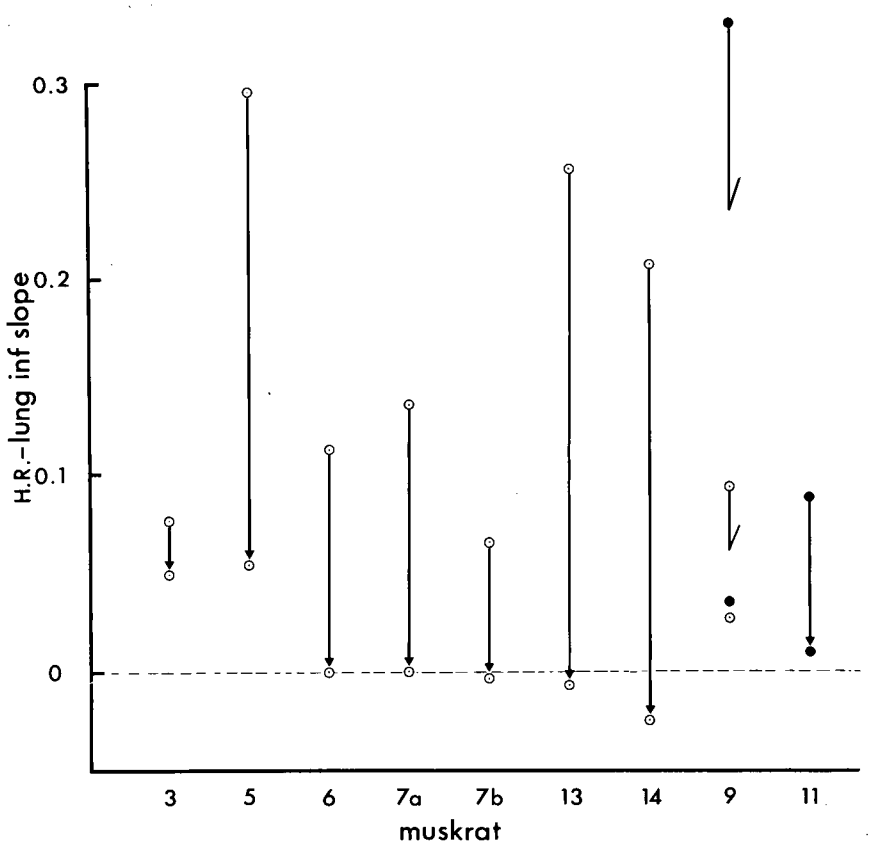

300
200
100
0
H.R. beats/min.
0
200
400
600
M.V. ml./min.

Figure 25. Loss of the cardiac response to artificial ventilation by steaming in 9 muskrats. Each vertical line represents the extent of the response loss taken from the heart rate-minute volume slopes during nasal stimulation (Fig. 24). The normal response is given at the top and the post-steam values near the dotted line; indicating complete abolition. Open circles represent response to water flow stimulation and the closed circles to saline flow.


0.3
0.2
0.1
0
H.R.-lung inf slope
3
5
6
7a
7b
13
14
9
11
muskrat

4.4 Effect of Lung Deflation on Heart Rate and Blood Pressure.

The effect of lung deflation per se on normal heart rate was investigated in 9 artificially ventilated curarized muskrats. The animals were usually ventilated with oxygen and PaO_2 remained constant throughout the test periods. Stopping ventilation with the lungs inflated had little effect on heart rate but lung collapse from any inspiratory pressure caused an immediate bradycardia which usually persisted from 5 to 20 seconds or until ventilation was resumed (Fig. 26). Lung deflation caused heart rate to fall from 268±7 to 59±4 beats/min within 0.97±0.17 seconds (n = 28) and neither the latent period nor the level of bradycardia was affected by the inspiratory pressure over the range of 5 to 15 cm H_2O. Blood pressure changes in response to inflation (decrease) or deflation (increase) were dependent on the inspiratory pressure but stabilized within 5 seconds and were unaffected by bilateral sinus nerve section. Maintained lung inflation by clamping the tracheal cannula at full inspiration caused a bradycardia after a period which was proportional to the inflation pressure (Fig. 27). When inflation pressures were varied between 5 and 15 cm H_2O, the time to the first appearance of bradycardia increased from 6.8±1.8 (n = 5) to 35.0±7.0 (n = 8) seconds respectively. The onset of bradycardia caused by deflation of the lungs however, was not changed when the inspiratory pressures were tested over the same range (0.76±0.15 and 1.28±0.20 (n = 8) seconds respectively).

5. Baroreceptor and Chemoreceptor Contributions to the Cardiovascular Responses.

An indication of baroreceptor activity was obtained from the degree of bradycardia which resulted from an increase in blood pressure caused by interarterial injection of adrenalin. Injection of 5 µg/kg of adrenalin

Figure 26. Cardiovascular effects of lung inflation and deflation. Top to bottom; arterial blood pressure (carotid), arterial blood oxygen, pneumotachogram (inspiration upwards), electrocardiogram and time trace (1 second marker). Inflation and deflation of the lungs were brought about by clamping the expiratory (upward arrow) and inspiratory (downward arrow) tubes of the respiratory valve. Inspiratory pressure was set at 12 cm H_2O throughout.

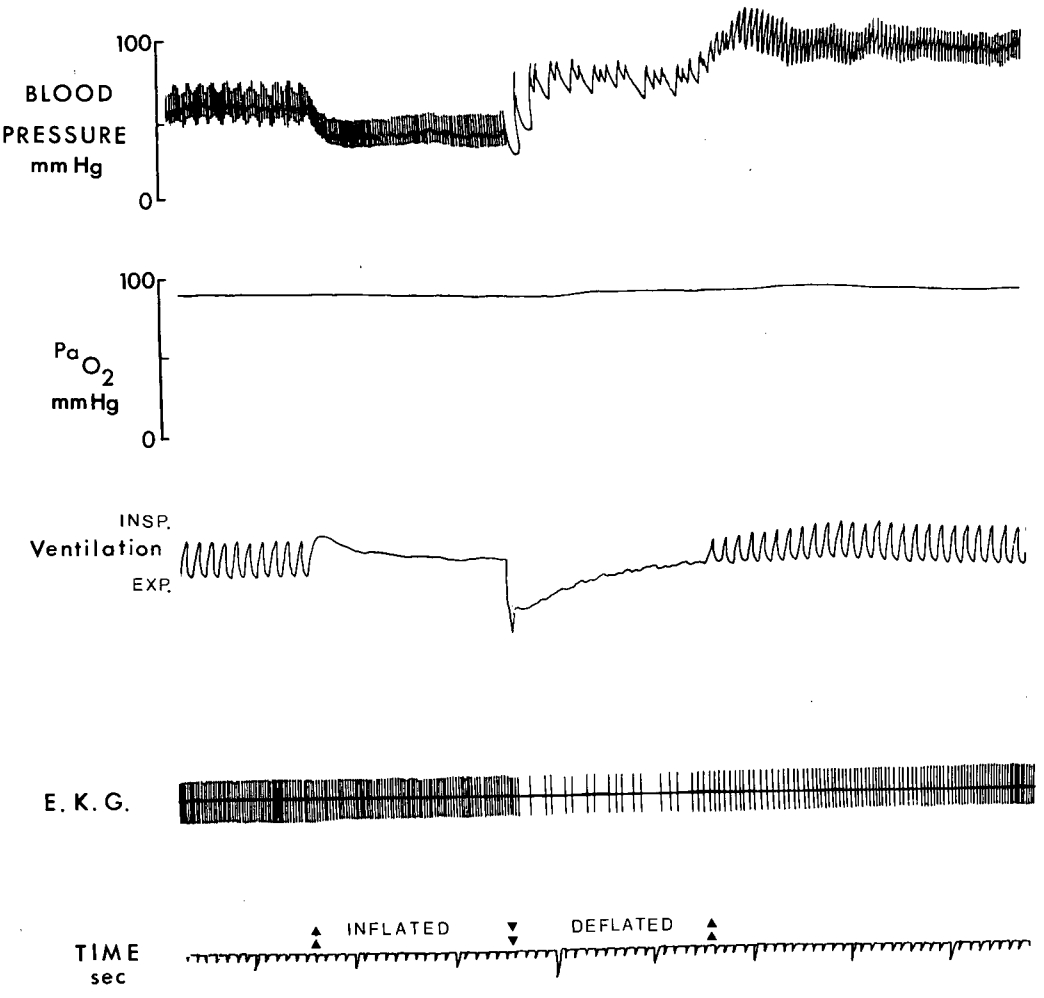
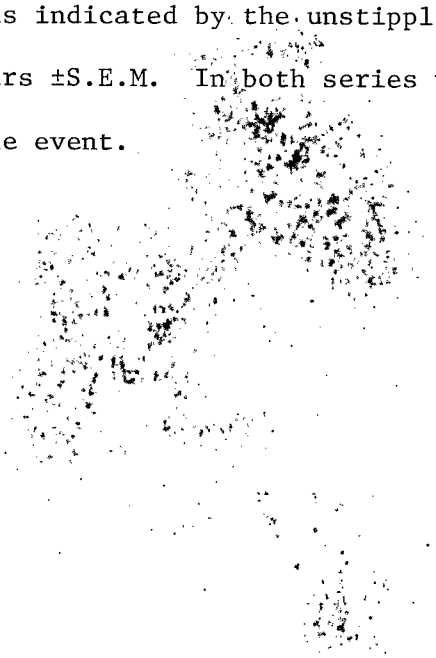
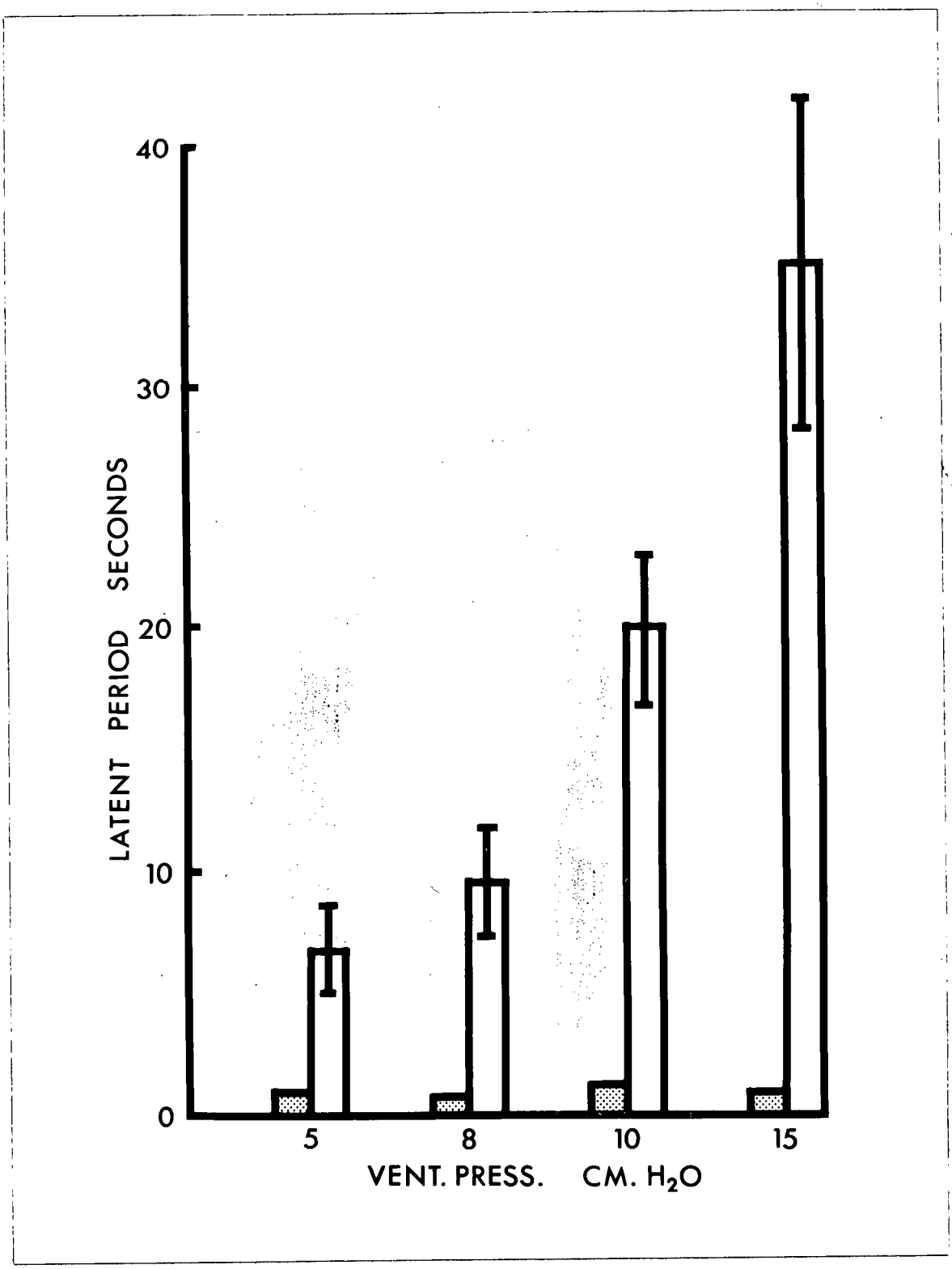

