ARTERIAL BARORECEPTOR CONTROL OF THE CIRCULATION
DURING FORCED DIVES IN DUCKS
(ANAS PLATYRHYNCHOS var.)

by

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When dabbling ducks are involuntarily submerged, arterial vasoconstriction produces a large increase in the peripheral resistance to blood flow which is balanced by a decrease in output of the heart, and arterial blood pressure is maintained. Arterial baroreceptors sense systemic blood pressure, and provide the afferent information to the baroreflex for pressure regulation. The effector limbs of the baroreflex are the same as those involved in the diving responses, and the baroreceptors are likely to be important in the integration of the cardiovascular responses to diving. The purpose of this study was to investigate the role of the arterial baroreceptors in maintaining blood pressure during diving, and in the initiation and maintenance of the diving responses.

Baroreceptor function was studied by diving ducks at various times after barodenervation, and by electrically stimulating the central end of one baroreceptor nerve in baroreceptor-denervated animals to simulate a controlled baroreceptor input before and during submersion.

Intact baroreceptor innervation was not necessary for the development of peripheral vasoconstriction, but loss of the baroreceptors modified the cardiac response to submersion
by impairing the vagally mediated bradycardia. There was no effect of baroreceptor nerve stimulation on peripheral resistance during diving, and the baroreflex operated via the heart rate in modulating blood pressure early in the dive. Later in the dive, stimulation was ineffective in altering either heart rate or blood pressure. Strong chemoreceptor drive results from decreased blood oxygen and increased carbon dioxide levels in the dive, and the results of experiments to determine the mechanism of baroreflex attenuation showed that an interaction between chemoreceptor and baroreceptor inputs may be at least partly responsible for reducing baroreflex effectiveness.

The main conclusion from this work is that the arterial baroreceptors contribute to the diving responses through modulation of heart rate, to help balance the fall in cardiac output against the baroreceptor-independent peripheral vasoconstriction in the first minute of forced dives.
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The experiments in Section I were performed in collaboration with D. R. Jones and W. K. Milsom.
GENERAL INTRODUCTION

The cardiovascular adjustments in diving animals during submersion are the result of an integrated set of reflex responses which reduce cardiac output and redistribute it to those organs, the heart and the brain, which cannot function without oxygen. Cardiac output may fall by 80-90 %., while vasoconstriction in peripheral vascular beds, such as skin, skeletal muscle, gut and kidney may elevate peripheral resistance by a factor of 10 or more. In the face of these changes, arterial blood pressure is maintained during diving, which ensures adequate supplies of nutrients to those organs which remain perfused. The barostatic reflex, or baroreflex, which buffers short-term changes in blood pressure, operates through the same effector arms as the diving response, and thus the arterial pressure receptors, or baroreceptors, may have an important role in control of the circulation during diving.

The systemic arterial baroreceptor reflex, or baroreflex, is the collective name for the changes in systemic vascular calibre and cardiac output occurring in response to changes in input from baroreceptors, located in the walls of large arteries, which sense arterial blood pressure in the central circulation. The baroreflex operates as a negative-feedback system in the awake, intact animal, rapidly correcting moderate displacements of blood pressure
from the current mean. The baroreceptors themselves are sensory nerve endings interleaved with the structural elements of the arterial walls of the baroreceptor zones. When the vessel walls are stretched circumferentially, due to a rise in blood pressure, these nerve endings respond by generating potentials which trigger impulse firing in the baroreceptor nerve fibres (Brown, 1980). These afferent fibres enter the brainstem where they ultimately exert their influence on the vasomotor and cardiomotor neuron pools which control cardiac output and the hydraulic resistance of the vascular tree (Spyer, 1981). An increase in vessel wall stretch at the baroreceptor zones, as blood pressure rises, results in reflex reductions in cardiac output and in the peripheral resistance to blood flow. Blood pressure is thus restored to the level before the disturbance. The direction of vaso- and cardiomotor effects is reversed in the baroreflex responses to a fall in blood pressure.

Paul Bert, in 1870, established the importance of cardiovascular adjustments in prolonging the underwater endurance of divers when he found that ducks developed a pronounced bradycardia during forced submersion and chickens did not. The neural mechanisms subserving the diving responses was not addressed directly until Huxley (1913) obtained a diving bradycardia in decerebrate ducks, thus delimiting this cardiac reflex pathway to the brainstem. This work was later confirmed and extended by Andersen...
That the vagus is the efferent pathway for cardiac retardation in diving ducks was first demonstrated by Richet (1899), who used the cholinergic blocking drug atropine to prevent bradycardia. This observation has since been confirmed, by vagotomy and pharmacological means, in all other vertebrate divers which have been examined (see Butler and Jones, 1982 for summary). Some authors have reported a residual bradycardia after atropinization in ducks (Hollenberg and Uvnas, 1963; Folkow, Nilsson and Yonce, 1967) and coypu (Folkow, Lisander and Oberg, 1971) which was eliminated in ducks by sympathetic beta-adrenergic blockade (Folkow et al., 1967). The engagement of the sympathetic cardiac nerves in control of the avian heart during diving is controversial, however, since the results of Folkow et al. (1967) could not be confirmed by Butler and Jones (1971). On the other hand, there is no doubt that the peripheral vasoconstriction accompanying submergence results from increases in sympathetic vasoconstrictor-fibre tone, as indicated by pharmacological studies in birds (Djojosugito, Folkow and Yonce, 1969; Kobinger and Oda, 1969; Andersen and Blix, 1974; and Butler and Jones, 1971) and in mammals (Folkow et al., 1971).

The subject of the afferent pathways which entrain the diving response has generated a great deal of controversy in the years since the work of Bert and Richet, with much of the
disagreement probably stemming from species differences. The importance of apnea in the diving response in birds was recognized by the early investigators, but the reflexogenic zone for apnea was not located until Andersen (1963a, b) and Butler and Jones (1968) found, in dabbling ducks, that stimulation of receptors in and around the nostrils, nasal cavity and internal respiratory passages of the head with water initiated breath-holding. Feigl and Folkow (1963) concluded that apnea alone was not enough, however, to produce bradycardia in dabbling ducks; there must also be subsequent reduction in arterial oxygen tension and increased CO2 tension, sensed by chemoreceptors in the bloodstream, for the full expression of this response. This was confirmed by Bamford and Jones (1974).

Hollenberg and Uvnas (1963) described experiments in which the tissues surrounding the carotid bodies in ducks were ligated and divided surgically in an attempt to denervate a possible arterial chemoreceptor site. They found that this procedure prevented most of the bradycardia, but they believed that they had also denervated arterial baroreceptors. No indication was given in their work that the authors had tested their denervates to confirm the loss of either chemoreceptor or baroreceptor function. Nevertheless, later work by Jones and Purves (1970) firmly established the role of vagally innervated carotid body chemoreceptors in producing forced-dive bradycardia in ducks,
and this was confirmed by Holm and Sorensen (1972). The fall in heart rate during forced submersion in dabbling ducks is thus due to altered blood gas tensions, sensed by peripheral chemoreceptors, subsequent to the cessation of breathing initiated by trigeminally-mediated receptors in the glottis and narial region of the beak.

Most early diving studies focused on the change in heart rate as an index of the extent of the diving response, because rate is measured easily and is a major component of the response. Irving, Scholander and Grinnell (1942) appear to be the first workers to have measured blood pressure during diving, reporting that, in the seal, pressure is maintained well in the face of the large fall in heart rate. These authors noted that the rate of fall in pressure during the long diastolic periods was much slower in the dive than occurred predive; they also found that pressure recorded from a small artery in the toe decreased markedly during diving. From these results, Irving et al. (1942) concluded that peripheral vasoconstriction must be closing off large portions of the circulation to maintain pressure as the heart slowed. Vasoconstriction during diving has since been documented in forcibly-submerged ducks (Johansen and Krog, 1959; Johansen and Aakhus, 1963; Hollenberg and Uvnas, 1963; Butler and Jones, 1971) although maintenance of blood pressure in the dive was variable in these experiments. Hollenberg and Uvnas (1963) reported increased pressure in
short dives of 1-2 min, while Butler and Jones (1971) and Johansen and Aakhus (1963) reported pressure drops in dives of the same length. Eliassen (1960), however, reported no change in either systemic or pulmonary arterial pressures in diving birds. McKean (1982) also reported maintained blood pressure in force-dived beaver and nutria, and showed in one trace of abdominal aortic blood pressure during a dive that there was no change in pressure during an extended diastole of 23 sec (McKean, 1982, Figure 3).

Given that the diving response employs the same efferent pathways as the baroreflex, it is reasonable to suppose that arterial baroreceptors play an active role in generating and maintaining cardiovascular adjustments to diving. Johansen and Aakhus (1963) considered this hypothesis when they measured blood pressure and heart rate during forced dives, but proposed that, since there was no consistent rise in systolic pressure prior to the onset of bradycardia in their experiments, arterial baroreceptor stimulation would not have been sufficient to bring the baroreflex into play and, therefore, the bradycardia was not baroreflex-induced. Hollenberg and Uvnas (1963) considered that, in their presumed chemo- and barodenervated ducks, the reduction in bradycardia was due to elimination of peripheral chemoreceptor input, and not to loss of the baroreceptors. However, it is doubtful that their experimental procedure did, in fact, ablate baroreceptor input.
Andersen (1966), in a thorough review of the circulatory adaptations to diving, suggested that the baroreceptors could play a role in generating the bradycardia during diving, and that the baroreflex may operate in response to subtle pressure rises which might not have been easily detectable by the methods then available. Folkow et al. (1967) attempted to evaluate baroreceptor contributions to circulatory control in ducks during diving by rapidly withdrawing blood from the central circulation (unloading the baroreceptors), and also by rapidly injecting the same blood volumes into the circulation (loading the baroreceptors). Neither of these manoeuvres produced detectable reflex heart rate effects in the dive, and the authors felt that input from the baroreceptors was therefore not important to balance cardiac output and peripheral resistance in maintaining dive blood pressure. However, later in the same paper they stated that in atropinized or vagotomized animals, which cannot lower heart rate in a dive, "the balance of the baroreceptor reflexes seems to prevent the intense vasoconstriction, and the animal will drown if kept submerged" (Folkow et al., 1967, p.359), implying a role for the baroreceptors in control of peripheral resistance. Kobinger and Oda (1969) also speculated that baroreceptors were of secondary importance in the dive response, since in experiments in which the peripheral vasoconstriction (and thus possible early-dive hypertension) was prevented by bretylium-induced
blockade of peripheral norepinephrine release, bradycardia
still occurred to nearly the same degree as in the untreated
control animals. If, in untreated animals, a baroreflex-
driven bradycardia occurred consequent to the hypertension
induced by vasoconstriction at the beginning of the dive,
this cardiac response should have been entirely eliminated by
preventing the vasoconstriction.

In a brief report, Holm and Sorensen (1970) stated that
combined peripheral chemo- and baroreceptor deafferentation
prevented bradycardia in 1 min dives in ducks. The authors
did not present evidence from any tests to confirm either
chemo- or barodenervation, nor did they state their methods
of denervation. They also contended that, since stimulation
of the peripheral chemoreceptors with cyanide or nicotine did
not produce a bradycardia in either the spontaneously
breathing or paralyzed intact animal, these receptors could
not be responsible for the diving bradycardia. The same
authors, in a 1972 paper, reversed their position on the role
of the chemoreceptors, concurring with Jones and Purves
(1970) that chemoreceptors are of primary importance in
generating the cardiac response to diving. In their 1972
paper, Holm and Sorensen presented details of their combined
chemo- and barodenervation procedure, which consisted of
cutting all small nerve branches leaving the distal vagal
ganglion (nodose ganglion). They believed that there were
baroreceptors in what they called the "carotid trifurcation"
(presumably that area of the common carotid artery at the base of the neck in birds which gives rise to the caudal thyroid and carotid body arteries, and to the artery supplying nearby sections of the airway, though this is not made clear), innervated, according to their Figure 1, by a small nerve branch from the nearby nodose ganglion. Holm and Sorensen (1972) also state that there is an innervation of the carotid body, and a connection to the nodose ganglion, from cranial nerve IX, the glossopharyngeal: the latter contention is not supported by other authors who have explored the anatomy in this area (Jones and Purves, 1970; Abdel-Magied and King, 1978).

In a pharmacological study of the pressor response to diving in ducks, Butler and Jones (1971) speculated from indirect evidence that baroreceptors may have a role in the cardiovascular adjustments to diving. They found that, in atropinized ducks, blood pressure rose by 50 % in the dive. The authors suggested that, had the same amount of vasoconstriction occurred in the vagally blocked animals as developed in untreated animals, pressure would have risen by much more than 50 % in the dive. They proposed that the baroreflex acted to reduce the pressure rise in atropinized ducks by limiting vasomotor outflow.