100
BLOOD
PRESSURE
mm Hg
0
100
Pa_{O_2}
mmHg
0
INSP.
Ventilation
EXP.
E. K. G.
INFLATED
DEFLATED
TIME
sec

Figure 27. Effect of inspiratory pressure on the latent period before onset of bradycardia caused by lung inflation and deflation. Inflation is indicated by the unstippled bars; deflation by the stippled bars ±S.E.M. In both series ventilation at 1 hz preceded the event.




40
30
20
10
0
LATENT PERIOD SECONDS
5
8
10
15
VENT. PRESS. CM. H_2O

caused the mean blood pressure to rise from 63±5 to 118±5 mm Hg (n = 6). A mean pressure of 115±4 mm Hg (n = 6) was required to elicit the cardiac barostatic response and at this pressure heart rate fell to 90±12 from a control rate of 253±6 beats/min. Bradycardia was maintained as long as the blood pressure was elevated in spite of continued artificial ventilation (Fig. 28). Denervation of the carotid sinus baroreceptors by sinus nerve section eliminated the barostatic reflex but had no effect on bradycardia caused by nasal stimulation or lung deflation (Fig. 28, right).

Carotid body chemoreceptor stimulation due to injection of 80-200 µg/kg potassium cyanide into the carotid artery caused hyperpnea and tachycardia in spontaneously breathing muskrats and transient bradycardia in curarized artificially ventilated animals. Bilateral sinus nerve section abolished both respiratory and cardiac responses to the injection of cyanide. Artificial ventilation of curarized muskrats with anoxic-normocapnic gas caused bradycardia when PaO_2 reached an average of 63±6 mm Hg (n = 6). Heart rate fell from an initial rate of 277±11 to 76±7 beats/min at a PaO_2 of 29 mm Hg (Fig. 29). Mean arterial pressure was little affected in the initial period of anoxic-normocapnic ventilation but rose when bradycardia occurred. Bilateral section of the sinus nerve delayed chemoreceptor driven bradycardia until PaO_2 had fallen to a partial pressure of 34±4 mm Hg (n = 9).

To further study the contribution of the chemoreceptors during simulated diving conditions, PaO_2 was continuously monitored in paralyzed muskrats which were asphyxiated with and without nasal water flow, and also when artificial ventilation was maintained during water flow. Five muskrats were prepared for this study by situating a temperature regulated oxygen electrode in a loop formed by cannulation of the right carotid artery. Asphyxiation

Figure 28. Abolition of baroreceptor reflex by sinus nerve section. Top to bottom, carotid artery blood pressure (mm Hg), arterial blood oxygen (mm Hg), pneumotachogram (inspiration upwards), electrocardiogram and time trace (1 second marker). Left, normal response to injection of 5 µg adrenalin into arterial loop; right, response to 5 µg adrenalin following bilateral section of sinus nerves. Recording also includes responses to 10 second period of asphyxia in the expiratory position (right). Ventilation at 8 cm H_2O.