This line of investigation was further pursued by Blix, Gautvik and Refsum (1974) in a study in ducks in which they investigated the effects of alpha-blockade with
phenoxybenzamine on both the cholinergic actions of the vagus on the heart, and on the diving response. Phenoxybenzamine did not significantly affect vagal control of the heart when given at a dose which produced complete vasoconstrictor fibre blockade, so their results during diving were not due to vagolytic effects of the pharmacological agent used. In the dive, the degree of bradycardia was reduced when vasoconstriction was prevented by alpha-blockade and blood pressure fell, in contrast to the situation in untreated animals which maintained blood pressure in the dive. The authors concluded that bradycardia is secondary to, and a consequence of, vasoconstriction: blood pressure rises as peripheral resistance increases at the start of the dive, and the baroreflex is activated to lower the heart rate. In ducks in which peripheral vasoconstriction in the dive was presented by pretreating the animals with reserpine to deplete catecholamines, bradycardia was also markedly reduced (Andersen and Blix, 1974). When a bolus injection of noradrenaline was administered intravenously about 20 sec into the dive, a large drop in heart rate occurred, accompanied by a pressure rise. This was presented as further evidence of the proposed primary role of the baroreceptors in generating diving bradycardia, and the authors went so far as to say that the chemoreceptors were relatively unimportant in the control of responses to forced diving.
Arterial baroreceptors have been implicated in the cardiac limb of the circulatory responses to smoke inhalation in the rabbit. These responses are initiated by input from the trigeminal nerve (White and McRitchie, 1973; White et al., 1974), and bear a striking resemblance to the diving response, which also has a trigeminal component. In rabbits, smoke causes apnea, and the resulting cessation of lung receptor input along with continued trigeminal activity produces a marked bradycardia and an increase in peripheral resistance, as well as a rise in arterial pressure. In the absence of carotid sinus and aortic baroreceptor input, the degree of bradycardia is reduced to roughly one half of the response in the intact rabbit, although loss of the baroreflex does not affect either peripheral resistance or blood pressure responses to smoke inhalation. White et al. (1974) and McRitchie and White (1974) proposed that the trigeminal input in response to smoke inhalation, besides affecting sympathetic vasoconstrictor and cardiac vagal motor neurones directly, also facilitated the baroreflex which then made a secondary contribution to the bradycardia in response to the pressure rise generated by the vasoconstriction. Such a mechanism may also operate during diving in ducks.

A direct approach to the role of the baroreceptors in diving was taken by Jones (1973). He first established unequivocally that, in ducks, there is only one set of arterial baroreceptors, located in the walls of the ascending
aorta, and innervated bilaterally by the aortic nerves (the "depressor" nerves of Nonidez (1935)) arising from the caudal poles of the nodose ganglia. When these nerves were cut in ducks, there was no cardiac response to drug-induced rises in blood pressure (Jones, 1973), a widely accepted test of baroreflex function in mammals (Smyth, Sleight and Pickering, 1969; Faris et al., 1980). Jones (1973) barodenervated ducks by bilateral aortic nerve section, and dived the animals 30-50 days after surgery. Bradycardia in these chronic barodenervates developed to the same extent as in intact and sham-operated animals in 2 min forced dives. The degree of vasoconstriction in the hind limb vascular bed was, however, reduced in denervates to less than half that seen in intact animals in the dive. The large fall in heart rate in the dive therefore was not matched by a proportional increases in vasoconstriction, and blood pressure fell in the dive. The author concluded from these experiments that the baroreceptors were not, as earlier workers had suggested, responsible for the dive bradycardia as a secondary response to peripheral vasoconstriction, but that peripheral resistance in intact animals rises in response to unloading of the baroreceptors due to falling blood pressure as a chemoreceptor-driven bradycardia develops.

Angell James, Daly and Elsner (1978) established that carotid sinus baroreceptors in seals have similar discharge characteristics to those in terrestrial mammals. The authors
attempted to determine whether the baroreceptor-cardiac interval reflex was reset, and whether the "gain" of this reflex changed during diving. They examined the relationship of the beat-to-beat interval to arterial blood pressure during elevations in pressure induced by phenylephrine injections, before, during and after flooding the face with water. This was intended as a "closed-loop" analysis of baroreflex function, since the animals were intact except for arterial catheters. In diving seals, since bradycardia occurred with maintained blood pressure, the authors concluded that the baroreceptor-cardiac reflex was reset in a dive so that it operated around a new level of heart rate (and probably a new level of peripheral resistance as well) to preserve barostasis. The closed-loop sensitivity of the baroreceptor-cardiac interval reflex appeared to increase in dives in their experiments, since a given pressure change in the dive produced a much larger change in cardiac interval than did the same pressure change at predive. The problem with such an analysis is that it does not give a clear picture of the regulation of blood pressure in the dive; it simply shows that there is greater engagement of the cardiac limb of the barostatic reflex during submersion, when the degree of vasoconstriction is manipulated artificially. In the same paper, Angell James et al. (1978) presented results of experiments in spontaneously breathing, anaesthetized animals in which the carotid sinuses had been isolated from
the circulation and a pressure ramp applied to the sinus baroreceptors while heart rate and systemic blood pressure were monitored ("open-loop" experiments). In these experiments the slope of the line relating systemic blood pressure to carotid sinus sac pressure was steeper than that relating heart rate to sac pressure indicating that, in the non-diving seal, peripheral resistance must also be changing as sinus pressure changes. That is, systemic pressure effects of altering the carotid sinus pressure could not be explained entirely on the basis of baroreflex-induced heart rate changes. When the procedure was repeated during dives, a 10 times increase was observed in the slope of the line relating carotid sinus pressure to pulse interval, but it is unclear whether these dives were performed in anaesthetized or awake animals. The authors conclude from both open- and closed-loop experiments that the baroreflex-heart rate response is facilitated during diving in the seal, and that the baroreceptors contribute to both the cardiac and vasomotor limbs of the diving response.

Millard (1980) injected pressor and depressor agents intravenously into ducks to determine the blood pressure-heart rate relationship before and during diving, and found that the slope of the pressure-heart rate line was reduced by half in the dive, indicating an inhibition of the baroreflex. This conclusion is opposite to that reached by Angell James et al. (1978) using the same method in the seal. In another
study involving acutely barodenervated ducks, Jones et al. (1982) estimated, by means of a participation index for central and peripheral receptor groups in the dive response, that the baroreceptors contributed less than 10% to changes in either heart rate or hind limb resistance during a 2 min dive. It is obvious that some of the controversy surrounding the role of the baroreceptors in diving stems from concentrating on the behaviour of one or the other limb of the baroreflex, and not on the overall regulation of the blood pressure itself; the picture is further complicated by the fact that both the baroreflex and the dive response act through the same effector systems.

In an attempt to reexamine the role of the baroreceptors in the control of the cardiovascular system during diving, Jones and West (1978) in preliminary experiments, and Jones et al. (1983, series I) in a more extensive study, implanted stimulating electrodes on the central cut end of one aortic nerve in ducks and sectioned the contralateral vagus to complete barodenervation. The baroreceptor nerve was then stimulated to engage the baroreflex before and during diving in these acute barodenervates. The advantage of this procedure over other methods for baroreflex activation in the dive was that the same input to the baroreflex mechanism could be applied in different circulatory states; the use of pressor tests or other means of altering baroreceptor input used by previous workers almost certainly did not result in
identical inputs to the central nervous baroreflex pathways under the different cardiovascular conditions before and during diving.

Both Jones and West (1978) and Jones et al (1983) recorded blood pressure and heart rate, and monitored hind limb vascular resistance as an index of the behaviour of total peripheral resistance during dives. In their barodenervates, resting blood pressure was elevated due to increased heart rate (to greater than 400 beats per min) and hind limb vascular resistance. Diving provoked a further increase in blood pressure, apparently due to a reduction in the degree of bradycardia in the denervates since dive vasoconstriction was unaffected after barodenervation. This reduction in the cardiac response to diving after acute barodenervation was in contrast to the full dive bradycardia reported by Jones (1973) in chronically barodenervated ducks. Predive stimulation of the aortic nerve produced a fall in both heart rate and peripheral resistance, resulting in a reduction in mean blood pressure. Stimulation with identical parameters during the dive caused less of a drop in blood pressure because baroreflex effects on both heart rate and resistance were significantly reduced. These results led the investigators to suggest that the ability of the baroreflex to control the circulation in diving is inhibited. Their findings concurred with Millard (1980) for ducks, but were contrary to the conclusion reached by Angell James et al.
(1978) on the basis of pharmacological and open-loop baroreceptor-stimulation studies.

The aortic nerve stimulation experiments outlined above were carried out in unilaterally vagotomized animals in which heart rate had more than doubled from the intact rate. It is likely that this large increase in heart rate was the result of the combination of vagotomy and aortic nerve section, and so would have been at least partly a consequence of the barodenervation procedure used and not solely the result of the loss of baroreceptor input. Butler and Jones (1971) have pointed out the variability of ipsilateral vagal control of the heart in ducks, and it seems probable that animals with one vagus removed would not be able to express full cardiac control. In preliminary tests, Jones et al. (1983 series I) found that unilateral vagotomy alone did not produce elevated heart rates in the 3 animals tested, but this procedure did affect the proportional drop in heart rate in the dive.

Chronically barodenervated ducks develop a full dive bradycardia, but lose the ability to maintain dive blood pressure (Jones, 1973; Lillo and Jones, 1982b) due to a reduction in the degree of peripheral vasoconstriction (Jones, 1973). This response to diving is in contrast to that in the acutely barodenervated animals, which lose a portion of the cardiac response but retain the ability to fully vasoconstrict the periphery. If these changes in the dive response with time are not taken into account, the
interpretation of results from the two groups will lead to different conclusions about the role of baroreceptors in diving.

It is clear from this summary that conflicting views about the role of baroreceptors in diving have resulted from differences in methods of activating the baroreflex, in analyzing responses of one or other effector limb of the baroreflex rather than the functional maintenance of blood pressure per se, and, in those studies employing barodenervation, differences in methodology of denervation. The time post-denervation at which the animals are dived also appears to be a factor, with chronically barodenervated ducks responding differently from acutely denervated animals. A more detailed study of the effects of baroreflex activation during diving in both acute and chronic barodenervates is required, as well as an analysis of the progressive effects of barodenervation on the cardiovascular system, in order to clarify the function of arterial baroreceptors in controlling the cardiovascular responses to forced submersion. The development of a standardized barodenervation procedure, and a standard method of activating the baroreflex by electrical stimulation of the baroreceptor afferent pathway appear to be the most profitable means of attacking this problem, I have chosen these approaches for the work in this study.

I aim, in this thesis, to investigate the hypothesis that the arterial baroreceptors play a role in control of the
cardiovascular system during forced submergence in ducks, and to attempt to define this role. The experiments in Section I have been designed to analyze the acute effects of baroceptor deafferentation by bilateral aortic nerve section on the dive response in vagally intact animals, and to examine the cardiovascular effects of baroreflex activation by continuous and intermittent stimulation of one aortic nerve during diving.

In Section II, I set out to analyze the cardiovascular adjustments occurring during the transition from the acute to the chronic barodenervated state, in order to establish the resting circulatory conditions during this transition. It was important to ensure during these experiments that the only factors implicated in the circulatory responses to the experimental procedure were those related to barodenervation alone, and therefore the experiments were designed to try to eliminate possible complicating effects of surgery or anaesthesia on the results. A procedure was developed to perform barodenervation after full recovery from the surgical manipulations involved in preparing the animals for experimentation. The structural adaptations of the circulatory system to the increased pressure load subsequent to barodenervation were examined as a possible contributing factor to the cardiovascular changes in chronic barodenervates.

The experiments in Section III were done to investigate
the changes in the cardiovascular responses to diving over
the time course of the transition from acute to chronic
barodenervation. The primary aim of this study was to obtain
a continuous record of these changes, in order to determine
the mechanisms behind the two distinct types of diving
response seen in acutely and chronically barodenervated
ducks. In Section IV, the aortic nerve stimulation
experiments of Section I were extended to examine the
responses in chronically barodenervated ducks. A protocol was
presented for nerve stimulation before, during and after
diving using a biological index for setting stimulus
parameters to enable results obtained at different times
after denervation, and in different animals, to be compared.
This is not a trivial problem in this type of long-term
experiment, given the changes which can occur in the
relationship between electrodes and nerve in the first few
weeks after implantation. Also in this section, an attempt
was made to investigate possible mechanisms of interaction
between the chemoreflex and the baroreflex which may underly
the alterations in the operation of the baroreflex during
forced dives.

In attempting to analyze the role of the baroreceptors
in circulatory control during submersion, I have focused on
baroreceptor function from two related perspectives: the
contribution of baroreceptor input to the maintenance of
blood pressure during diving, and the role of these receptors
in the initiation and maintenance of the cardiovascular adjustments to diving.
SECTION I

Cardiovascular Responses to Aortic Nerve Stimulation During Diving in Acute Barodenervates

INTRODUCTION

The efferent limbs of the baroreflex, cardiac output and peripheral resistance, are both influenced by inputs other than those from baroreceptors during diving, including input from central and peripheral chemoreceptors (Jones, Milsom and Gabbott, 1982). Separation of the influences of these afferent pathways from the effects of the baroreceptor input on the cardiovascular system during diving is difficult when all of the reflex pathways operate in concert in an intact animal. A preparation in which the only missing factor is the baroreflex, which can then be invoked at any time by the investigator to test its effects, provides a method for separating effects of the baroreceptors from those of other inputs during a dive. This is accomplished in the experiments in this section by electrically stimulating one aortic nerve in baroreceptor-deafferented animals.

The experiments presented as Series 1 in Jones et al. (1983) were done in ducks with the left aortic nerve sectioned, in which complete barodenervation was effected by
interruption of the contralateral vagus. These experiments indicated that baroreceptors do play a role in modulating the cardiac response in the dive, but the results in these animals may have been complicated by the use of unilateral vagotomy to complete the denervation. Specifically, cutting one vagus nerve would have reduced input from the carotid body chemoreceptors by half, and the efferent parasympathetic control of the heart during diving may have been impaired.

Hindlimb vascular resistance was measured in these experiments, by a constant-flow perfusion technique, as an indicator of the behaviour of peripheral resistance during diving. There is a possibility that blood flow in the hind limb during diving could be shunted through the web or other non-nutritive arterio-venous connections (Djojosugito et al., 1969), and if this were the case, changes in hindlimb perfusion pressure in a constant-flow system would not be a good index of true changes in peripheral resistance during diving.

The experiments in this section were designed to assess the cardiovascular responses to diving in animals acutely barodenervated by sectioning both left and right aortic nerves at once, leaving the carotid body innervation intact. Total peripheral resistance changes are estimated from cardiac output measurements made with electromagnetic flow probes monitoring pulmonary arterial flow.
METHODS

Experiments were done on 8 male and female white Pekin ducks (*Anas platyrhynchos* var.) obtained from a breeding colony established on the campus of the University of British Columbia. Body weight of these animals was $2.8 \pm 0.1$ kg (mean ± 1 standard error of the mean). Before any experimental procedures were carried out, animals were held indoors for at least 1 wk under artificial lighting with a 12 hr light-dark photoperiod, at a temperature of 20-22 degrees C, the same temperature as the laboratory.

Surgery for barodenervation and electrode implantation was carried out 24-48 hr before experiments. Animals were restrained ventral side upwards on an operating table, and were given intramuscular injections of up to 50 mg/kg sodium pentobarbital (Nembutal; Abbott Laboratories, Montreal, Quebec). The feathers covering the clavicular air sac were removed, a 10 cm incision was made longitudinally through the skin in the midline and the air sac membrane was exposed. The membrane was opened in the midline to reveal the great vessels and associated structures of the anterior thorax, which are illustrated in Figure 1. In male birds, the large syrinx was retracted to one side to view the aortic nerves; in females the smaller syrinx did not need to be moved.
Figure 1. Ventral view of the large arteries in the anterior thorax of a female white Pekin duck. The innervation of thoracic structures in this region is shown schematically.
The aortic nerve on the left side is prominent in the sheet of fascia which forms a web between the root of the common carotid artery, the brachiocephalic arch and the dorsal body wall. The nerve is accompanied by a small artery in its course from the left nodose ganglion to the aortic trunk, and the nerve was dissected free of the artery and the sheet of fascia. A ligature of 5-0 surgical silk was tied around the nerve as far distal to the nodose ganglion as possible, and led through a pair of miniature stimulating electrodes. These electrodes consisted of two solid silver wire (0.2 mm thick) rings of approximately 1 mm inside diameter, embedded 1 mm apart in epoxy resin. An opening was formed through the centre of both rings and the epoxy encapsulating them, to provide an enclosed electrode arrangement which minimized stimulus current leakage to surrounding structures. The electrode leads were formed from a twisted pair of flexible stranded copper wire insulated with a polyvinyl chloride jacket and soldered to the silver rings before encapsulation. The nerve was sectioned distal to the ligature, one free end of the tie was passed through the electrode lumen, and the central cut end of the nerve was drawn through the electrodes. The knot in the ligature was sufficient to prevent the nerve end from slipping out of the lumen. The electrode body was then fixed to the dorsal body wall with tissue cement (Histoacryl blue; Bohringer, Mannheim, West Germany), care being taken not to let cement
flow onto the nerve itself. The wire leads were anchored to muscles adjacent to the electrode site and, after leaving a strain-relief loop inside the chest, were sutured to the skin of the neck outside the incision. At this point, the nerve was given a brief electrical stimulation to check the effectiveness of the electrodes; an immediate reduction in heart rate occurred in a successful preparation.

Some difficulty in identifying the right aortic nerve without extensive dissection was encountered due to the presence of several small nerves leading towards the heart from the right side (Figure 1), and a preliminary study in 4 animals was made to identify the right baroreceptor nerve electrophysiologically. Electrical recordings from whole-nerve and few-fibre preparations showed that only one vagal branch in this area carried efferent information with a discharge pattern corresponding to the pressure pulse, and with similar characteristics to those described for aortic baroreceptors by Jones (1973). Activity in this branch had the same relationship with the cardiac cycle as did activity recorded from the readily identifiable left aortic nerve. Once functionally identified, the baroreceptor nerve on the right was traced from the nodose ganglion to the root of the aorta in each preparation. Having established the anatomy in these animals, the correct nerve could then be identified on the right side and sectioned in the barodenervates without extensive dissection.
The right aortic nerve runs in the same sheath as, but separate from, the vagus, posteriorly from the nodose ganglion to a point lateral to the descending aorta. Here it separates from the vagus and courses ventrally and medially over the anterior aspect of the right pulmonary artery and towards the aortic root (Figure 1). The nerve was exposed by retracting the descending aorta medially, a short length was dissected free from surrounding tissues, and the nerve was cut between the descending aorta and the right pulmonary artery.

Electromagnetic blood flow probes (Biotronex Laboratories; Silver Springs, Maryland) were placed around both pulmonary arteries in 6 animals and around one pulmonary artery in 2 animals. In these 2 animals, a piece of plastic tubing with the same internal diameter as the flow probe was put around the other pulmonary artery. The probes were selected for a fit which provided a reduction of about 10-20% in vessel cross-sectional area. Probe leads were sutured to the muscles of the ventral body wall and led outside the incision in the chest. The clavicular air sac was repaired and the skin was closed over the repaired air sac.
Experimental Procedure.

On the day of the experiment, arterial and venous catheters were implanted, under local anaesthesia (Xylocaine, 2% without epinephrine; Astra Pharmaceuticals Ltd., Mississauga, Ontario), in the ulnar artery and vein in one wing. The cannulas were made of polyethylene (Clay-Adams PE 90 arterial, PE 50 venous; Becton-Dickinson Co., Parsippany, New Jersey). Blood pressure was recorded through the arterial cannula with a Statham P23Db (Statham Laboratories Inc., Hato Rey, Puerto Rico) or Bio-Tec BT70 (Bio-Tec Instruments, Pasadena, California) pressure transducer. The transducer was set at the level of the heart and calibrated with a saline column of adjustable height. Blood flow in the pulmonary arteries was recorded with a Biotronex BL 610 pulsed-logic flowmeter, and cardiac output was obtained as either the sum of the flows in both pulmonary arteries, or as twice the flow in one pulmonary artery in the two animals with only one implanted flow probe. The electrical baseline for zero flow was established in diastole during diving. The flowmeter system was calibrated with the probes in situ at the end of each experiment, after the animals were killed with an intravenous overdose of sodium pentobarbital. In the calibration procedure, each pulmonary artery was exposed and cannulated above and below the probe and saline at known flow rates was run through the vessel segment with the attached probe. Langille and Jones (1975) have established that this
procedure gives the same results for the same probe when either blood or saline is used for the calibration fluid in ducks. Heart rate was obtained from the electrocardiogram (ECG), recorded conventionally with bipolar wire electrodes placed subcutaneously.

The effectiveness of the barodenervation procedure was checked with a pressor test. Epinephrine (Parke-Davis Canada Inc., Scarborough, Ontario) was injected intravenously in a 0.2-0.5 ml bolus of saline to make a total dose of 2 ug/kg. If no bradycardia accompanied the ensuing pressure rise of 20-50 mmHg, the animal was considered to be bereft of functioning baroreceptors.

Arterial blood pressure, heart rate derived from a cardiac ratemeter driven by the ECG signal, and pulmonary flows were written out on a heat-writing chart recorder (TR888, Techni-Rite Electronics Inc., Warwick, Rhode Island) writing on rectilinear coordinates. All variables were also recorded on an 8 channel FM tape recorder (Hewlett-Packard, San Diego, California) for later analysis. Analysis of 1 ml blood samples taken from the arterial cannula was done with an IL13 blood gas machine (Instrumentation Laboratories, Inc.; Lexington, Massachusetts) with the thermostat adjusted to keep the electrodes at 41° C, the normal body temperature of the animals. Calibration of the pH, oxygen and carbon dioxide electrodes was done with precision buffer solutions and gas mixtures of known composition, before each blood
sample was inserted.

On the day of the experiment, after cannulation, the animals were lightly restrained ventral side up on an operating table. Predive recordings were made 1-2 min before the dive with animals at rest, breathing air. The head was then positioned beak downwards in the mouth of a large funnel and held there in a padded clamp. Involuntary submersion was accomplished by plugging the funnel spout and pouring 10°C water quickly into the funnel to cover the head. At the end of the dive the funnel was drained by unplugging the spout, and the head was released from the clamp. Few of the acute barodenervates in these experiments could tolerate dives longer than 2 min, so a dive length of 1 min 30 sec to 2 min was used for all experiments. Recording continued for 2 min postdive, after breathing had resumed.

In experiments in which the aortic nerve was stimulated, the electrodes were driven by monopolar square pulses from a Grass S4 stimulator (Grass Instruments; Quincy, Massachusetts) via a stimulus isolation unit to prevent stimulus artefacts from appearing on either the ECG or blood flow traces. Stimulus parameters ranged from 1-20 Hz, 1-3 ms, and amplitude of the pulse voltage was set in each experiment at a level which gave large falls in blood pressure before submergence. This was done to emphasize stimulus-induced changes in cardiovascular variables during the dive. Two stimulus patterns were used in each animal,
with some variation in stimulation parameters: the aortic nerve was stimulated continuously from before to 2 min after submergence; or stimulation was intermittent, consisting of 20 sec pulse trains given predive, at 1 and 2 min in the dive, and at 1 and 2 min postdive. Cardiovascular variables were measured 10-15 sec after the start of each stimulus pulse train, in the intermittent-stimulation experiments. Several dives were done in each animal, separated by at least 45 min to 1 hr. Blood samples were obtained before and at 90 sec in the dive, and were analyzed immediately. At the end of the day, the animals were sacrificed, barodenervation was confirmed during post-mortem examination, and the flow probes were calibrated as outlined above.

Analog data recorded on the FM tape system were fed into a Digital PDP Lab 8e computer (Digital Equipment Corp.) running a custom program which produced mean values of blood pressure and flow over preselected time periods. After summing the pulmonary blood flows to obtain cardiac output, total peripheral resistance was calculated as the quotient of mean arterial pressure and cardiac output at a given time. The total pressure drop across the vascular beds in the body was assumed to be equivalent to the arterial pressure for this calculation; venous pressure was not measured in these experiments, since Jones (1973) has shown that it is less than 10% of mean arterial pressure in ducks during diving.

Data from barodenervates dived without stimulation were
compared with data from either type of stimulated dive at the
given times using a one-way ANOVA (SPSS, ONE-WAY). Within
each group, data were compared at different dive times using
a two-factor analysis with repeated measures over time (UBC
Computing Centre program, ANOVAR). Where F values for these
tests were significant ($P \leq 0.05$), pairs of means were
compared with the least significant difference test (Snedecor
and Cochran, 1967). All numerical data in this study are
presented as means ± 1 standard error of the mean.
RESULTS

1) Continuous Stimulation

Cardiac output decreased progressively during diving in the denervated animals represented in Table 1, so that by 1 min 30 sec of submergence, total blood flow dropped to 24% of the predive value. This was largely due to a decrease in heart rate to 30% of predive; stroke volume fell by less than 25% of predive. TPR increased by 9.2 times in the dive, but with a faster time course than the cardiac response. As a result, blood pressure early in the dive was significantly above predive, and not until 1 min in the dive did pressure begin to fall coincident with the start of the bradycardia. By the end of the dive, blood pressure was not significantly different from the predive level.