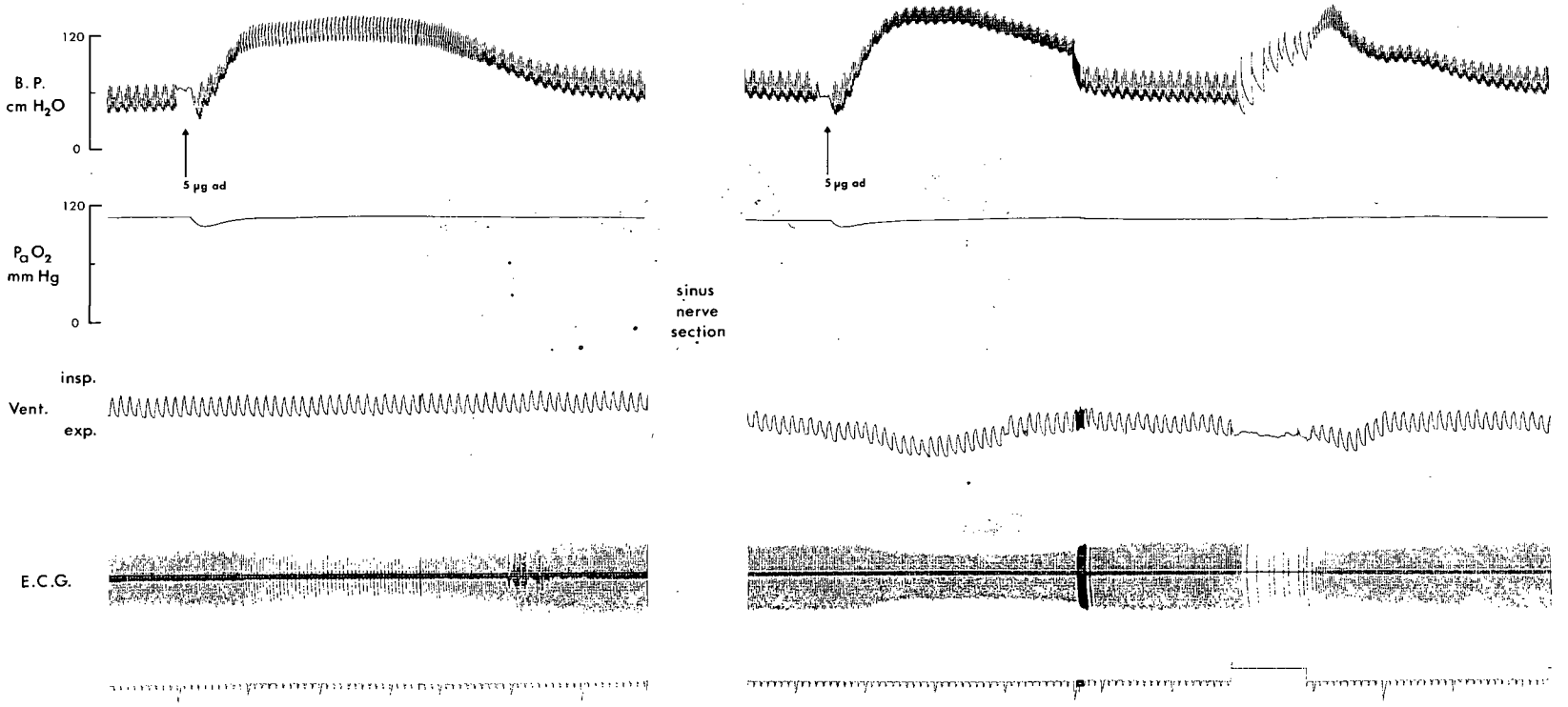
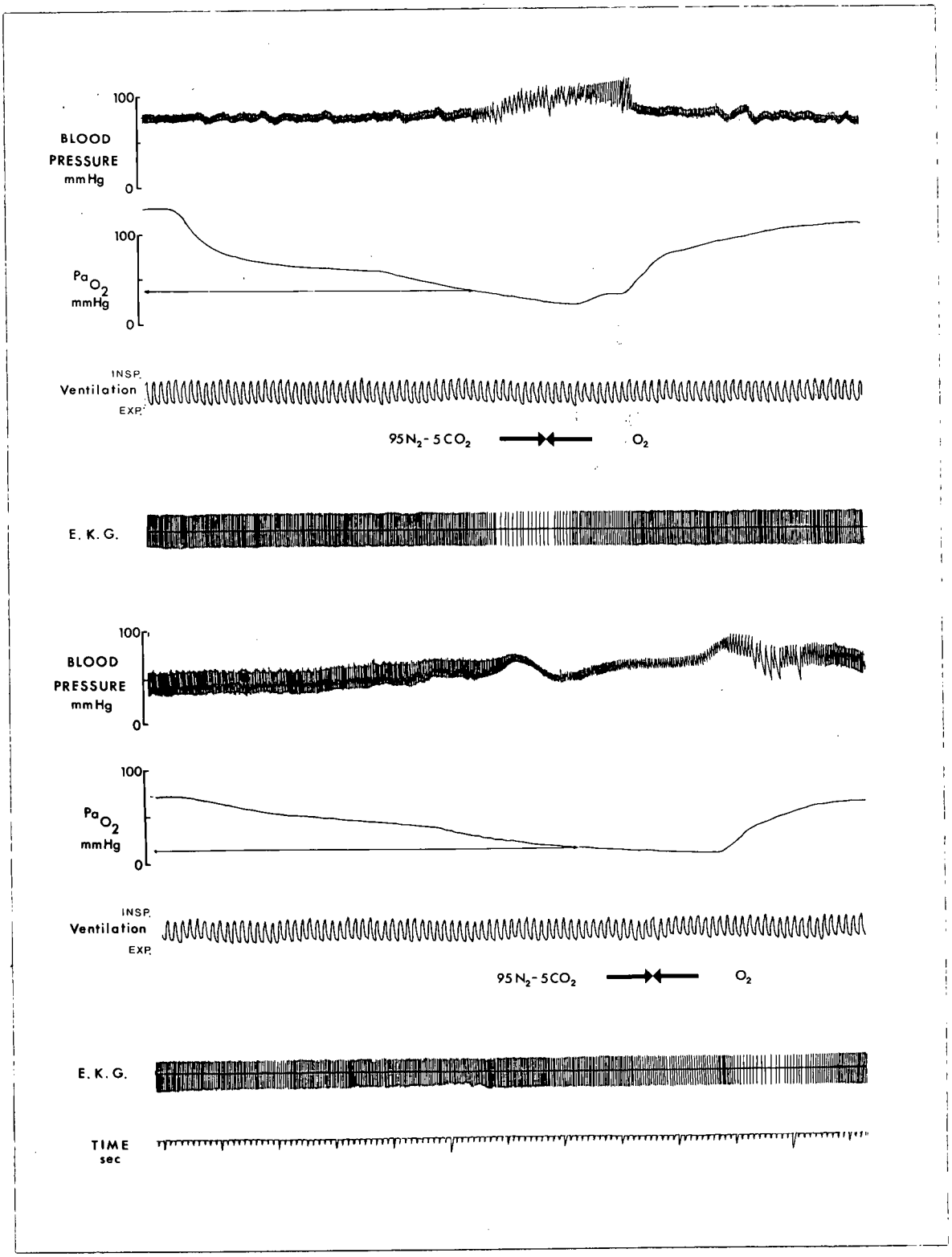

120
B. P.
cm H_2O
0
5 µg ad
120
P_aO_2
mm Hg
0
sinus
nerve
section
5 µg ad
insp.
Vent.
exp.
E.C.G.

Figure 29. Effect of sinus nerve section on the responses to hypoxia. Top to bottom, carotid arterial blood pressure, carotid arterial blood oxygen, pneumotachogram (inspiration upwards) and EKG. A, normal response; B, following bilateral sinus nerve section. Artificial ventilation was set at 10 cm H_2O and 1 hz. Ventilation with 5% CO_2 in nitrogen and 100% oxygen are indicated by the arrows. The onset of hypoxic bradycardia is indicated by the horizontal line. Time, 1 second marker.


100
BLOOD
PRESSURE
mmHg
0
100
Pa_{O_2}
mmHg
0
INSP.
Ventilation
EXP.
$95N_2$-$5CO_2$
O_2
E. K. G.
100
BLOOD
PRESSURE
mmHg
0
100
Pa_{O_2}
mmHg
0
INSP.
Ventilation
EXP.
$95N_2$-$5CO_2$
O_2
E. K. G.
TIME
sec

was brought on by clamping the tracheal cannula after expiration. Figures 30 and 31 compare the cardiac response to asphyxia with that when artificial ventilation was maintained during water flow stimulation and when water flow and asphyxia were combined. Bradycardia caused by asphyxiation alone did not begin until 8.3±0.6 seconds (n = 18) after tracheal clamping and heart rate continued to fall to a rate of 78±8 after one minute. The response to water flow stimulation was immediate (latent period 0.55±0.07 seconds, n = 10) with or without ventilation and heart rate fell to 53±7 (apneic) and 110±5 beats/min (ventilated) after 10 seconds of stimulation.

In view of the pronounced cardiac response to water stimuli in the anaesthetized muskrat, the rate of decline of arterial blood oxygen was greater than expected when compared to the asphyxic controls and the relative gradual fall found in PaO_2 in other animals of comparable diving ability (Irving et al., 1941b; Clausen and Ersland, 1968; Ferrante, 1970). Nevertheless, when the trachea was clamped PaO_2 began to fall sooner and more rapidly than if water was begun simultaneously with asphyxiation and the PaO_2 throughout the one minute trials were significantly different (95% confidence limit) if the control was normalized to 100% (Fig. 32). Normalization was required because of the variability of control PaO_2 to moderate levels of artificial ventilation. The latent period before a measured fall in PaO_2 was 7.3±1.2 (n = 15) and 11.4±1.5 (n = 14) seconds respectively for the asphyxic and water stimulated asphyxic responses. Figure 33 illustrates the relationship of heart rate and PaO_2 based on the data given in Figure 32. The graphs underline the dependence of heart rate on blood oxygen tension to about 40 mm Hg when the animal is asphyxiated. However, during water stimulation of the nares, cardiac function is apparently totally independent of PaO_2 during the entire experimental period.

Figure 30. Response of arterial blood oxygen tension to asphyxia in a curarized muskrat. A, nasal water flow (32 ml/min) coupled with artificial ventilation at 8 cm H_2O and 1 hz; B, asphyxia; C, nasal water flow with asphyxia. Initiation and termination of events are indicated by the arrows. Time marker is 60 seconds. The dip in PaO_2 at the onset of bradycardia (C) was artifactual and due to the flow sensitive nature of the oxygen electrode.