When continuous stimulation of the aortic nerve was begun before the dive, blood pressure fell by 61%. Stimulation did not affect either heart rate or stroke volume significantly, so this decrease in blood pressure was due to stimulus-induced vasodilation. Early in the dive, blood pressure increased despite continued stimulation, and remained significantly above the predive value for the rest of the dive, in contrast to the pressure response during non-stimulated dives, which decreased as the dive progressed. The time course and proportional decrease in cardiac output and heart rate were the same in stimulated dives as in dives
TABLE 1

Effects of continuous aortic nerve stimulation on cardiovascular variables during diving in acute barodenervates. Arterial pressure (MAP), cardiac output (CO), heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR) are expressed as mean ± 1 S. E. M. for 8 observations in 8 ducks. The (+) signs indicate significant (P ≤ 0.05) differences at each dive time between unstimulated dives (DEN) and dives in which the aortic nerve was continuously stimulated (STIM). Asterisks (*) indicate significant changes in DEN or STIM dives from the respective predive value.
<table>
<thead>
<tr>
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</tr>
<tr>
<td>mmHg</td>
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</tr>
<tr>
<td>DEN</td>
<td>137</td>
<td>213</td>
<td>142</td>
</tr>
<tr>
<td>STIM</td>
<td>54</td>
<td>107</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>±8</td>
<td>±13</td>
<td>±11</td>
</tr>
<tr>
<td></td>
<td>±4</td>
<td>±10</td>
<td>±9</td>
</tr>
<tr>
<td>CO</td>
<td>864</td>
<td>280</td>
<td>141</td>
</tr>
<tr>
<td>ml/min</td>
<td>517</td>
<td>303</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>±64</td>
<td>±50</td>
<td>±24</td>
</tr>
<tr>
<td></td>
<td>±70</td>
<td>±55</td>
<td>±25</td>
</tr>
<tr>
<td>HR</td>
<td>294</td>
<td>130</td>
<td>99</td>
</tr>
<tr>
<td>beats/min</td>
<td>±16</td>
<td>±18</td>
<td>±15</td>
</tr>
<tr>
<td>SV</td>
<td>2.26</td>
<td>2.15</td>
<td>1.43</td>
</tr>
<tr>
<td>ml</td>
<td>±0.22</td>
<td>±0.36</td>
<td>±0.24</td>
</tr>
<tr>
<td>TPR</td>
<td>0.22</td>
<td>0.76</td>
<td>1.47</td>
</tr>
<tr>
<td>P. R. U</td>
<td>±0.026</td>
<td>±0.052</td>
<td>±0.025</td>
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Arterial blood gas tensions and pH during diving in acute barodenervates. Oxygen tension (PaO\textsubscript{2}), carbon dioxide tension (PaCO\textsubscript{2}) and pH (pHa) are presented as mean ± 1S. E. M. for 8 observations in 8 animals. Dive blood samples were taken 90 sec after submergence. Asterisks (*) represent significant differences between respective dive and predive values, for unstimulated dives (DEN) and dives in which the aortic nerve was stimulated either continuously (CONT STIM) or intermittently (INT STIM).

<table>
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<th>PREDIVE</th>
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<tbody>
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<td></td>
<td>DEN</td>
<td>INT</td>
<td>CONT STIM</td>
<td>DEN</td>
</tr>
<tr>
<td>PaO\textsubscript{2}</td>
<td>81.4 ± 1.3</td>
<td>85.2 ± 5.2</td>
<td>83.9 ± 5.0</td>
<td>33.4 ± 1.8</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>PaCO\textsubscript{2}</td>
<td>25.6 ± 0.5</td>
<td>25.1 ± 1.1</td>
<td>26.0 ± 1.2</td>
<td>38.5 ± 2.3</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>pHa</td>
<td>7.57 ± 0.01</td>
<td>7.56 ± 0.02</td>
<td>7.58 ± 0.04</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>*</td>
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without stimulation, but total peripheral resistance was prevented from rising as much as in animals dived during stimulation.

During the 2 min periods after dives without stimulation, heart rate remained significantly above the predive value, although by 10 sec postdive peripheral resistance dropped to the same level as at predive and remained there. Primarily as a result of the high postdive heart rate, blood pressure remained elevated throughout the recovery period, with the highest pressure recorded at 1 min postdive. Continuous stimulation during recovery did not significantly alter levels of heart rate, cardiac output or peripheral resistance from the unstimulated levels up to 1 min postdive, and affected only heart rate after this time. However, blood pressure was reduced significantly from the unstimulated level throughout the recovery period.

Arterial blood oxygen tension and arterial pH in acute barodenervates were significantly less at 90 sec in the dive than at predive, while arterial carbon dioxide tension rose significantly during diving (Table 2). pH also decreased significantly in the dive. Continuous stimulation of the aortic nerve had no effect on the dive levels of blood gases or pH.
2) **Intermittent Stimulation**

Table 3 shows that reductions in both cardiac output (by 34 %) and total peripheral resistance (by 22 %) occurred during intermittent stimulation of the aortic nerve before diving, and together, these cardiovascular changes resulted in a decrease in mean arterial pressure to less than half of the prestimulation value. Predive stroke volume was not affected by stimulation, so the decrease in cardiac output was due almost entirely to a 29 % fall in heart rate.

During the dive, proportionate effects of intermittent stimulation on blood pressure were progressively reduced, with pressure falling to 37 % and then to 27 % of predive in response to bouts of stimulation at 1 min and 2 min into the dive, respectively. This decrement was partly due to the loss of the response of peripheral resistance to stimulation. Aortic nerve stimulation remained effective on the heart in the dive via cardiac rate but not stroke volume. Heart rate dropped by 49 % early in the dive when the prestimulation rate was still high, and by only 19 % during stimulation at 2 min in the dive, when the bradycardia was maximal. As in the continuous stimulation experiments, intermittent stimulation produced no significant differences in dive blood gases or pH (Table 2).
TABLE 3

Effects of intermittent stimulation of the aortic nerve on cardiovascular variables during diving in acute barodenervates. Variables are the same as in Table 1 and values are expressed as mean ± 1 S. E. M., for the number of observations in brackets under each value, in 8 animals. The (+) sign indicates a significant difference between the prestimulation value (PRE) and the value during a 20 sec stimulation period starting at the indicated time in the dive (STIM). Asterisks (*) indicate significant differences in PRE or STIM values from their respective predive values.
<table>
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<th>PREDIVE</th>
<th>DIVE</th>
<th>RECOVERY</th>
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<tr>
<td></td>
<td>1:00</td>
<td>2:00</td>
<td>1:00</td>
</tr>
<tr>
<td></td>
<td>DEN STIM</td>
<td>DEN STIM</td>
<td>DEN STIM</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmHg</td>
<td>180 ±10</td>
<td>219 ±16</td>
<td>172 ±14</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
<td>(12)</td>
</tr>
<tr>
<td>CO</td>
<td>850 ±84</td>
<td>278 ±63</td>
<td>145 ±17</td>
</tr>
<tr>
<td>ml/min</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>HR</td>
<td>296 ±18</td>
<td>106 ±19</td>
<td>71 ±8</td>
</tr>
<tr>
<td>beats/min</td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>SV</td>
<td>2.58 ±0.24</td>
<td>2.71 ±0.46</td>
<td>2.00 ±0.16</td>
</tr>
<tr>
<td>ml</td>
<td>(16)</td>
<td>(15)</td>
<td>(10)</td>
</tr>
<tr>
<td>TPR</td>
<td>0.27 ±0.053</td>
<td>1.32 ±0.40</td>
<td>1.41 ±0.19</td>
</tr>
<tr>
<td>F. R. U.</td>
<td>0.18 ±0.023</td>
<td>1.39 ±0.31</td>
<td>1.60 ±0.32</td>
</tr>
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<td></td>
<td>(10)</td>
<td>(10)</td>
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</table>

Note: * indicates significant difference from baseline.
Intermittent stimulation of the aortic nerve 1 min into the recovery period produced the same proportional changes in blood pressure, heart rate and cardiac output as did end-dive stimulation, while total peripheral resistance responded at this time with nearly the same proportional change as before the dive. Of all the cardiovascular variables, peripheral resistance had most nearly returned to the predive value by 1 min postdive, the other variables taking several minutes to recover. By 2 min postdive, the heart rate response to stimulation had also recovered fully and so, by this time, the proportional blood pressure response to stimulation was restored.
DISCUSSION

Acute baroreceptor denervation, performed by sectioning the aortic nerves bilaterally, reduced the degree of diving bradycardia compared with that in intact animals. Denervation, however, did not eliminate the larger portion of the cardiac response to diving, as would be expected if the baroreceptors were responsible for the bradycardia, although ablation of the baroreceptor-heart rate reflex did prevent dive heart rate from decreasing to the same degree as in intact animals (Butler and Jones, 1971). Andersen and Blix (1974), on the basis of pharmacological evidence, concluded that the baroreflex is primarily responsible for diving bradycardia in ducks. These authors treated ducks with reserpine, a catecholamine depleting agent, in order to prevent the neurogenic vasoconstriction accompanying diving. When these reserpinized animals were dived, a slight bradycardia and a large drop in blood pressure occurred, and intravenous injection of norepinephrine in the dive produced an increase in blood pressure and an immediate fall in heart rate. The authors concluded that diving bradycardia must therefore be secondary to peripheral vasoconstriction, and was a consequence of baroreflex activation as peripheral resistance began to rise early in the dive. In the only data that the authors show from these experiments (Andersen and Blix, 1974, Figure 2), the heart rate dropped somewhat as
blood pressure fell in the dive; if their contention is true, then this drop in pressure should have produced a dive tachycardia mediated by the baroreflex. The authors' experiments demonstrate that the baroreflex is active in the dive, but their evidence is not conclusive for a primary role for baroreceptors in this response.

Continuous stimulation of one aortic nerve provides a fixed level of baroreflex activation against which the cardiovascular effects of diving could be assessed. That is, tonic outflow from sympathetic and parasympathetic neurones produced by constant stimulation of the baroreceptor nerve would be modulated by inputs from the other receptor groups involved in diving, and these modulations of the baroreflex would then be apparent in the changes seen in cardiac output and peripheral resistance responses during and after forced dives. During continuous stimulation in resting animals, the baroreceptor-heart rate reflex did not appear to contribute to blood pressure control, since heart rate did not change significantly. The large fall in blood pressure during stimulation occurred through operation of the peripheral resistance arm of the baroreflex. The proportional change in total peripheral resistance from predive to end-dive was the variable most affected by continuous nerve stimulation during submersion, with the baroreceptor-hind limb resistance reflex limiting the resistance rise to 6.6 times, compared with a 9.2 times increase in non-stimulated dives. Despite the
reduction in vasoconstriction caused by stimulation, blood pressure during stimulated dives remained above predive while in non-stimulated dives, blood pressure was the same at end-dive as predive. Baroreceptor nerve stimulation throughout the dive could not prevent blood pressure from rising, and this must be a reflection of the influences of inputs from other receptor groups on the cardiovascular system.

These findings point up the necessity of making the effects of baroreflex activation on the blood pressure itself the central point of baroreflex investigations in diving: previous studies of baroreceptor function have emphasized the relation between various effector components of the baroreflex, such as heart rate or vascular resistance, and blood pressure, rather than the role of baroreceptors in controlling blood pressure per se. For instance, Angell James, Daly and Elsner (1978) have suggested that, in seals, the relationship between cardiac interval and mean arterial pressure is reset towards bradycardia, and previous suggestions have been made that the vasomotor-pressure relationship is also reset in mammals (Angell James and Daly, 1972). Millard (1980) has suggested that the baroreceptor-cardiac relationship in ducks is depressed during diving, contrary to claims made by Angell James et al. (1978) that the cardiac limb of the baroreflex operates more efficiently in diving seals. Concentration on pressure-heart rate and pressure-vasomotor relationships, which are likely to be
reset in the dive by baroreceptor-independent processes activated by trigeminal input from the facial area (Andersen, 1963a,b; Butler and Jones, 1968; Angell James and Daly, 1972), apnoea and ensuing inactivation of pulmonary afferents and central respiratory neurones (Jones, 1976), and chemoreceptor inputs (Jones and Purves, 1970; Jones et al., 1982), will result in a misleading picture in which the importance of the baroreceptors in the dive response may be over-emphasized.

Intermittent stimulation of the aortic nerve in animals at rest produced larger falls in blood pressure than did continuous stimulation, with the cardiac and peripheral arms of the baroreflex contributing equally to the pressure response. In the dive, the ability of short trains of stimulation to affect blood pressure was reduced as the dive progressed. The operation of the baroreflex was modified in the dive so that only the baroreceptor-cardiac reflex continued to be effective (although attenuated), while the peripheral resistance arm was rendered ineffective. The results of these experiments lead to the conclusion that the baroreflex operates via heart rate to control cardiac output in regulating blood pressure, after the cardiovascular adjustments (which occur in the absence of baroreceptors) have been established in the dive.

Of the two baroreflex activation paradigms used in these experiments, intermittent stimulation is probably more likely
to produce useful information about baroreflex function during diving. Continuous stimulation of the aortic nerve to give the large decreases in blood pressure observed in these experiments simulates an input from the baroreceptors which would represent a large and continuing pressure rise throughout the length of the dive. Under these circumstances the baroreflex would be engaged to a much greater degree than could be expected in an intact animal during diving, and thus provide an unrealistic picture of baroreflex function. The difference between the two protocols is also reflected in the different proportional contributions of cardiac output and total peripheral resistance to pressure changes during stimulation before diving, which may have resulted in part from the lack of a standardized method of setting nerve stimulation parameters in the two protocols. The technique of brief activation of the baroreflex at selected times before and during diving simulates the type of experiment in which pressor agents were used to alter blood pressure and thus to change baroreceptor input, but with the advantage that nerve stimulation provides a controlled level of input to the central baroreflex pathways without affecting cardiovascular variables directly.

The present experiments show that, in ducks with the innervation to the carotid body region intact, sectioning the aortic nerves produces a completely barodenervated preparation. These results confirm the work of Jones (1973),
who stated that all of the afferent fibres from systemic arterial baroreceptors in ducks are carried in the aortic nerves. Abdel-Magied et al. (1982) and Abdel-Magied (1984) have claimed, on the basis of ultrastructural evidence, that in chickens there is a baroreceptor zone in the walls of the common carotid arteries near the carotid bodies. These authors, however, presented no functional evidence to back up their claim. It was reported by Estavillo and Burger (1973) that afferents from cardiac receptors are present in these nerves in chickens, but if this is the case in ducks, these receptors do not participate in the baroreflex.