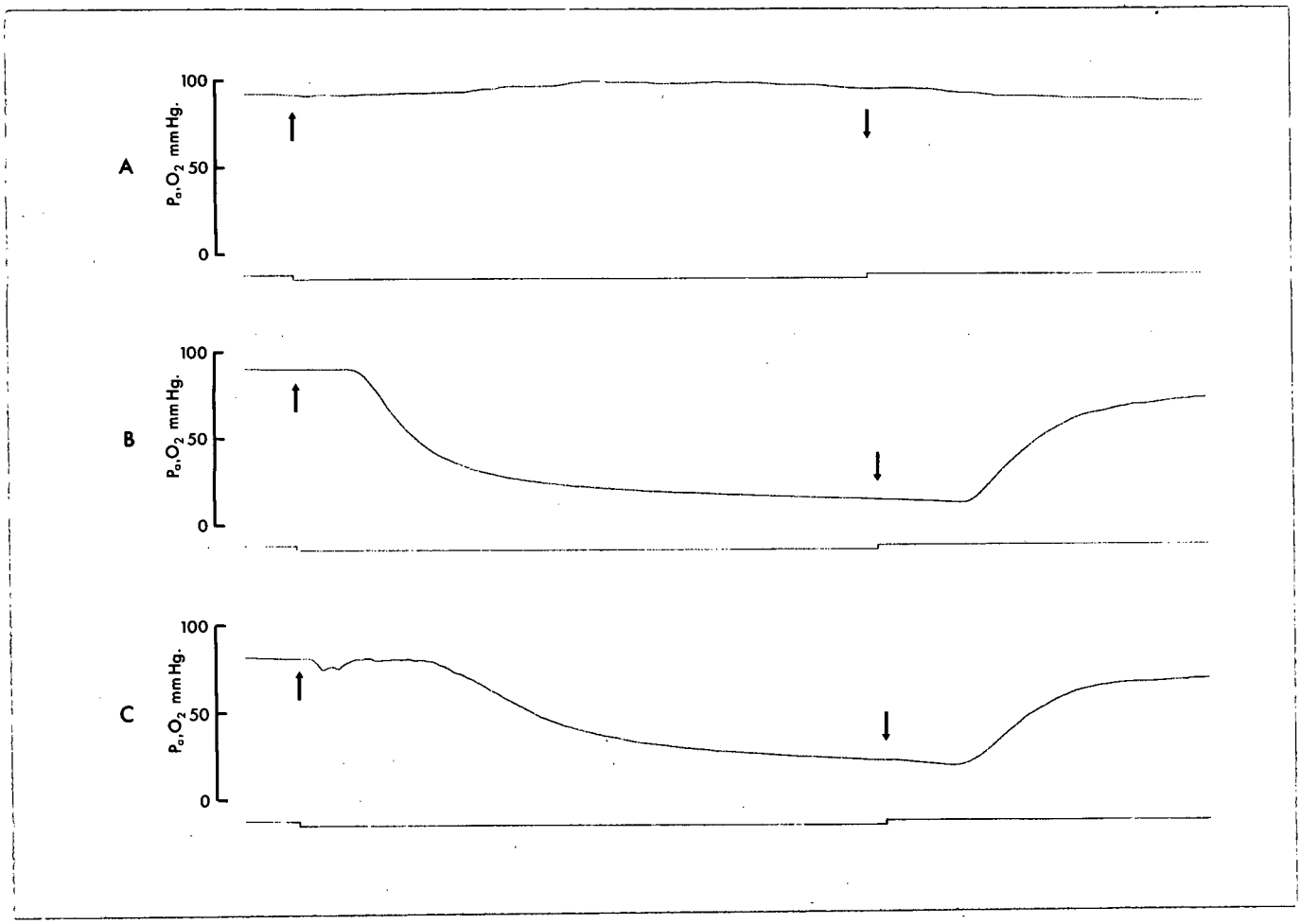

A
B
C
100
50
0
P_a, O_2 mm Hg.

Figure 31. Mean heart rate during asphyxia and water flow stimulation with and without artificial ventilation in five curarized muskrats. a, asphyxia brought on by tracheal clamping (n = 18); w, nasal stimulation with water flow of 32 ml/min coupled with artificial ventilation at 12 cm H_2O and 1 hz (n = 16); w & a, nasal water stimulation during asphyxiation (n = 22). All trials were 60 seconds in duration, beginning and ending at the markers.

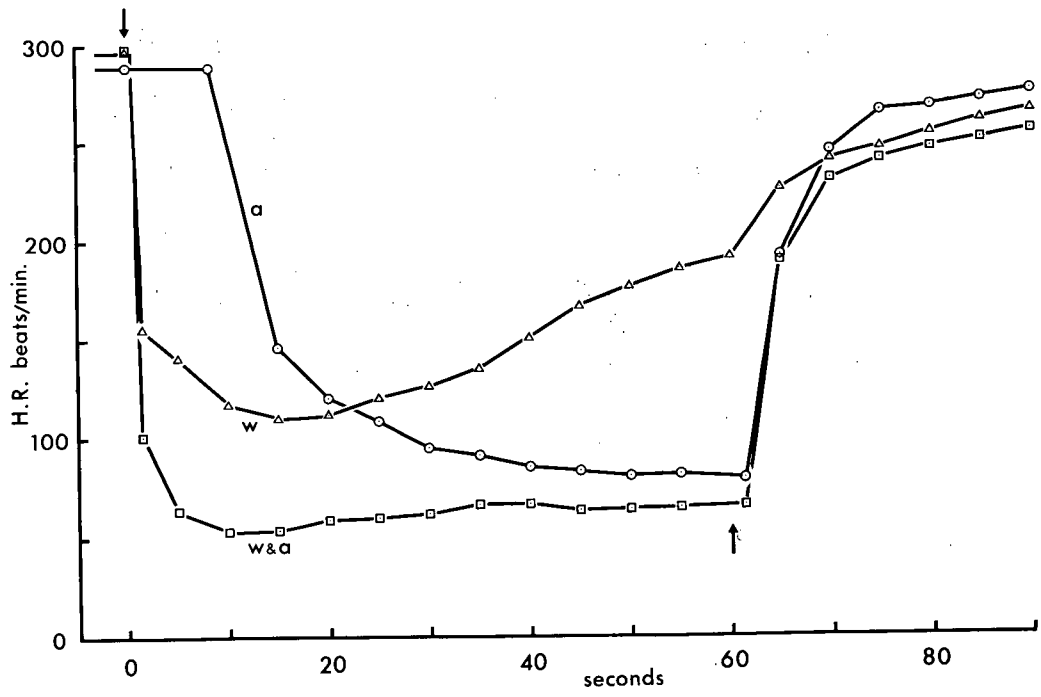

300
200
100
0
H.R. beats/min.
a
w
w&a
0
20
40
60
80
seconds

Figure 32. Response of arterial blood oxygen tension to water flow induced asphyxia in five muskrats. w, nasal water flow (32 ml/min) coupled with artificial ventilation at 8 cm H_2O and 1 hz (n = 10); w & a, nasal water flow during asphyxiation (n = 14); a, asphyxia (n = 15). Events were initiated and terminated at the arrows. All control values are normalized to 100%.

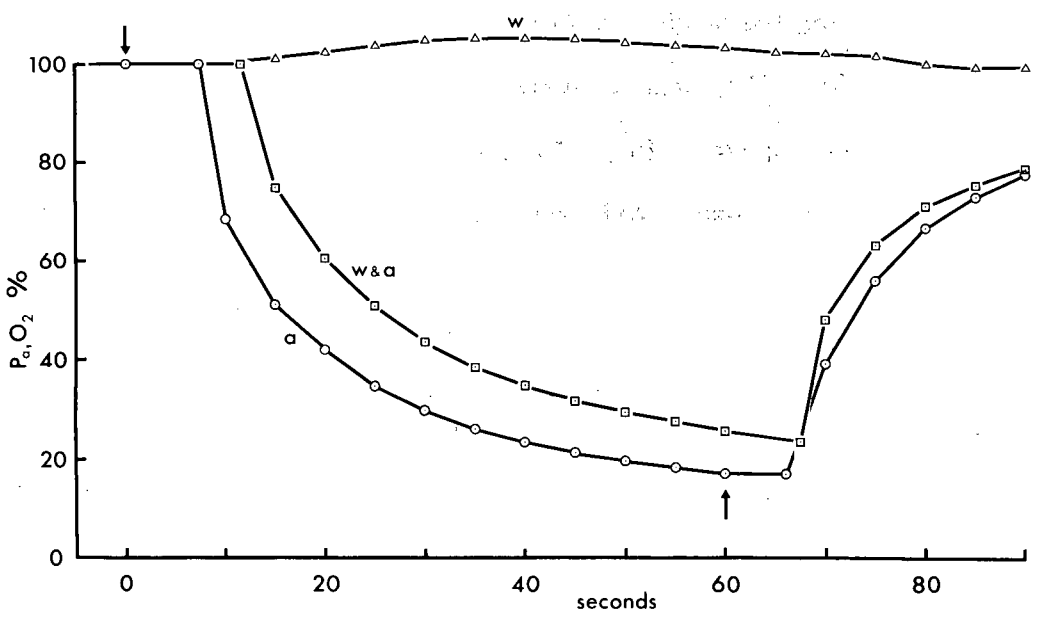
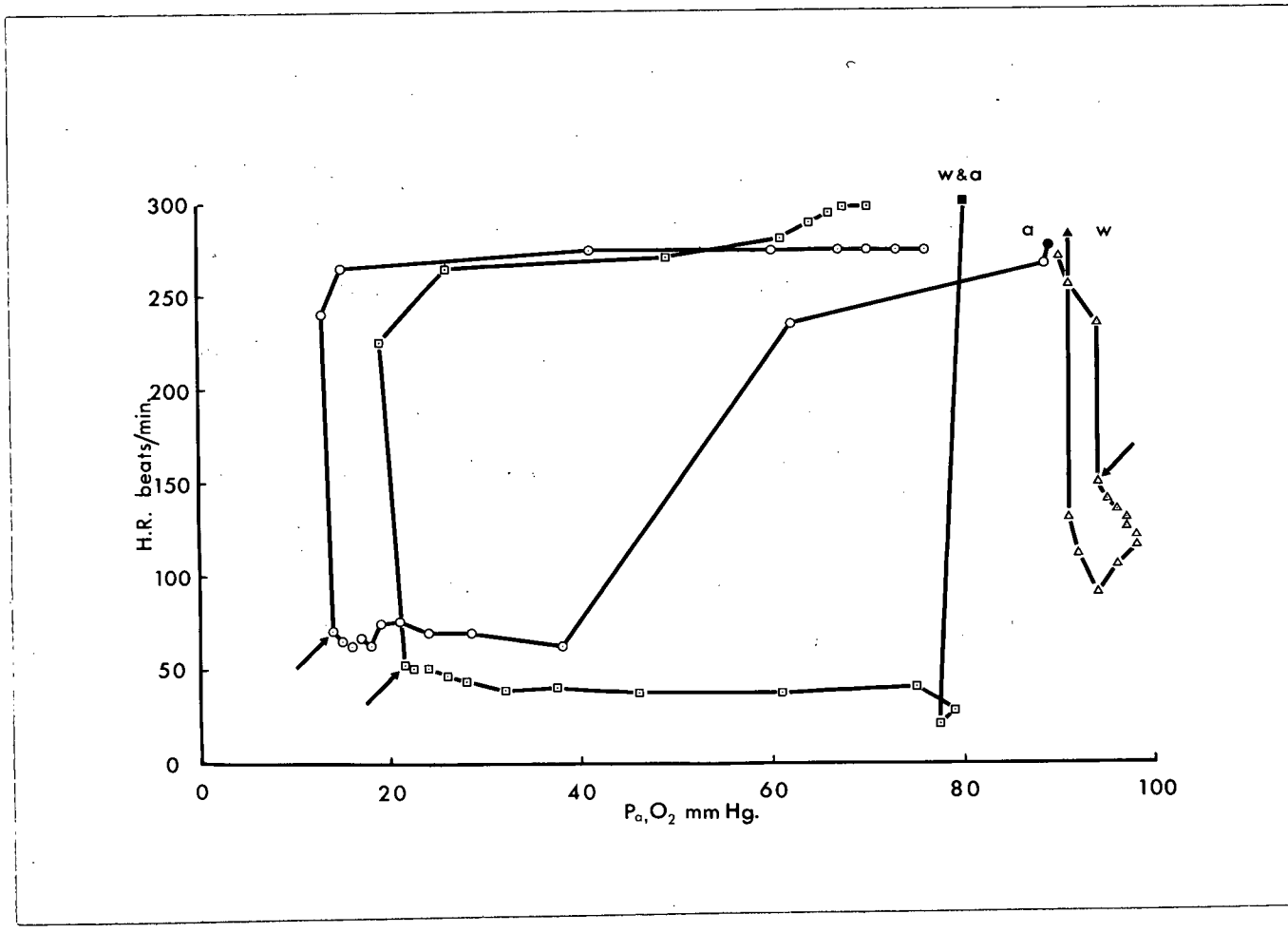

w
w&a
a
100
80
60
40
20
0
P_a, O_2 %
0
20
40
60
80
seconds

Figure 33. Plot of heart rate and arterial blood oxygen during asphyxia and nasal water flow. Points are as in Fig. 38 (5 muskrats). w, nasal water flow (32 ml/min) coupled with artificial ventilation at 8 cm H_2O and 1 hz; w & a, nasal water flow during asphyxiation; a, asphyxia. Points are plotted at 5 second intervals during 60 second events starting from control (closed markers) and ending at arrows.


w&a
a
w
300
250
200
150
100
50
0
H.R. beats/min.
0
20
40
60
80
100
$P_{a,}O_2$ mm Hg.

Discussion

The results from the present study show that even though the muskrat is not noted for its underwater endurance, it displays cardiovascular reflexes that are as profound as any found among marine mammals. The animal normally inhabits shallow waters and in the wild is rarely observed to dive for periods longer than 30 seconds although a tolerance of 12 to 17 minutes underwater has been reported (Irving, 1939; Errington, 1963). In this study, provoked submersions lasting up to 10 minutes were observed in Ondatra zibethica osoyoosensis which still far exceed that predicted from calculated oxygen stores if resting metabolism was maintained during the dive. Based on a blood oxygen capacity of 25% (Irving, 1939), a lung volume of 20 ml and a resting metabolic rate of 0.73 ml/gm hr (McEwan et al., 1974) the upper limit of diving time is about 2.7 minutes. Like the shallow diving seal however, the muskrat generally exhales air prior to diving and thus apneic endurance time without cardiovascular adjustment would reduce this estimate to approximately 2.3 minutes and near the diving endurance times found in terrestrial mammals of equal size (Irving, 1939). As a result it is not surprising that changes in heart rate and peripheral blood flow in the muskrat are dramatic (Lord, unpublished) and reflect an unusual ability to conserve oxygen.

In contrast to the well documented lability of heart rate in the marine mammal (Scholander, 1940; Irving et al., 1942; Van Citters et al., 1965; Elsner et al., 1966b), the resting muskrat displayed a regular EKG which was rapid and without any sign of the sinus arrhythmia which is so prominent in the seal. Sinus arrhythmia was found to occur only in the first few seconds of recovery from diving. The maximum cardiac response to submersion in the

freely diving animal averaged from 8 to 17% of the resting heart rate in a series of dives ranging up to one minute. Restrained dives on the other hand, yielded responses of 19 to 27% of the control rate but the difference between the two types of dives was largely a result of a significantly lower resting rate in the restrained animals. Nevertheless, while the stabilized underwater rate during forced diving was statistically higher than in the unrestrained animals, this indicates that the muskrat responds quite differently to restraint than either the seal or the porpoise. In fact, a greater response occurs during forced diving in the seal (Elsner, 1965; Harrison et al., 1972) while the porpoise displays surprisingly weak cardiac responses under these conditions (Elsner et al., 1966b).

In the freely diving seal, bradycardia has been variously measured at 5% (Harrison, 1960), 12% (Harrison and Tomlinson, 1960), 10% (Murdaugh et al., 1961), 14-28% (Harrison et al., 1972), 18% (Kooyman and Campbell, 1972) and 5-8% (Dykes, 1974a) of the pre-dive rate. In most cases however, the duration of these dives was much longer than those reported here and heart rate likely reflects to a greater degree, direct asphyxic responses. This is particularly evident in the elephant seal whose heart rate falls from 80 to 4 beats/min after a 40 minute dive while it reaches only one half the resting rate after one minute underwater (Van Citters et al., 1965). Terrestrial species such as man (Kawakami et al., 1967; Heistad and Wheeler, 1970; Whayne and Killip, 1967; Corriol and Rohner, 1968), cats (Lisander, unpublished observations) and the white rat show responses which are far slower in onset and much less intense than in aquatic or marine species. In the rat, submersion bradycardia reaches just 29-44% of the control rate after 30 seconds of dive time (Lin and Baker, 1975; Huang and Peng, 1976).

The relationship between the cardiac and vasomotor responses to diving is still a matter of speculation but it is clear that both are important particularly in prolonged dives. Species in which intense cardiac responses are recorded also show the most prominent increases in peripheral resistance (Irving et al., 1941b; Ferrante and Frankel, 1971; Folkow et al., 1971) yet the vasomotor response does not depend on the development of bradycardia (Murdaugh et al., 1968). Even though there may be an innate dissociation of the two reflexes in diving, heart rate is presumed to be an accurate reflection of the total diving response (Irving et al., 1941b; Leivestad, 1960; Blix et al., 1975; Blix et al., 1976a).

It has been suggested that the major role of the bradycardic response to submersion is not related directly to the conservation of oxygen since diving without a cardiac response in the seal does not affect short term diving ability (Murdaugh et al., 1968). Rather, these workers point out, that the reflex may be necessary to equate cardiac output with a reduced area of tissue perfusion. Irving et al. (1941b) however, claim that bradycardia per se in the seal accounts for a saving of approximately half the oxygen store in longer dives. This conclusion is supported by White and McRitchie (1973) who demonstrated that oxygen conservation in unanaesthetized rabbit is only effective when both cardiac and vasoconstrictor responses to nasopharyngeal stimulation are intact. They suggested that because vagal blockade results in a faster decline of blood oxygen tensions during stimulation, bradycardia aids in oxygen conservation by reducing pulmonary blood flow. Peripheral resistance changes in the unanaesthetized nutria are abrupt and have been shown to increase 10 fold in 10 seconds of submersion (Folkow et al., 1971) while in the seal the response is maximal after less than 20 seconds (Irving et al., 1942; Elsner et al., 1966a) suggesting that the rapid onset of

bradycardia is closely followed by a rapid increase in peripheral resistance. This view is also supported by work in the rabbit in which the cardiovascular effects to smoke inhalation were found to be fully established after 7-10 seconds of stimulation (White and McRitchie, 1973).

While it proved difficult in this study to isolate the external narial contribution from the internal nasal reflexes by denervation alone, one can safely conclude that narial receptors precipitate the train of diving reflexes in the muskrat. Apnea and bradycardia were caused by water lapping the nares of the conscious animal; a reflex which does not depend on the anterior portion of the maxillary division since bilateral section of this nerve at the level of the zygomatic arch had no effect on the responses. Studies on the innervation and stimulation of nerves leading to the nares indicate that the nasociliary nerve may be a major contributor to the reflexes originating from this area. On the other hand, abolition of these reflexes by anaesthesia explains the low sensitivity of the nasociliary nerve to electrical stimulation. Sensory innervation and motor control of the nares resides in the nasociliary but like the sphenopalatine and nasopalatine branches of the maxillary division which supply the nasal mucosa, it is not easily accessible and consequently its involvement in the reflexes was not tested directly. It is interesting to note that visual input seems to play no role in the generation of the responses during forced dives in the muskrat, but this should not be assumed to be the case in the wild. In marine mammals visual cues are known to have a profound effect on heart rate (Elsner, 1969).

While the afferent limb of the narial reflexes probably lies in branches of the maxillary or ophthalmic divisions of the trigeminal nerve and includes central suprabulbar connections found to be involved in reflexes at the conscious level (White et al., 1974), the findings do not necessarily conflict

with those of Dykes (1974b) who demonstrated that facial neurotomy in the seal alters behavioural responses but not the time course of bradycardia to submersion. Even in the absence of the narial reflexes, secondary sensory pathways within the nares and in the nasal passages would most likely produce responses no different from those initiated by narial receptors.

Variations in water temperature had no noticeable effect on the diving responses in the muskrat. This is consistent with results from ducks (Andersen, 1963a; Butler and Jones, 1968) and seals (Dykes, 1974b) but not in man in which bradycardia prompted by face immersion is temperature dependent (Kawakami et al., 1967; Song et al., 1969; Whayne and Killip, 1967; Moore et al., 1972). The results however, do not appear to agree with Thornton et al. (1978) who found a greater cardiac response in force dived muskrats when water temperature was lowered from 32 to 2^{o}C. Nevertheless, since these animals were immersed in water prior to the head being submerged, responsiveness to the colder temperature may have been increased. As in the anaesthetized dog (Angell James and Daly, 1972a), no temperature dependency of the reflex caused by nasal irrigation was found in the muskrat.