The present experiments were done in animals barodenervated by bilateral aortic nerve section, performed under general anaesthesia, and observations were made 1-2 days after surgery. It is possible that the effects of surgery or anaesthesia had not completely worn off by the time the experiments were begun, which would have confounded the results. In addition, the resting cardiovascular variables in this series of experiments were different from those observed in unilaterally barodenervated animals in which denervation was completed by ablation of the contralateral vagus (Jones et al., 1983; Series 1 experiments), the most prominent of these differences being the lack of hypertension at rest in the animals with bilateral aortic nerve section, and the very high resting heart rate of unilaterally vagotomized barodenervates. These
and other differences may stem either from the denervation procedures used, or from the combined effects of surgery or anaesthesia, masking the true effects of barodenervation. In neither of these studies were observations made on the animals before denervation procedures were begun, and so it was not possible to compare cardiovascular variables obtained after barodenervation with those in the same animals in the intact state. "Sham" recordings were done in unilaterally barodenervated animals before vagal section, although animals in this state do not represent true shams, since one aortic nerve had been sectioned. The effects of unilateral vagotomy alone were also examined in the series 1 experiments of Jones et al. (1983), and cardiovascular differences from intact animals were found both at rest and in dives. These problems are addressed in the next section, where a procedure is presented for artefact-free barodenervation.

The experiments presented here show that the cardiovascular responses to diving still occur 1-2 days after arterial baroreceptor denervation. The baroreflex is functional during diving, but its effectiveness in altering blood pressure is reduced due to an attenuated baroreceptor-heart rate reflex and complete loss of baroreceptor influence on peripheral resistance.
SECTION II

Cardiovascular Changes in the Transition From Acute to Chronic Barodenervation

INTRODUCTION

Ducks which have been barodenervated acutely, by unilateral aortic nerve section in combination with contralateral vagotomy, are hypertensive (Jones et al., 1983, series 1). This was not true, however, of the acutely barodenervated animals in Section I, and this difference may result from variations in the methodology of the denervation procedure. There is, in addition, a lack of control data for the effects of acute barodenervation on the cardiovascular system. In the experiments of the previous section, no sham denervations were performed, nor were measurements made before bilateral barodenervation. In the Series 1 experiments of Jones et al. (1983), measurements made before unilateral vagotomy, in animals with the contralateral aortic nerve sectioned, were labelled "sham" but these do not constitute genuine shams because the cardiovascular effects of cutting one aortic nerve were never assessed. Unilateral vagotomy itself may also affect both resting heart rate and the absolute and proportional heart rate responses during diving.
It is conceivable that the results of the acute barodenervation experiments in Section I could have been compromised by incomplete recovery from surgical trauma and general anaesthesia 1-2 days after surgery, an effect of the experiment which would be unrelated to the loss of the baroreceptors themselves. General anaesthetic agents used in acute experiments in mammals have deleterious effects on cardiovascular reflexes invoked during and possibly for a short time after general anaesthesia, and this factor has been considered a serious complication in relating the results from such experiments to the reflex responses in awake, spontaneously behaving animals (Vatner and Braunwald, 1975; Vatner, Franklin and Braunwald, 1971). In bilaterally barodenervated ducks, stimulating the aortic nerve to check electrode function during the implantation procedure, while the animals were still under general anaesthesia, produced less of a cardiac response than when the animals were given the same stimulus 1-2 days later, demonstrating an acute depression of this response by the anaesthetic.

To address these problems, a preparation is presented in this section in which the baroreceptors were denervated instantaneously, by the use of snares placed around both aortic nerves during surgery under general anaesthesia. The snares were withdrawn after complete recovery from surgery, and after control observations were made in the intact state. In this way, each animal acted as its own control for the
effects of barodenervation per se.

The forced diving responses of acutely barodenervated ducks (Section I; Jones et al., 1983) differ markedly from those in chronically denervated animals (Jones, 1973; Lillo and Jones, 1982b). The time course of the cardiovascular adaptations occurring in the first few weeks after loss of arterial baroreceptors has not been documented in ducks, but might provide some insight into these differences in the diving responses of the two groups. Therefore, once artefact-free barodenervation had been achieved, my aim was to monitor cardiovascular variables in the denervated animals until blood pressure rose to values similar to those in the chronically barodenervated ducks reported in earlier studies.

Documentation of cardiovascular adjustments following denervation must include at least the measurement of arterial pressure, heart rate, and blood flow for the estimation of changes in peripheral resistance. In order to make these measurements over a period of several weeks in the present study, the adaptation of already existing acute instrumentation techniques and the development of new ones for chronic recordings were required. In the acute experiments of Section I, total peripheral resistance was assessed from measurements of central arterial pressure and cardiac output obtained from electromagnetic flow probes on the pulmonary arteries. These probes introduced several problems, including partial occlusion of the arteries (about
10-20 %) to get the required fit for a good signal-to-noise ratio, the occasional susceptibility of the microvolt-range blood flow signal to interference from some levels of aortic nerve stimulation, and the proximity of the probes, particularly on the right pulmonary artery, to the aortic nerves in their pathway to the aortic root. The latter problem was of particular concern due to the requirement that these nerves function normally for some time after the operation to implant the instruments. For these reasons I decided to measure blood flow to the hind limb, using ultrasonic flow probes. The behaviour of this vascular bed, supplied by the ischiatic artery, has been well documented at rest and during diving, and flow changes here have been proposed as a good index of the behaviour of total peripheral resistance in forced dives in both intact and barodenervated ducks (Butler and Jones, 1971; Jones, 1973).

Heart rate in chronically barodenervated ducks at rest is significantly higher than that in resting intact animals but drops to the same end-dive level in chronic denervates as in intacts (Jones, 1973), whereas resting hind limb peripheral resistance doubles in chronic barodenervates (Jones, 1973) compared with that in intact ducks (Butler and Jones, 1971) at rest. However, in the dive, peripheral resistance in chronic barodenervates rises to only half the level in intact animals (Jones, 1973). Therefore, while both cardiac and peripheral adaptations to loss of baroreceptors
occur in the circulation in its resting state, in the dive
only the ability to vasoconstrict the periphery is altered in
chronically barodenervated ducks.

There are several possible mechanisms which could be
responsible for the long-term adaptation, and the change in
response during diving, of the peripheral resistance. One
possibility is that there is a change in the neuroeffector
relationship at the resistance sites in the hind limb
vasculature, which should be evident in a different hind limb
resistance in the barodenervate than in the intact animal for
a given level of activity in the hind limb vasoconstrictor
fibres. This hypothesis is investigated by comparing the
hind limb vascular resistance responses to electrical
stimulation of a portion of the vasomotor outflow tract to
this bed in intact and barodenervated animals.

A second possibility is that, in hypertensive animals,
structural changes in the arterial tree due to a maintained
increase in the pressure loading of the vascular walls could
produce wall hypertrophy, and thus altered vascular
resistance, analogous to the changes occurring within a few
days of the induction of hypertension in mammals (Folkow,
1982). Any structural changes, if they are to contribute
significantly to the increased peripheral resistance seen in
chronically barodenervated ducks, ought to be identifiable by
histological comparison of vascular tissue from normo- and
hypertensive animals. This hypothesis is investigated by
obtaining hind limb arterial wall cross-sectional areas over a range of vessel diameters in both groups of animals.

A third possibility is that the differences in vascular resistance at rest and during diving after barodenervation are the result of a change in the output profiles of vasomotor discharge from the bulbar neurones involved in vascular control, as the medullary regulatory pathways adapt to long-term lack of baroreceptor input. However, before this hypothesis can be examined, the effects of chronic barodenervation on the vasculature must be ascertained.

The rise in heart rate in acutely barodenervated ducks may result from an increase in cardiac sympathetic tone, a withdrawal of vagal tone, or both. The relative roles of sympathetic and parasympathetic control of the heart after acute barodenervation were investigated by comparing resting heart rates in intact and denervated ducks before and after beta-adrenergic receptor blockade.
1) **Bilateral Barodenervation**

a) Preparation.

Eight adult white Pekin ducks were used in these experiments. The age of these animals ranged from 18 wks to 1 yr and body mass ranged from 2.4 to 3.7 kg (mean mass 3.1 ± 0.2 kg). Only females were selected for this study because the syrinx joining the trachea to the primary bronchi is much smaller than that of the males and does not obstruct the view of the great vessels in the upper thoracic area when the clavicular air sac is opened. All animals were kept indoors in the holding facilities described in Section I. Animals were held for at least 3-4 days before surgery, and were allowed food and water *ad libitum*. Some intact animals lost weight during the holding period before surgery because they stopped eating, so body weight was monitored in all animals, and those that did not eat were not used in this study.

For anaesthetization on the day of surgery an animal was placed on its back on the operating table and lightly restrained. The posterior tibial vein was located below the metatarsal-tibial joint of the lower leg, and a polyethylene cannula (PE 10) was inserted into the vein with the aid of an intravenous catheter placement unit. The cannula was then taped to the foot. Sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Mississauga, Ontario; 65 mg ml⁻¹) was
delivered undiluted, in an initial bolus of 40 mg; this was followed with further doses as required throughout the surgical procedure. The initial dose produced surgical anaesthesia in most birds (evaluated by loss of response of the nictitating membrane to touching the cornea) within 15-30 min. The total dose for each animal was between 35 and 45 mg kg\(^{-1}\) body weight over the course of 1-2 hours.

When the animal reached a surgical plane of anaesthesia, the feathers were removed from the area of skin covering the clavicular air sac, and the skin and air sac were punctured in the midline as described in Section I. Just before puncturing the air sac, two 4-0 silk sutures were placed on either side of the puncture site, to facilitate drawing together the two membrane edges for later repair. The aortic nerves were separated from accompanying blood vessels using fine stainless steel needles, but were not disturbed from their positions in the surrounding fascia. Waxed surgical silk snares were then passed around the nerves with the aid of a grooved director which prevented the sliding silk from catching or tugging on the nerves. The free ends of thread were twisted together in their respective pairs, formed into strain relief loops inside the thoracic cavity, and led outside the incision.

The air sac membrane was carefully repaired by using the previously placed sutures to draw the centres of the cut membrane edges together, then stitching with 4-0 silk on an
atraumatic needle. An airtight seal, confirmed by watching the membrane move with each breath, was achieved. The skin was closed over the repaired air sac, and a stitch placed through the centre of the air sac suture line was used to draw the outer surface of the membrane against the underside of the skin incision. This was done to prevent the natural breathing movements of the animal eventually producing a cavity between the membrane and the overlying skin which could fill with fluid during the healing process and become a possible site for infection. Following skin closure, the free ends of the snares were knotted and sutured securely to the skin to one side of the incision. In some animals a wide-spectrum veterinary antibiotic (Ampicillin, Ayerst Laboratories, Montreal, Quebec; 250 mg) was injected intramuscularly near the wound site after surgery. The wound healed within a few days, and the animals left the wound site alone during preening.

Within 1-2 days of the operation to implant the snares, a blood flow probe was implanted around one ischiatic artery, usually the left, under local anaesthesia. An incision was made in the skin to expose the posterior margin of the iliotibialis lateralis muscle. The ischiatic artery was reached by tunnelling between this muscle and the underlying tissue. Parks ultrasonic blood flow probes working on the Doppler principle (Parks Medical Electronics Inc., Beaverton, Oregon) were used in this study, and required extensive
modification before they could be implanted (see Appendix). The correct probe size was chosen to be a loose fit on the artery after the exposed arterial segment had been bathed in local anaesthetic for a few minutes to maximize its diameter by completely relaxing the smooth muscle in the vessel wall. After the probe had been placed around the vessel and the two halves of the probe body tied together, saline was injected into the lumen to displace air bubbles which, if not dislodged, hinder healing as well as acoustic transmission processes. Experience with this type of flow probe has shown that local tissue growth into the crystal cavities in the lumen is rapid and results in stable flow traces within a few days. The vessel and probe were then pushed back into the position originally occupied by the artery and the angle of the leads emerging from the probe was adjusted to prevent twisting of the probe body on the vessel. The overlying muscle was returned to its former position and its posterior margin was reattached over the emerging probe leads. The connector on the free ends of the probe leads was pushed under the skin to emerge from a small incision near the hip joint and the entire length of the leads was then covered by suturing the thigh incision. The connector incision was then sutured and the connector itself was anchored to the skin over this incision by loose stitches placed at each corner of the connector housing.

Polyvinyl chloride tubing (PVC; Bolab, Lake Havasu City,
Arizona) was used for intravascular cannulae. This tubing was treated internally with a heparin-containing solution (TD-MAC; Polysciences Inc., Warrington, Pennsylvania) to bind heparin to the tubing walls in order to prevent blood clotting in chronic preparations. A length of tubing was filled with a 1:1 mixture of TD-MAC solution and toluene for 60 to 90 sec, then the mixture was withdrawn and the tubing was flushed slowly with 20 ml of avian saline containing 500 I. U. of heparin per ml. The tubing was air-dried overnight. This procedure provided cannulae which would remain patent for up to three weeks when flushed every second day. After exposure of the ulnar artery and vein in the wing, under local anaesthesia, small side branches of these vessels were freed from surrounding tissue and 12 cm lengths of PVC tubing of appropriate diameter were inserted 8-10 cm so that the tips lay within the brachiocephalic trunk or the vena cava, respectively. Side branches were used for cannulation rather than the more usual occlusive cannulation of the major wing vessels, because it was desirable to preserve as much of the normal wing circulation as possible in these chronic animals. Both arterial and venous cannulae had side holes cut 1-2 mm back from the tips. The exposed cannula ends were kept very short and could be secured to the underside of the wing with small pieces of tape when not in use. After placement of the cannulae, the incision was closed, cleaned and covered with a gauze pad taped in place.
These cannulae remained open and trouble-free in spite of repeated wing movements, such as stretching and flapping, made by the birds in the course of normal behaviour. Between observation days, the cannulae were filled with a concentrated solution of heparin (1000 I. U. ml⁻¹) in avian saline.

b) Experimental Procedure.

On the day of the experiment, usually 5-7 days after snare implantation, the animal was lightly restrained in a supine position on the operating table, and blood flow and pressure were recorded. Laboratory noise was minimised and a cardboard shield around the table was used to prevent movement in the room from disturbing the animal and influencing steady-state cardiovascular measurements. The baroreflex was tested by raising the blood pressure with intravenous injections of 0.1-0.2 ml boluses of saline carrying 25-50 ug of phenylephrine (Neosynephrine hydrochloride, Winthrop Laboratories, Aurora, Ontario), and monitoring the cardiac response to this pressure rise. Animals were then dived by forcibly submerging the head for 2 min 30 sec, and if pressor tests and dive responses were normal, the animals were considered acceptable for barodenervation.
Aortic nerve destruction was accomplished bilaterally by freeing the ends of the snares from the skin and withdrawing the threads gently until both snares were entirely free of the body. Cardiovascular responses to this instantaneous denervation procedure were recorded for up to 1-2 hrs after denervation, then no further measurements were made until the following day. Pressor tests were not performed in acute barodenervates within the first 24 hrs after denervation because of the danger of exacerbating any damage to small blood vessels which may have occurred as a result of the snare withdrawal disturbing new tissue growth in the chest.

At 24 hrs postdenervation, the animals were again tested for a baroreflex and, if there were no cardiac responses to increased blood pressure, data collection was begun. Animals were monitored for up to 16 days postdenervation.

c) Recording, Data Collection and Calibration Procedures.

Phasic blood pressure, phasic flow and an event marker signal were displayed on an 8-channel recorder and stored on tape as described previously. Mean pressure and mean flow were also written out on the chart, as well as, in some experiments, the ECG waveform from bipolar subcutaneous electrodes. Mean pressure was obtained in most experiments by electrically filtering the phasic pressure signal with a
low-pass filter using a time constant suitable for the prevailing heart rate (usually greater than 1 sec). This mean pressure was compared, for several animals, with that obtained from the "area under the curve" method in which areas were computed from fast chart traces of phasic pressure. Mean pressures obtained simultaneously by electrical filtering and by calculating areas from the same pressure trace agreed within 4% at several different heart rates, so the filtered mean was accepted as representative of true mean pressure.

The pressure transducer was clamped at the same level as the heart when the duck was in position on the table, and the pressure trace was calibrated by setting pressure at the transducer to known levels with a fluid-filled syringe connected in closed circuit with a sphygmomanometer dial gauge. This gauge was calibrated against a mercury manometer. The pressure recording system gave linear chart pen displacements over the range of 0-350 mmHg. The transducer-tubing combination had a damped natural resonant frequency of greater than 40 Hz, as determined by an impulse test (the "Hansen pop-test", McDonald, 1974). The damping factor was not estimated.

In the barodenervation experiments, pneumatic occluders were implanted on the ischiatic artery downstream of the flow probes in the first two animals, to establish a true zero flow for comparison with that obtained towards the end of
diastole at end-dive. These showed good agreement, and occluders were not used in later experiments. It was found that a brief cessation of flow in the ischiatic artery could be easily produced by compressing the artery against the femur near the hip with pressure applied by a thumbnail, to confirm the setting of the baseline flow trace during the daily setup procedures at the start of a recording session. At the end of the experiments, the animals were anaesthetized with sodium pentobarbital, the flow probe site was exposed, and the probes were calibrated in vivo, in situ or in vitro, by running blood through the probe at known flow rates as described in the Appendix. Hind limb vascular resistance was calculated as the quotient of mean arterial pressure and ischiatic blood flow.

d) Experimental Protocol and Data Analysis.

As described above, control data were collected before barodenervation. After denervation, cardiovascular variables were observed in barodenervates up to 16 days, but no animal was observed on each day for the entire experimental period. Data have therefore been grouped to make equal numbers of observations in each day group. The animals became used to the experimental environment within one or two observation sessions before denervation, and thereafter cardiovascular
variables settled to baseline values at rest within 15-20 min of being placed on the table. In each recording session, observations were made over the course of 4-6 hours and if pressor tests were performed, no further observations were made for at least an hour afterwards.

Mean arterial pressure, heart rate and hind limb vascular resistance values from the recording sessions were analyzed using analysis of variance in a blocked design (Zar, 1984). Each individual was treated as a single block and these blocks were analyzed with repeated measures over time, and with replicated measurements on the same experimental days. In the case of significant F-values (P < 0.05), Tukey's multiple means comparison test was used to detect significant differences between pairs of means. This test is considered to be more robust than the LSD test under these circumstances (Zar, 1984).
2) **Effects of Beta-Receptor Blockade on the Cardiac Responses to Diving and Barodenervation**

Five female white Pekin ducks (mean body mass $3.0 \pm 0.2$ kg) were implanted with aortic nerve snares as described previously. After recovery from surgery, heart rate was measured and the baroreflex was tested by pressure rises induced with phenylephrine injections. One hour later, each animal was given a 1 mg bolus dose of the beta-antagonist propranolol (Inderal; Ayerst Laboratories, Montreal, Quebec). The effectiveness of beta-blockade was tested with 0.2 mg doses of the beta-agonist isoproterenol (Isuprel hydrochloride; Winthrop Laboratories, Aurora, Ontario), which causes a tachycardia in untreated ducks. This tachycardia was eliminated after propranolol treatment. Barodenervation was accomplished by pulling the snares and was confirmed by repeated pressor tests. One to two days after barodenervation, heart rates were recorded before and after propranolol treatment, and the success of the beta-blockade was checked by monitoring the cardiac responses to injected isoproterenol.
3) **Hind Limb Sympathetic Stimulation**

Three ducks were used in experiments to locate sympathetic outflow pathways involved in control of the hind limb circulation. These animals were implanted with arterial and venous cannulae for blood pressure recording and drug injection respectively, and ischiatic artery flow probes were implanted. The animals were positioned ventral side down on the operating table and sodium pentobarbital (Somnotol, 35-45 mg kg$^{-1}$ body weight) was given intravenously to induce general anaesthesia. The skin overlying the hip and surrounding musculature was removed, and the iliotibialis cranialis and iliotibialis lateralis muscles were separated from their origins on the side of the ilial ridge anterior to the hip. Reflecting the mass of these muscles ventro-laterally exposed the iliotrochantericus caudalis muscle, which when separated from its synsacral origin, exposed the ala preacetabularis, a lozenge-shaped hollow surface in the lateral synsacrum. Acess to the lumbar and ischiatic nerve plexuses and the lumbo-sacral sympathetic ganglion chain was gained by removing the floor of this hollow with bone rongeurs (see Figure 2). Extensive electrocautery was required for hemostasis of the exposed tissues, particularly in the spongy bone of the synsacrum.

After removing the floor of the ala preacetabularis, access to the inter-ganglion nerves lying along the ventrolateral aspect of the fused synsacral vertebrae was
Figure 2. Schematic diagram of the sympathetic ganglion chain on the left side in the pelvic region of the duck. The site of sympathetic nerve stimulation which produced maximum hind limb blood flow reduction is shown by the schematic stimulating electrodes, and the site of nerve section is shown by the drawing of the scissors.
obtained by ventrally displacing the kidney and freeing each nerve from surrounding fascia between adjacent ganglia. For stimulation of these nerves, a ligature of 5-0 silk was tied around the nerve close to the more rostral of a pair of ganglia, the nerve was cut between the ligature and the rostral ganglion, and the free end of the ligature thread was used to draw the longer cut end of the nerve, still attached to the more caudal ganglion, through a pair of stimulating electrodes similar to, but smaller than, those described for aortic nerve stimulation in the previous section. This procedure was performed first on the connective between the last two thoracic ganglia (located between vertebral segments 21 and 22), then repeated at each succeeding segment in the caudal direction to segment 28, the sixth lumbosacral ganglion. At each site, various stimulus patterns were tried while hind limb flow and blood pressure were monitored. The most consistent and greatest degree of vasoconstriction was obtained at the connective between sympathetic ganglia at lumbosacral segments 2 and 3, just rostral to the first spinal nerve contributing to the ischiatic plexus from which the ischiatic nerve to the hind limb arises (Figure 2).

Once this site had been established, a second set of sympathetic stimulation experiments was done in 2 baroreceptor-intact and 4 bilaterally barodenervated ducks. The denervated animals were stimulated at 14 days post-denervation, and procedures for denervation and instrumenting
the animals in both groups were the same as described in previous sections. The animals were anaesthetized throughout the experimental procedure. Biphasic square wave pulses generated by two Grass S48 stimulators connected in tandem to a pair of Grass PSIU constant-current stimulus isolation units (Grass Instruments, Quincy, Massachusetts) were applied to the electrodes through a connector which allowed monitoring of the stimulus current and voltage waveforms on a differential oscilloscope (Tektronix 5113, Beaverton, Oregon). The pulses within each biphasic pulse pair were separated by 0.5 ms, pulse duration was 0.5 ms, and the stimulus intensity for any given run was set by stimulating at a fixed frequency in the range of 20-30 Hz while increasing the current on successive bouts of stimulation until no further decreases in hind limb flow occurred (the rationale for this choice of stimulus parameters and waveform are discussed in the Appendix). Once the maximum intensity was found, selected stimulation frequencies to cover the range of 0.2-30 Hz were applied in random order. Usually two runs were possible in each animal. Stimulus response curves were then erected for both groups, by plotting sympathetic stimulus frequency against the proportional change in calculated hind limb vascular resistance during stimulation.
4) **Hind Limb Artery Structural Analysis**

Four adult female white Pekin ducks of approximately 25 wks of age were used in experiments to compare arterial wall cross-sectional areas in the hind limbs of intact and barodenervated animals. Two animals with intact baroreceptor innervation were sacrificed, while two were barodenervated 14 days before being killed. Body weights of the intact animals were 3.1 and 2.9 kg; the barodenervates weighed 2.7 and 3.2 kg.

All animals were sacrificed by an overdose of sodium pentobarbital (150 mg injected into the peritoneal cavity). As soon as possible after death the central circulatory area in the thorax was exposed by puncturing the clavicular air sac, and the descending aorta was severed at its junction with the brachiocephalic trunks. A large-bore metal cannula was inserted and tied into the descending aorta, facing towards the lower body, and the posterior vena cava was also exposed. The aortic cannula was connected to a reservoir of warm avian saline containing 0.25 % sodium nitrite for vasodilation, held two meters above the animal, and blood was flushed from the vessels of the lower body. Perfusate was drained from the punctured right atrium by suction. 2 litres of saline, perfused over 5-6 min, was enough to clear most of the blood from the tissues. Perfusion was then begun with 10 % formalin buffered with phosphate buffer to pH 7.2 (Culling, 1974), also containing 0.25 % sodium nitrite, from a
reservoir at the same height as the saline tank. Formalin perfusion continued for 10 min, then the posterior vena cava was clamped, the line to the fixative reservoir was left open, and the pressure head was maintained in the lower body tissues overnight while fixation proceeded. Some venous leakage occurred but there was still fluid left in the reservoir after 10 hours, so it was assumed that vascular pressure had been maintained over this time period. This was important to ensure fixation of the vessels at a physiological diameter. After fixation the hind limbs of the animals were removed at the hip, skinned, the feet removed, and the limbs stored in buffered formalin at room temperature until sectioning.

As much of the arterial tree as possible was dissected free of the leg musculature, and short pieces (approx. 1 mm long) of representative arterial segments of various diameters were cut from the tree. A segment was placed on the stage of a freezing microtome with the long axis of the segment as nearly perpendicular to the direction of travel of the blade as possible, and cross-sections were cut at 40-60 um thickness. These sections were examined as wet mounts under either a compound microscope (Wild Model M11, Heerbrugg, Switzerland) or a dissecting microscope (Wild Model M5), depending on diameter.