The finding that the afferent limb of the internal nasal reflexes in muskrats resides in the maxillary and internal laryngeal nerves is somewhat different from that found in sheep, rabbits and dogs. In sheep, cardiorespiratory responses induced by pulsatile water flow introduced into a tracheal cannula were abolished by section of the external (glottal) and superior laryngeal nerves (Tchobroutsky et al., 1969) but in rabbits nasal reflexes to irritant vapours seem to be carried in the olfactory and trigeminal nerves (Allen, 1928). On the other hand, respiratory and cardiovascular responses to nasal water flow in the dog are abolished by cutting the maxillary and ethmoidal nerves (Angell James and Daly, 1972a); the latter innervating the

mucosa of the nasal sinuses and similar in sensory function to the sphenopalatine branches referred to earlier. The location of the afferent limb on the medial aspect of the eye explains the oculo-cardiac reflex prompted by pressure on the eyeball (Aserinsky and Debias, 1961; Ganderia et al., 1978b). In the duck the most likely contributors to the nasal reflex are the ophthalmic division of the trigeminal (Andersen, 1963c) or the glossopharyngeal nerve (Bamford and Jones, 1974), the latter of which is considered to be stimulated by water drawn to the glottis in forced dives. Blix et al. (1976b) on the other hand, believe that the trigeminal as well as the glossopharyngeal nerves are involved in the elicitation of the diving responses.

Reflexes originating from the nasopharyngeal area are known to evoke respiratory and cardiovascular responses similar in magnitude to those found during diving. Experimentally these reflexes may be excited by fluid flow through the nasal passages (Tchobroutsky et al., 1969; Angell James and Daly, 1972a) and a variety of noxious vapours (Allen, 1928; 1929; Ebbecke, 1944; Angell James and Daly, 1969a; White and McRitchie, 1973). Even though water is not taken into the nares of the normally diving mammal, the reflexes are presumed to prevent further inhalation of water and set in motion the same oxygen sparing mechanisms and thus provide a latent afferent limb distinct from the narial reflex limb, which potentially offers a defense against drowning. Bamford and Jones (1974) and Leitner et al. (1974) have considered the possibility that the glottis of the duck may be regarded as a true reflexogenic site for diving apnea but conclude that the glottal receptors probably only contribute to the respiratory reflex initiated by trigeminally innervated receptors.

In addition to the differences in the afferent arms of the narial and nasal reflexes, evidence given here also points to dissimilarities among their

central connections. Anaesthesia or decerebration has a profound effect on the responses to submersion but with the exception of the rabbit, probably does not have a conspicuous effect on the cardiac and respiratory reflexes originating from within the nasal passages. Oddly apnea did not occur in the anaesthetized muskrat when dived but nevertheless the animal displayed a pronounced bradycardia caused by water stimulation of the nasal airways. Water stimulation of these areas including the glottis caused an apneic reflex which excluded water from the lungs. On the other hand, curarized and artificially ventilated animals were still able to give striking cardiac responses to submersion but lost the reflex if the nares was covered and water was not allowed to enter the internal nasal passages. The anaesthetized muskrat however, showed no cardiac responses during submersion if it was permitted to breathe freely through an exposed cannula, confirming that the narial reflex is abolished by anaesthesia.

The independence of the nasal reflexes from the higher brain centres in the muskrat is not surprising in view of the work by Huxley (1913) and Andersen (1963) on the diving duck. In both cases the authors reported only a slight decrease in reflex activity after decerebration or anaesthesia and there was no evidence that either procedure altered the afferent connections. Postural effects have long been regarded as a possible contributor to the diving reflexes; a claim first advanced by Huxley (1913) and later supported by Koppanyi and Dooley (1929) in muskrats. Both accounts reported that postural changes induce apnea and bradycardia. Other investigations however, fail to confirm these conclusions and find no marked responses on ventiflexing the head of the duck (Reite et al., 1963) or seal (Irving et al., 1935). The results reported here suggest that while bradycardia may be initiated by quickly ventriflexing the head of the muskrat, the response is shallow and

transient. Moreover, whether the animal begins the normal dive from in the water or on a perch, it appears not to ventriflex the head sufficiently to evoke a response that would contribute to an oxygen sparing adjustment.

The exclusion of water from the nasal passages of the muskrat is of course, a feature common to all diving mammals. But while the anaesthetized animals took water in to the level of the glottis, their intolerance to saline suggests that the afferent limbs of the narial and nasal reflexes are triggered by different types of receptors. Unlike the narial receptors those in the nasal passages are osmosensitive, slow adapting and can be found in terrestrial species (Harding et al., 1976; Harding et al., 1978).

The maintained responsiveness of the pharyngeal receptors in spite of an increasing heart rate throughout water stimulation implies that some central adaptation process must exist which is similar to that in the cat. Berger (1977) has demonstrated that the same central units which he believes are involved in the slowly adapting Hering-Breuer reflex, are also activated by the stimulation of the inferior laryngeal nerve and suggests that integration of these reflexes takes place at the medullary level. Receptors in the lower pharyngeal area are known to be related to apnea in the fetus (Barcroft, 1946) as well as in the adult (Boushey et al., 1972; Boushey et al., 1974; Storey and Johnson, 1975; Berger, 1977) and to be effective must be slowly adapting. Nasal receptors responding to water flow are considered to be free nerve endings situated in the epithelial mucosa or lamina propria (Cauna et al., 1969) but it is likely that these are mixed with other types (Boushey et al., 1974).

The dissimilarity of the contributions of the maxillary and inferior laryngeal nerve to the respiratory and cardiac responses indicates that the two afferent limbs have different central connections and that glottal-

pharyngeal input is more involved with respiratory control than with heart rate. Based on nerve blockade, the maxillary division accounted for 68.5% of the cardiac response to nasal water flow while the inferior laryngeal branch contributed the majority of the respiratory response (55.5%). These differences are not easily explained in terms of the freely diving animal because the means of stimulation was severe and unlikely to occur in the natural state of diving. At best, under the latter conditions, the effect on heart rate of internal nasal stimulation can be seen only as a marginal contribution to the muskrat and perhaps only underlines the close relationship between the respiratory and cardiovascular centres. It is obvious from the results given here that apneic tendencies, although transient, which can be initiated with as little as 50 µl of water may be an essential mechanism against water inhalation in aquatic species.

A surprising observation in the unrestrained dives was the apparent dependency of the cardiac response on the dive duration. In one animal the initial cardiac intervals on submergence seemed to indicate the length of dive to follow thus giving rise to speculation that the muskrat matches the degree of response with dive time. A similar relationship has been noted by Kooyman and Campbell (1972) who concluded that the Weddell seal prepares itself for diving activities. Jones et al. (1973) however, believe that the seal adjusts dive duration to correspond to the degree of response set early in the dive. The results given here show that rapid flight dives were invariably associated with a weaker cardiac response rather than the "maximum" initial bradycardia recorded from those dives in which the muskrat swam and remained under the covering wire before resurfacing.

These data, while confined to one animal, were highly predictable and point to a cardiovascular adjustment which is not a purely conditioned

response insofar as the animal fails to respond consistently to a given stimulus. Relative to the seal this suggests some form of poorly conditioned control of heart rate; either a full response is evoked when an oxygen sparing adjustment is required or a partial response occurs and heart rate is lowered to approximately 60-100 per minute. Since oxygen conservation is not required in shorter dives, it may be that the reduced response represents a conditioned overruling of the primary reflex. In either of the two types of diving found here, it is obvious that resurfacing was not prompted by the depletion of oxygen stores even if established heart rate was high. It is noteworthy however, that the distinction drawn between the two types was dependent on the "open" water available to the muskrat and thus the response may not have resulted from anticipation of diving time *per se* but rather from the nature of the dive.

Anticipatory reflexes to diving have been observed in some pinnipeds (Murdaugh *et al.*, 1961; Kooyman and Campbell, 1972; Jones *et al.*, 1973) and the trained porpoise (Elsner, 1965) and when present are much more prominent than those noted in the muskrat. In the freely diving duck anticipatory reflexes include a tachycardia, which evidently results from hyperventilation prior to immersion (Butler and Woakes, 1976; 1979). The results shown here indicate only an occasional bradycardic response before immersion of the nares but even then do not necessarily imply volitional control of heart rate since many stimuli evoke similar responses in the conscious animal. By the same token, anticipatory increases in heart rate before resurfacing are a common occurrence in most aquatic mammals (Irving *et al.*, 1941a; Murdaugh *et al.*, 1961; Kooyman and Campbell, 1972). Although this was not found in the muskrat, its presence in higher vertebrates suggests some overruling of the primary and

secondary reflexes by conditioning through higher centres and is reminscent of the modified cardiac response found in transient diving in the muskrat.

The cardiac response of the muskrat during short voluntary dives also contrasts to the labile nature of heart rate in the seal (Harrison and Tomlinson, 1960) and porpoise (Elsner et al., 1966b) in which it is sometimes not greatly affected by brief submersions or in feeding dives (Jones et al., 1973). On resurfacing, recovery of heart rate was always coincident with the re-establishment of the breathing pattern but when the animal was prompted to rapid successive dives heart rate did not increase appreciably between dives. The abnormal course of recovery was considered to be due to continuing apnea and thus underscores the importance of respiratory activity on the cardiovascular reflexes, particularly in the recovery period.