Inner and outer diameters of cross-sections were measured with an ocular micrometer (Wild 15XSK), which was
calibrated at each of the different magnifications used in both microscopes with a micrometer slide (Bausch and Lomb, 2000 um scale). The smallest linear dimension which could be resolved with this setup was about 10 um. Only those sections which were circular in shape, or nearly so, were measured. Sections which had been cut at an angle to the vessel axis and thus appeared elliptical, or those from a vessel which had been compressed into a non-circular cross-section when fixed, were discarded. The inner diameter was measured across the lumen of the section, at the inner edges of the epithelium. The outer diameter was taken across the outer borders of the media, easily distinguishable in unstained wet mounts as having higher contrast than the surrounding adventitia (see arrows in Figure 7). Wall cross-sectional area was calculated by subtracting the circular area enclosed by the lumen from the total area derived from the outside diameter.
RESULTS

1) **Bilateral Barodenervation**

No sham denervations were performed in the experiments presented here, since the cardiovascular variables had all returned to the same levels as those in unoperated animals by 5-7 days after the operation to implant aortic nerve snares. The responses of the cardiovascular system to elevations in blood pressure induced by the intravenous injection of phenylephrine are summarized in Table 4 and illustrated in Figure 3. The peripheral vasoconstriction caused by 50 ug bolus doses of phenylephrine produced a mean blood pressure increase of 51.5 mmHg (1.03 mmHg ug⁻¹ phenylephrine) in controls, provoking a mean fall of 59.5 beats min⁻¹ in heart rate. Baroreflex-heart rate sensitivity was -1.16 beats min⁻¹ mmHg⁻¹. The cardiac response to this test was abolished by aortic nerve destruction: the same dose of phenylephrine produced a larger mean pressure increase (60.6 mmHg; 1.21 mmHg ug⁻¹ phenylephrine) in barodenervates, with no significant change in heart rate. The larger number of observations in denervates was due to repeated pressor tests in the same animals on different days after denervation to check for the return of any baroreflex response due to regeneration of the baroreceptor innervation. This did not occur over the observation period, and the denervation technique was considered successful in eliminating
TABLE 4

Effects of intravenous phenylephrine on blood pressure (MAP) and heart rate (HR) before and after barodenervation. Values are means ± 1 S. E. M., for 31 observations before (INT) and 87 observations after denervation (DEN) in 8 ducks. The (~) symbol indicates a significant difference between INT and DEN values (t-test with unequal n; P ≤ 0.05) and the (#) symbol means that the value during the pressor test is significantly different (t-test, equal n) from pretest.

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<thead>
<tr>
<th></th>
<th>PRETEST</th>
<th>PHENYLEPHRINE</th>
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<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>HR</td>
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<tr>
<td></td>
<td>mmHg</td>
<td>beats/min</td>
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<tr>
<td>INTACT</td>
<td>161.9 ±4.7</td>
<td>152.8 ±4.5</td>
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<tr>
<td></td>
<td>~#</td>
<td>~#</td>
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<tr>
<td>DEN</td>
<td>195.2 ±7.1</td>
<td>284.9 ±10.5</td>
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Figure 3. Arterial blood pressure (ABP) traces from the same animal before (INT) and after bilateral barodenervation (DEN). At the arrows, bolus injections of 50 µg phenylephrine were given intravenously.
baroreceptor input.

Chart recordings of blood pressure during pressor tests are shown in Figure 3 for the same animal before and at 2 days after barodenervation. In the control test (Figure 3a) the cardiac response does not begin until well after the pressure has started to rise, and is not expressed fully until after the pressure peak has been reached, the latency from beginning of pressure change to first cardiac response being 700-800 ms. The tip of the arterial cannula was within a few mm of the baroreceptor site and should have picked up pressure changes at the same time as the receptors themselves. The lack of a cardiac response in the denervated animal is evident in Figure 3b, and a slower pressure decline, as well as a reduction in pulse pressure after brodenervation can also be seen.

The immediate cardiovascular effects of bilateral aortic nerve destruction are illustrated in the chart recordings in Figure 4. Phasic arterial pressure and hind limb blood flow are shown, along with cardiac frequency obtained by triggering a ratemeter from the pressure pulse, before and for 8 min following withdrawal of both aortic nerve snares at once. No obvious changes in heart rate or blood pressure occurred within the first minute after barodenervation in this animal, but a rapid increase in stroke flow occurred in the hind limb bed, as seen by the widening of the flow trace. Stroke flow then moderated after about 1-2 min, but the mean
Figure 4. Immediate effects of bilateral barodenervation on cardiovascular variables. Traces from top to bottom: arterial blood pressure (ABP), instantaneous heart rate derived by a cardiac ratemeter triggered from the pressure signal (HR), and hind limb blood flow (HLF). At the arrow marked DEN, snares around both aortic nerves were withdrawn.
flow increased as rising heart rate drove the cardiac output up and blood pressure began to rise. Over the next few minutes, mean flow fell, heart rate continued to rise, and hypertension developed. These trends continued until heart rate and peripheral resistance reached plateaus above and below the predenervation levels respectively at about 15-20 min after nerve section, and hypertension was maintained.

This pattern of cardiovascular change was seen in all but two of the animals after denervation. In these two, a marked but transitory bradycardia and hypotension developed upon denervation, lasting for 10-15 sec, then the circulatory changes followed the same time course and pattern as those presented in Figure 4. All animals began to drink copiously within a few minutes of barodenervation, taking in 0.5-1 litre within the first hour, and continued to drink more fluids throughout the rest of the study than before denervation.

Loud noises, talking, touching the animal, or altering the lighting in the laboratory all produced transient changes in cardiovascular variables, but these were not consistent in either direction or magnitude. For example, tapping the operating table would produce first an increase in blood pressure, then when repeated a few minutes later, would result in a decrease. The variability of heart rate, blood pressure and hind limb vascular resistance increased after barodenervation, as illustrated by the approximate doubling
in the standard errors of these cardiovascular variables at rest (Table 4).

Figure 5 represents mean arterial pressure, heart rate and hind limb vascular resistance before and up to 16 days following barodenervation. The rise in blood pressure illustrated in the recording in Figure 4 is shown in Figure 5 to have increased by 20% at 24 hrs after denervation, and pressure continued to rise progressively with time to a plateau level at 11-16 days. The pressure rise was due in acute barodenervates to a 70% increase in heart rate, since peripheral resistance was unchanged at this stage. With time, the heart rate fell to a level intermediate between the intact and acute levels, and by 11-16 days postdenervation, heart rate was still 40% above the intact level. As heart rate declined, however, hind limb vascular resistance gradually increased to double the control value and this contributed to the maintained hypertension. By the end of the 16 day observation period the increased peripheral resistance combined with the continued tachycardia resulted in a maximum blood pressure rise of 75 mmHg, representing an increase of 40% over the predenervation level. Observations were not prolonged beyond 16 days after nerve section because blood pressure by this time had reached the same level as that reported by Jones (1973) in ducks 30-50 days post-denervation. The term "chronic", when used to describe barodenervates in this study, refers to animals in which
Figure 5. Cardiovascular adaptations to bilateral barodenervation. Arterial blood pressure (MAP), heart rate (HR) and hind limb vascular resistance (HLVR) are given as mean ± 1 S. D. for 15 observations in 8 animals. Asterisks (*) indicate significant differences from the predenervation level (Pre) in each day group. The range of days postdenervation included in each group is given under the bars.
blood pressure was the same as in the barodenervates of Jones (1973), and "acute" refers to the condition of barodenervates within a few days of complete denervation, in which blood pressure had not yet risen to the level in chronically denervated animals.
2) **Effects of Beta-Receptor Blockade on the Cardiac Response to Barodenervation.**

Mean heart rate in untreated baroreceptor-intact ducks was 180 ± 15.9 (S. E. M.) beats min⁻¹, and this did not change after beta-blockade (172 ± 8.2 beats min⁻¹). Acute barodenervation produced a significant increase in heart rate of 32 %, to 267 ± 20.4 beats min⁻¹, but administration of propranolol in these animals caused heart rate to fall to a level (198 ± 12.6 beats min⁻¹) not significantly different from that before denervation.
3) **Hind Limb Sympathetic Stimulation**

The site of access to a substantial portion of the sympathetic vasoconstrictor fibres innervating the hind limb vasculature was the paravertebral ganglion chain connective joining lumbosacral segments 2 and 3 in the duck. Stimulation at this location gave the most intense vasoconstriction obtained at any site, the response developing within 1-2 sec of the start of a stimulus pulse train, and reaching a plateau level within 5-6 sec at all frequencies of stimulation. Various combinations of stimulus intensities and frequencies always produced pure vasoconstriction here. Maximal stimulation at locations rostral or caudal to this site produced less intense vasoconstriction, and in two animals used in the preliminary location experiments, vasodilation resulted from stimulation of the connective between lumbosacral segments 1 and 2. At this location, low intensities and high frequencies of stimulation (50-100 uA, >10 Hz) produced vasodilation while higher intensities and lower frequencies resulted in vasoconstriction.

When the interganglionic connective was cut between lumbosacral segments 2 and 3, hind limb vascular resistance decreased by 0.3 to 2.9 P. R. U. in baroreceptor-intact (control) animals, and by 1.4 to 2.84 P. R. U. in barodenervated animals. This decrease represents an average proportional change of 26 % in the denervates, compared with only 9 % in control animals. Stimulation of this connective
in two intact animals and 4 barodenervated animals, at
stimulus intensities ranging from 100-600 μA and frequencies
from 0.2-30 Hz produced the frequency-response curves of
Figure 6. At the highest stimulation frequency, resistance
to flow in the hind limb increased nearly 7-fold in both
groups. The pre-stimulation resistance was higher in the
barodenervates but there were no differences in the
frequency-response curves, on a proportional basis, between
the groups. In both control animals and barodenervates, the
hind limb response to sympathetic stimulation, and to 50 μg
intravenous boluses of phenylephrine were abolished following
intravenous injections of the alpha-adrenergic antagonist
phentolamine (Regitine, Ciba-Geigy Canada Ltd., Calgary,
Alberta) at doses of 1.7-2.0 mg kg\(^{-1}\) body weight. Alpha-
blockade lasted for at least 30 min in this preparation,
during which time hind limb resistance was reduced by 10-15
%. No vasodilation occurred during sympathetic stimulation
after alpha-blockade.
Figure 6. Effects of sympathetic ganglion chain stimulation on hind limb vascular resistance (HLVR) in intact and barodenervated ducks. Resistance changes at each stimulation frequency are expressed as:

\[
\% = \frac{\text{stim HLVR}}{\text{prestim HLVR}} \times 100
\]

Error bars represent 1 S. E. M. 6 observations in 2 intact animals and 6 observations in 4 barodenervates are presented.
- intact
- barodenervate 14-18 days

sympathetic stim freq. Hz

% prestim

HLVR
4) Hind Limb Artery Structural Analysis

The two baroreceptor-intact (control) animals had arterial blood pressures of 162 ± 7 and 156 ± 8 mmHg (mean ± S. E. M.), while pressures in the two barodenervates were 195 ± 12 and 206 ± 15 mmHg (n=5 for each animal in both groups). There appeared to be no gross differences in the arterial trees dissected from the 4 hind limbs of each group. The same general branching structure and vessel segmentation was common to both groups, with minor differences in the locations of branches smaller than 400-500 um in diameter. Most of the sections were circular in cross-section (Figure 7), with a sharply delineated separation between the tunica media and the tunica adventitia. No crenellation of the intima was observed in any of the sections, nor did the medial lamellae vary in appearance among sections of similar diameters from different animals, indicating that the intravascular pressure level during fixation was high enough to have prevented significant vessel wall collapse, and thus the appearance of these vessels in cross-section was likely to have been a good representation of their in vivo state. In none of the sections was a substantial subendothelium apparent in the intima, even in the largest arterial sections from the ischiatic artery examined under high magnification. Within the media in sections of all diameters from both groups, a highly organized lamellar structure was evident, particularly when sections were viewed under polarized
Figure 7. Photographs of sections of the ischiatic arteries from a normotensive (a, INT) and a hypertensive (b, DEN) duck. Sections were cut at 60 um thickness, and the scale bars represent 500 um in each photograph. The arrows represent the measuring points between which inside diameter (I. D.) and outside diameter (O. D.) were taken.
light, but without the use of specific staining techniques, it could not be determined whether these lamellae were composed of smooth muscle alone, or a combination of smooth muscle and elastic or collagenous fibres. Consequently, no differences in lamellar makeup could be distinguished between sections from intact and hypertensive animals in this study.

Scatter plots of internal diameter versus wall cross-sectional area for the entire range of arterial diameters measured in this study are presented in Figure 8a (intact) and 8b (barodenervated). Diameters ranged from 85 to 2200 μm, and the shapes of the plots in the two groups indicates a non-linear relationship between diameter and area: larger arteries in both groups had heavier walls in proportion to their diameters, than did smaller arteries. A striking feature of these data is the clumping of points at larger diameters in both groups; this is the result not of missing values, but of the natural segmentation of the vessels as branching occurs in the vascular tree.