Since the muskrat typically dives for only short periods, the primary reflexes are likely to account for almost all of the cardiovascular responses brought on in a normal escape or foraging dive. The intensity of these reflexes leaves one to speculate that the chemoreceptors may function largely to maintain the pre-established responses rather than to induce further changes in dives of this length. This reasoning is supported by the observation here that a substantial bradycardia was maintained for at least one minute when ventilation in the curarized but unanaesthetized animal was continued during submersion. While this may be true in the muskrat, seal and other divers it clearly does not apply to all mammals. In the rat, for example, carotid body denervation virtually abolishes the entire cardiac response to submersion (Huang and Peng, 1976). In dives exceeding one minute, however, chemoreceptor reinforcement of the cardiovascular adjustments is probably much greater. Because heart rate increases during diving when

chemoreceptor input is reduced or withdrawn, while in the freely diving animal it continues to fall, suggests it may be that muskrats cannot maintain the initial responses but must rely on a secondary mechanism in long periods of asphyxia. If blood pressure remains relatively constant in the freely diving muskrat as it does in the seal (Murdaugh et al., 1961; Angell James et al., 1976) then baroreceptor driven responses are unlikely to be involved. Thus indirectly, it may be concluded that the greater cardiac response seen in unrestrained diving in the muskrat probably reflects facilitation of the trigeminal reflex by chemoreceptors such as the case in the dog (Angell James and Daly, 1973) and seal (Angell James et al., 1978).

It is well established that activation of lung receptors by hyperventilation can initiate tachycardia and systemic vasodilatation (Anrep et al., 1936a, b; Daly and Scott, 1958; Daly et al., 1967; Daly and Robinson, 1968). While this also appears to be the case in the muskrat, the effect of ventilation on heart rate is far more evident under conditions which favour bradycardia as forced ventilation even at high minute volumes in the anaesthetized animal resulted in only marginal increases in heart rate. Consequently high vagal tone seems to be necessary for the complete expression of respiratory effects on heart rate. This conclusion is supported by Anrep et al. (1936b) and Levy et al. (1966) who observed that sinus arrhythmia occurs in the dog only when vagal tone was present and subsequently found that the phenomenon was based largely on the diminution of vagal tone during the inspiratory phase. Their claim that the lung inflation reflex disappears in the presence of strong vagal activity however, does not apply to the muskrat.

As in dogs (Angell James and Daly, 1978a; Gandevia et al., 1978a; Aserinsky and DeBias, 1961), rabbits (White et al., 1974) and seals (Daly et

al., 1977) artificial ventilation in the muskrat tends to reverse the bradycardic response to nasal stimulation; the degree of which is approximately proportional to the minute volume. Unlike ducks (Bamford and Jones, 1976), no level of ventilation was found to fully overrule the cardiac response to apneic stimulation. Apneic stimulation given simultaneously with normal or above normal tidal volume ventilation most often yielded a 1:1 relationship between heart rate and the frequency of ventilation and is seen to reflect the peripheral component of sinus arrhythmia first investigated by Anrep et al. (1936b). The purpose of such a reflex is speculative but in the diver there seems little doubt that the re-establishment of normal heart rate and blood flow distribution is greatly accelerated upon resurfacing by pulmonary input. Thus it may be that the reflex permits a more rapid repayment of an incurred oxygen debt and a shorter period at the surface between dives. At least in the muskrat, pulmonary input also seems to be necessary to sustain normal heart rate as lung deafferentation resulted in significant decreases in both artificially ventilated and spontaneously breathing animals.

Based on sequential elimination of the respiratory and cardiac responses to artificial ventilation by the inhalation of steam, the receptors initiating the apneic response to maintained inflation are different from those causing cardioacceleration since the abolition of the Hering-Breuer reflex occurred first. There also seems to be little doubt that the same receptors which cause tachycardia also have a stimulating effect on respiration since even moderate tidal volumes tended to pace spontaneous breathing. Epithelial "irritant" receptors are known to promote reflex stimulation of breathing but they may also respond to maintained lung inflation and deflation (Mills et al., 1970). The inspiratory-exciting reflex resembles that found in cats (Larrabee and Knowleton, 1946; Glogowska et al., 1972) and rabbits (Davies

and Roumy, 1977) which is claimed to reflexly improve lung filling by a positive feedback mechanism and supports the conclusions of Knowleton and Larrabee (1946) that the principal action of these receptors on respiration is to reinforce breathing rhythm established through central connections. The phrenic nerve response to artificial ventilation however, indicates that eupneic breathing in the muskrat evokes the inspiratory-exciting reflex at a lower receptor threshold than that found in other mammals.

Lung deafferentation also produced some striking changes in normal heart rate as well as the expected slowing of spontaneous respiration. During lung steaming heart rate declined before a loss in the cardiac response to ventilation was obvious but the cause of this was not clear. Repeated high minute volume ventilation was found to temporarily restore normal rate indicating that there may be an incomplete anatomical separation of the two groups of receptors which lead to reflex apnea and cardioacceleration. Unlike the muskrat, the slowing of heart rate caused by pulmonary deafferentation by either steam inhalation (Hainsworth et al., 1973) or by nerve section (Gandevia et al., 1978a) does not occur in the dog.

The cardiac response to nasal water flow in the muskrat is unaffected by constant lung inflation to pressures well beyond that required to initiate the Hering-Breuer reflex. This is a similar observation to that in dogs in which rhythmic inflation was a far more effective means of reversing vasomotor responses to trigeminal stimulation (Davidson et al., 1976; Angell James and Daly, 1978a). Constant inflation of the lungs however, did not always produce tachycardia in muskrats as shown in the dog (Hainsworth et al., 1973) and when present was never maintained for more than a few seconds. In fact, bradycardia developed with ongoing inflation after a period which was proportional to the inflation pressure. Baroreceptor denervation does not alter the

course of heart rate change in the dog during inflation of the lungs (Hainsworth et al., 1973; Hainsworth, 1974) even though increases in intrathoracic pressure are known to impede venous return and thereby tend to lower blood pressure and initiate the barostatic reflex. The "secondary" slowing of heart rate during lung inflation is considered to arise from pulmonary receptor adaptation since it is abolished by section of the pulmonary vagi (Anrep et al., 1936a).

In the absence of nasal stimulation the state of lung inflation has a marked effect on arterial blood pressure. Inflation causes an immediate hypotension whereas deflation results in an approximately equal change in pressure; both responses being independent of inhaled carbon dioxide and carotid sinus innervation. The hypotensive response to constant lung inflation is not unique to the muskrat since it is also found to occur in the beaver (Irving, 1937), dog (Hainsworth, 1974) and rabbit (Ott and Shepard, 1971). The slowing of heart rate in response to deflation on the other hand, has been the subject of a number of studies (Nahas, 1956; Angell James and Daly, 1969b; White and McRitchie, 1973) but its relationship to the vasomotor response has not been determined. In the muskrat, the cause of these responses was not tested further but it is presumed that both the bradycardic and vasomotor responses to static lung volume changes arise from pulmonary receptors since they are eliminated by a few breaths of steam (Hainsworth et al., 1973) and are unaffected when baroreceptor activity is held constant (Hainsworth, 1974). The bradycardic response to deflation in the rabbit, however, requires an intact barostatic reflex (White et al., 1974).

The barostatic reflex causing bradycardia in the muskrat occurred at about 115 mm Hg when hypertension was induced by adrenalin; a pressure which is considerably less than in the non-diving state in the seal. Baroreceptor

driven bradycardia in the diving mammal however, is probably triggered at a lower pressure than that indicated here for water applied to the face of the anaesthetized seal resets the barostatic reflex towards bradycardia and thus potentiates a reflex which may already contribute to the diving adjustments (Angell James et al., 1978b).

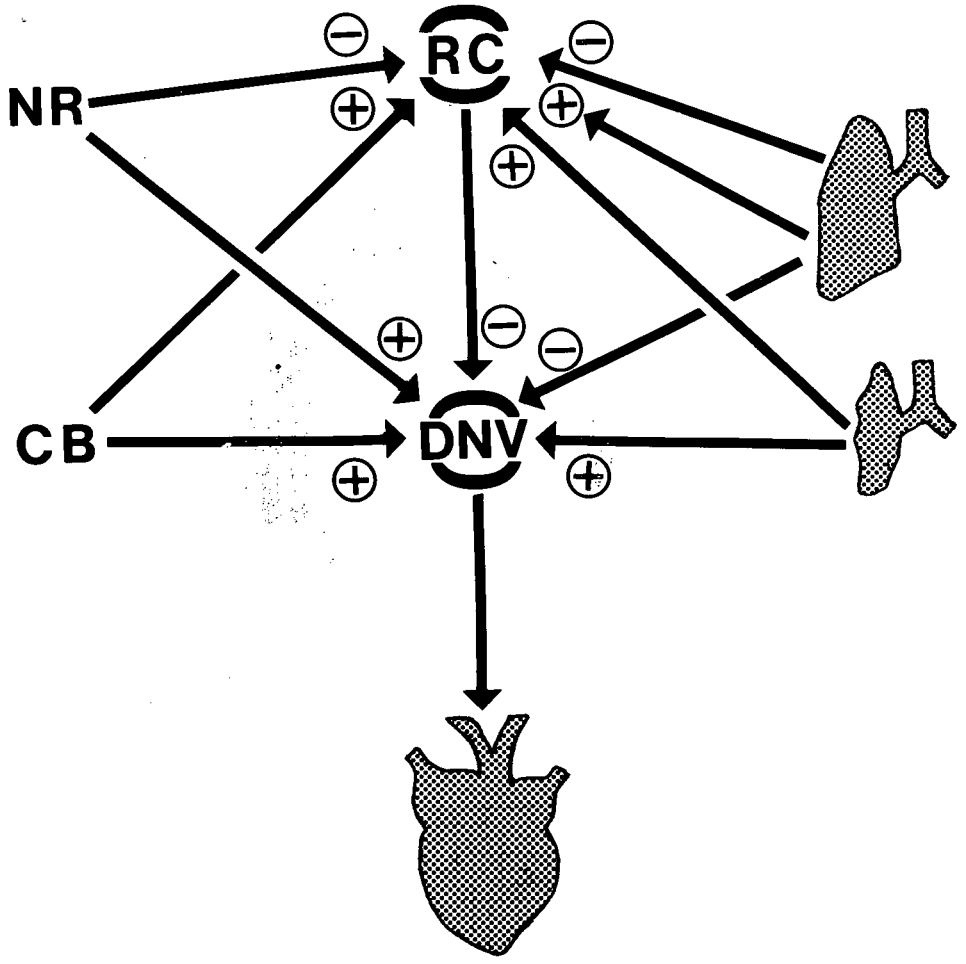
There is little doubt that chemoreceptors are involved in the cardiovascular responses in prolonged diving but whether this activity originates from sites other than the carotid body is not known. Bilateral sinus nerve section in the anaesthetized muskrat delayed chemoreceptor driven bradycardia from a PaO_2 of 63 mm Hg to 34 mm Hg indicating that the carotid bodies are by far the most chemosensitive units but not the only ones responding to hypoxia and hypercapnia. In prolonged stimulations of the nasal mucosa PaO_2 declined to 44 mm Hg within 20 seconds, so in spite of anaesthesia it is reasonable to assume that the chemoreceptors will contribute to the cardiovascular responses at this time. It should be pointed out however, that chemoreceptor bradycardia was investigated during artificial ventilation and therefore it is likely that this contribution will be made at an even higher PaO_2 during apnea.

The present results suggest that in the normal diving mammal the cardiac response to submersion could be an expression of at least three groups of receptors. Individually each group seems to have a direct influence on both the respiratory and cardioinhibitory centres (Fig. 34) and in the absence of other stimuli, each reflex is potentially able to evoke cardiac responses in the same order of magnitude as that observed in the freely diving muskrat (Table III). Lung deflation per se provoked bradycardia but failed to increase the cardiac reflex when coupled with nasal stimulation. No conclusion may be reached as to whether the deflation reflex facilitates the response to narial stimulation at the point of submersion. While the chemo-

Table III. Experimental tests causing bradycardia in the muskrat. Mean values of heart rate are shown before (control) and at a time of maximum response. Mean heart rate during diving of restrained animals is compared to response in other tests and the significant differences noted. X, difference between means is significant (P<0.05%). 0, difference is not significant (P>0.05%). n = number of observations.

	Control	Test	Significant difference	Notes
Restrained dive	266±3 (n = 66)	51±2 (n = 60)	-	
Unrestrained dive	310±3 (n = 102)	27±3 (n = 3)	X	Dives >40 sec
		73±3 (n = 50)	X	Dives <4 sec
Lung deflation	268±7 (n = 23)	59±4 (n = 23)	0	Paralyzed
Nasal water flow (32 ml/min)	277±5 (n = 34)	20±2 (n = 34)	X	Anaesthetized and apneic
Nasal saline flow	273±9 (n = 12)	38±3 (n = 12)	X	Anaesthetized and apneic
Nasal water flow with lungs deafferentated	195±21 (n = 10)	48±2 (n = 9)	0	Anaesthetized and apneic
Forced dive under anaesthesia	281±5 (n = 56)	39±2 (n = 39)	X	Non-apneic (40 sec)
		84±5 (n = 56)	X	Non-apneic (1-2 sec)
Water on nose	292±6 (n = 6)	76±2 (n = 6)	X	Paralyzed and ventilated
Hypoxia	277±11 (n = 6)	76±7 (n = 6)	X	Paralyzed and ventilated; PaO_2 = 44 mm Hg
Barostatic reflex	253±6 (n = 6)	90±12 (n = 6)	X	Paralyzed and ventilated

Figure 34. Neural mechanisms regulating respiration and heart rate in the muskrat. RC, respiratory centre; DNV, dorsal nucleus of the vagus; NR, nasal receptors; CB, carotid bodies. The inputs from the inflated lungs (right) to the respiratory centre refer to the Hering-Breuer reflex (top arrow) and to the effect of lung ventilation. The effect of inflation on heart rate appears to be direct since cardiac pacing by lung inflation occurred irrespective of apnea induced by nasal stimulation. Sudden lung deflation also seems to have direct links to both centres. Increases in respiratory activity were observed following deflation (deflation reflex) but this also caused a bradycardia. The diagram also illustrates the paradoxical effects of nasal and carotid body stimulation on respiration and heart rate. Although bradycardia always accompanied apnea during nasal stimulation, a decrease in heart rate also occurred under conditions in which respiratory rhythm was continued with forced ventilation. Carotid body stimulation alone increased respiratory activity but also yielded bradycardia during ventilation hypoxia when there was no conspicuous increase in respiration.


RC
NR
CB
DNV

receptor reflex no doubt facilitates the trigeminally induced responses later in the dive, the baroreceptor function in diving is speculative. Baroreceptors in the muskrat have been shown to cause bradycardia in response to drug induced hypertension but beyond a possible need to make slight readjustments to the more dramatic changes caused by the narial and chemoreceptor reflexes, no role for them is evident in diving. On the other hand, the cardiac response to lung ventilation seems to be necessary for the maintenance of normal heart function while its primary contribution to diving is confined to the recovery period.

SUMMARY

1. Heart rate in the muskrat was recorded during restrained and unrestrained diving. In dives which were 40 sec in duration, the unrestrained animals developed a relatively stable heart rate which was significantly lower (27±3) than in those trials in which they were restrained (56±4). There was no difference in the onset of bradycardia in the two types of dives. The unrestrained response represents a heart rate of about 9% of the resting rate and is similar to that found in free range diving in pinnipeds.

2. Anaesthesia failed to abolish the cardiac response to submersion and in fact, heart rate followed approximately the same course as in the forced dives after a significantly longer latent period (591±40 vs 300±10 msec). Breathing movements continued during these dives particularly when the muskrats were dived into saline.

3. Evidence was presented that the muskrat exhibits some form of poorly conditioned response in the initial phase of submersion. The animal does not show significant anticipatory responses to submersion or emersion such as those which characterize diving in the seal. It was concluded that the muskrat probably responds to the nature of the dive rather than to "anticipated" diving time.

4. It was concluded that while higher centre input plays a role in diving ability, the primary reflex to submersion originates in receptors located at the nares and is probably carried in its afferent limb by the nasociliary nerve. Division of the main maxillary nerve trunk which inner-

vates the narial flaps, failed to affect the cardiac response to submersion.

5. Anaesthesia blocks the external narial reflex and allows nasally inhaled water to initiate a nasal reflex. It is thought that the principal function of this reflex is to exclude water from the trachea but nasal stimulation in this manner also evokes striking cardiorespiratory responses. Bradycardia is the most prominent of these but transient apnea and vasomotor responses are also evident.

6. The nasal reflex originates in the glottis and pharynx and is carried by the inferior laryngeal and maxillary nerves. The former appears to provide the greater respiratory response (55%) while the latter initiates most (68%) of the cardiac response to nasal water flow. The laryngeal receptors which contribute to the afferent limb are slow adapting and osmosensitive.

7. The effect of respiratory activity on heart rate was shown and the significance of the interaction in regards to the diving mammal was discussed. The muskrat like the dog, demonstrates the fullest expression of the cardiac response during apnea. However, it may be at the point of resurfacing that central and peripheral input leading to cardiovascular changes have their greatest effect.

8. Lung deafferentation by steaming results in a significant decrease in normal heart rate suggesting that pulmonary input in the muskrat is required for the maintenance of normal cardiovascular function.

9. The pulmonary receptors initiating the Hering-Breuer reflex to constant inflation are distinct from those which increase heart rate caused by ventilation. The receptors which have this cardiac influence however, are probably the same as those which augment the respiratory cycle since both responses are lost simultaneously during progressive deafferentation by steam. Although the Hering-Breuer receptors are slow adapting, they have no direct effect on the cardiac response during submersion.

10. Bradycardia may be evoked by lung deflation per se. The slowing of heart rate began 0.97±0.17 seconds after deflation and this time period was independent of the inflation pressure.

11. Hypertension induced by adrenalin caused a barostatic reflex at 115±4 mm Hg indicating that blood pressure increases due to peripheral vasoconstriction during a dive would be limited at this pressure and tend to lower heart rate even further.

12. Bilateral sinus nerve section in the anaesthetized muskrat delayed chemoreceptor driven bradycardia from a PaO_2 of 63 mm Hg to approximately 34 mm Hg and indicates that the carotid bodies are the most chemosensitive units but not the only ones responding to changes in blood gas tensions.

13. Although chemoreceptors undoubtedly act to maintain the diving responses, it is likely that the external narial reflex accounts for almost all of the cardiovascular adjustments brought about in a normal foraging or escape dive since these are usually of short duration. In dives approaching one minute or longer, the chemoreflex probably plays a significant

role in these responses and to prompt the animal to resurface before the expiration of oxygen stores.

14. The present study shows that in the muskrat, the cardiac response to submersion results from at least three groups of receptors. These cause the primary narial reflex initiated by water contact at the nares, a lung deflation reflex and a chemoreflex both resulting from the ensuing apnea. The interaction of these reflexes was discussed.

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