Superposition of the two plots showed that they appeared very similar except at larger diameters. For a more detailed comparison, the data in each plot were partitioned by diameter into a low and a high range, replotted on enlarged scales, and examined for significant differences (Figure 9). For purposes of comparison, area and diameter on these limited parts of the curves were assumed to have linear co-relations, and regression lines were drawn
Figure 8. Relationship between inside diameter (I. D.) and wall cross-sectional area for hind limb arteries in intact and barodenervated (14 day) ducks. a) Data from intact animals (164 cross-sections from 4 hind limbs in 2 animals); b) denervates (163 sections, 4 hind limbs in 2 animals).
Figure 9. Inside diameters and wall cross-sectional areas for two diameter ranges of hind limb arteries in intacts (a) and barodenervates (b). Data in this figure have been abstracted from Figure 8 for regression analysis. Regression lines were fitted by the least-squares method.
a) INTACT

\[ y = 0.00033x - 0.052 \]
\[ r^2 = 0.92 \]

b) DEN

\[ y = 0.00035x - 0.067 \]
\[ r^2 = 0.95 \]

\[ y = 0.00079x - 0.452 \]
\[ r^2 = 0.98 \]

\[ y = 0.00118x - 0.777 \]
\[ r^2 = 0.91 \]
through the points on each graph using a least-squares computer program (S, Becker and Chambers, 1984). The regression fits are significant for all data sets (F-test), and there are significant simple linear correlations between area and internal diameter over these diameter ranges. The slope of the regression line of the data for larger arteries from barodenervated animals was significantly different from that for intact animals, the slope for barodenervates being steeper. This indicates that, in barodenervates, arteries over this admittedly limited diameter range have significantly thicker walls than do those of the same internal diameter in intact animals. There was no significant difference between data from intact and barodenervated animals in the area-diameter relationship over the smaller diameter range.
DISCUSSION

Blood pressure, heart rate, hind limb blood flow and hind limb vascular resistance 5-7 days after aortic nerve snares were implanted (Figure 5, Pre) were within the range of normal values reported for intact ducks (Butler and Jones, 1968; Djojosugito et al., 1969; Kobinger and Oda, 1969; Butler and Jones, 1971; Jones, 1973; Bech and Johansen, 1980; Millard, 1980; Lillo and Jones, 1982a, 1982b) and for ducks which had been sham-operated as controls in chronic barodenervation experiments (Jones, 1973; Lillo and Jones, 1982a, 1982b). The lack of circulatory differences between the control group in the present experiments and intact animals from other studies is therefore taken as evidence that control animals in this study had fully recovered from any after-effects of anaesthetic or the operation to implant the snares, before barodenervation was performed by removing the snares. It follows that any cardiovascular changes occurring in these animals, once barodenervated, were solely the consequence of baroreceptor loss.

The lag time of 700-800 ms from the beginning of the phenylephrine-induced pressure rise to the first slowing of the heart in ducks (Figure 3a) was not an artefact of measurement of the pressure, because the arterial cannula tip was within a few mm of the baroreceptor site, and delay in the writing of the pressure signal on the chart recorder was
negligible. This latency was about double the value estimated by Pickering and Davies (1973) in humans, for much smaller pressure rises, and such a long response time in the present experiments means that the slope of the line relating cardiac interval to pressure rise on a beat-by-beat basis is of no use in ducks as a measure of the sensitivity of the baroreflex-cardiac response, since pressure is obviously not regulated on a beat-by-beat basis. The most useful indicator of baroreflex-cardiac function was the magnitude of the change in heart rate for a given rise in arterial pressure, which was \(-1.16 \text{ beats min}^{-1} \text{ mmHg}^{-1}\). This was the same sensitivity as that reported by Millard (1980) in *Anas boscas*, another species of domestic duck. The fact that no return of the cardiac response to pressure elevations occurred over the 16 day observation period shows that the snare method of denervation used in this study prevented reinnervation of the arterial baroreceptors, and that no other receptor group assumed arterial pressure-related control of the heart in their stead.

The immediate cardiovascular adjustments to snare withdrawal in ducks (Figure 4) were less extreme than those noted by Ludbrook et al., (1985) in rabbits, in which transient bradycardia and hypotension occurred in 5 of 6 rabbits barodenervated by crushing one carotid sinus nerve with a snare. In their experiments, the other carotid sinus nerve and both aortic nerves had previously been sectioned
during the surgery to implant the snare. After an initial pressure and heart rate rise which peaked at 10-15 min post-denervation, Ludbrook et al. (1985) reported a net decline in both variables over the next week in rabbits, to a plateau significantly above the pre-denervation level. In ducks, however, the immediate rise in pressure (Figure 4) was not moderated, but continued to increase until 16 days post-denervation, while heart rate followed the same pattern of increase and decline (Figure 5) seen in rabbits.

There is at present no general agreement that arterial barodenervation always produces what earlier workers termed "neurogenic" hypertension (Heymans and Neil, 1958). Chronic hypertension consequent to total arterial barodenervation has been demonstrated in conscious dogs monitored 2-4 wk post-denervation (McRitchie et al., 1976), up to 1 yr (Scher and Ito, 1979), and for more than a year after denervation (Ito and Scher, 1981). Maintained hypertension in barodenervates has also been reported in rabbits to 8 days post-denervation (Alexander and de Cuir, 1970; Blomberry and Korner, 1979; Korner, Head and Badoer, 1984) and in sino-aortically denervated fetal sheep through the last third of gestation (Yardley et al., 1983). Cowley, Liard and Guyton (1973) reported that, in dogs monitored over 24 hour periods, extensive and meticulously performed barodenervation did not produce maintained hypertension over the course of a year or longer, although the lability of blood pressure was doubled
in these animals. The same result was reported by Norman et al (1981) and Buchholz et al. (1986) in rats monitored at 30 days post-denervation. Ito and Scher (1981) contended that the reported lack of chronic hypertension in sino-aortic denervates was due to incomplete barodenervation, while Cowley (1979) and co-workers maintain that "apparent" long-term hypertension in denervates was an artefact of the protocol used by other workers for monitoring blood pressure.

In rabbits, heart rate "resets" to nearly normal levels (Alexander and deCuir, 1970) after a period of barodenervation similar to that in the chronic denervation study of Jones (1973) in ducks, in which both heart rate and blood pressure remained markedly elevated. Similar chronic hypertension and tachycardia were also reported in ducks by Lillo and Jones (1982b).

In my study, barodenervated ducks kept in cages in a constant environment, handled daily, and lightly restrained on an operating table for up to 4-6 hours with minimum disturbance, showed continued increases in blood pressure at each successive recording session over the course of 16 days post-denervation (Figure 5). These animals appeared to be well adapted to, and relaxed in, the recording environment by the end of the control recording sessions before denervation, and the recording protocol and laboratory environment do not appear to have been important factors in the hypertension following barodenervation. In fact, both control and
barodenervated animals would often fall asleep for portions of the recording sessions, with slight changes in cardiovascular variables. On a given recording day, blood pressures and heart rates were elevated when the barodenervates were first put on the table for observation, but within 15-20 min these variables had stabilized to lower levels which were maintained throughout the rest of the recording session. In ducks, therefore, chronic hypertension seems to be a genuine result of loss of the baroreceptors, and not an artefact of the monitoring procedure. This hypertension was accompanied by a maintained increase in pressure lability, a feature of chronic barodenervates which has also been reported in dogs (Cowley, Liard and Guyton, 1973).

Heart rate in baroreceptor-intact ducks at rest is set by vagal activity alone, because when the sympathetic cardiac effectors were blocked with propranolol, no change in heart rate occurred. After acute barodenervation, however, heart rate was driven up by an increase in sympathetic cardiac outflow; no change in cardiac vagal drive occurred after barodenervation, because heart rate returned to the predenervation level in these animals after beta-blockade.

Blood pressure does not decrease as heart rate declines with time after denervation (Figure 5), so the increases in peripheral resistance occurring in the hind limb are likely to be representative of resistance changes throughout the
vascular beds of the body, and appear to be cumulative with time. One of the mechanisms discussed by Folkow (1982) in the genesis of mammalian hypertension is "structural resetting", or a decrease in the ratio of arterial wall thickness to lumen radius (w/r). If a vessel wall becomes thicker at a constant radius, as a consequence of structural adaptation to increased pressure (arterial wall hypertrophy in hypertension, Furuyama, 1962) then the same tonic level of sympathetic drive to the smooth muscles in that wall after hypertrophy should produce a greater degree of lumen reduction and thus result in higher resistance to flow than before the structural alteration. Since blood flow in a vascular bed will change in proportion to the fourth power of the change in radius of the vessels in that bed, only a small degree of wall hypertrophy, with no change in tonic sympathetic drive, would produce the doubling of hind limb resistance seen in the barodenervated animals of Figure 5.

No structural analysis of the vasculature in hypertensive birds has been reported, although there has been a large amount of work done in this area in mammals in recent years. The usual method for estimating changes in w/r in hypertensive mammals depends on the constant-flow perfusion of isolated hind-quarter vascular beds in age- and weight-matched normotensive and hypertensive rats, in which arterial inflow pressure, venous outflow pressure, and blood flow are measured. From these variables, pre- and postcapillary
resistances are calculated and the contribution of arteriolar resistance to the total resistance of the vascular bed is then estimated. Any differences in behaviour of the beds in the two groups is attributed to structural changes in the resistance vessels (Lundgren et al., 1974; Folkow, 1976; Folkow et al., 1977, 1978; Nordlander and Friberg, 1985).

The data presented in Figure 5 show that hind limb resistance is elevated in hypertensive ducks, but the morphological confirmation of such a functional resistance change is not easily accomplished, particularly if the majority of the structural adaptation is occurring at the arteriolar level, as Folkow (1982) and others have suggested. Most of the morphological studies in hypertensive mammals have been on larger vessels ranging from the aorta to the small arteries (Furuyama, 1962; Wolinsky, 1970; Wolinsky and Glagov, 1967; Short, 1966; and Friedman, Nakashima and Mar, 1971).

Several techniques have been used for morphometry of arterial sections with varying degrees of success. The results of Furuyama (1962) with regard to the measured wall thickness are dubious because he did not control for the effects of fixation on the dimensions of the material used in his study. Sectional wall thickness, for a fixed cross-sectional area, will depend on the amount of pressure in the vessel, which sets the wall tension at the time of fixation. In the study by Furuyama, some fixation was carried out by simple immersion, without pressurizing the vasculature, and
the result was a crenellated and definitely non-physiological lumen border, as pointed out by Short (1966). Lee (1986) has stated that both the physiological state of the vessels at the time of fixation (whether contracted or relaxed), and the variables selected for comparison between normal and pathological vessels, can induce errors in the conclusions reached from such studies. Immersion fixation causes the vessels to collapse and distort, so the method used in the present study was fixation by perfusion, at a reasonable physiological pressure, with a vasodilator in both the flushing fluid and the fixative to ensure that the vessels were maximally relaxed.

If wall thickening is occurring as a result of hypertension in barodenervated ducks, the most reasonable variable to measure for comparison with controls would be arterial wall cross-sectional area, because area, unlike wall thickness, is independent of the vessel diameter as long as the vessel is circular in cross-section. Theoretically, if a given vessel were to develop a thicker wall after being subjected to a higher pressure for a period of time, then the wall area should be greater than that of an equivalent vessel in a normotensive animal. This was the basis for my comparison between control and hypertensive animals. Even though one might expect the internal diameters of arteries from the two groups of animals to be different in vivo, due to the higher blood pressure in the denervates, it must be
appreciated that the vessels were initially part of the same population. This means that vessels from barodenervated animals would have had the same wall areas at the same internal pressures (and diameters) as those in the intact animals if no change in wall structure had occurred. I therefore selected internal diameter as the independent variable for plotting against wall cross-sectional area to compare vessel morphology in the two groups of ducks.

The area for a given internal diameter is greater in barodenervated ducks than in control animals, in the larger hind limb vessels (Figure 9), and therefore may contribute to the chronic rise in hind limb vascular resistance. According to the theory presented by Folkow (1982), this finding is the opposite to that predicted in mammalian hypertensives on the basis of physiological measurements: arterial hypertrophy should occur to a greater degree towards the arteriolar level. The technique used in the present study did not allow estimations of wall areas in vessels smaller than 80-100 um in diameter with any reliability, so a whole range of structural adaptations may have been missed. There is evidence in intact, normotensive ducks that conduit vessels in the size range examined in the present study make a 20-30% contribution to resting hind limb vascular resistance (Folkow et al., 1966), so in barodenervated animals with arterial wall thickening, the contribution of this segment of the arterial tree to resting resistance could be even
greater.

The site of stimulation of the sympathetic ganglion chain producing maximal vasoconstriction in the duck hind limb was between ganglia associated with lumbosacral segments 2 and 3. There is some disagreement about the correspondence of vertebral segmentation in birds and mammals (Pick, 1970; Baumel, 1979), but the site found most effective in ducks appears to be within one or two segments of that used in similar mammalian experiments, counting by segment number caudally from the first cervical vertebra. The site of sympathetic trunk stimulation used in cats by Folkow (1952), Kjellmer (1965), Folkow et al. (1971) and Lundvall and Jarhult (1976) in studying hind limb vasomotion was between ganglia at lumbar segments 4 and 5. In the dog, maximum hind limb vasoconstriction was produced by stimulating on either side of the ganglion at lumbar segment 2 (Sonnenschein and Weissman, 1978).

In ducks, the curve relating the degree of vasoconstriction in the hind limb to sympathetic stimulation frequency continued to rise in a smooth, graded fashion with increasing frequency, to a maximum resistance of 7 times the prestimulus level at 30 Hz (Figure 6). In the cat hind limb, however, resistance plateaus at 9.5-10 times the prestimulus level, at a stimulation frequency of approximately 10 Hz (Folkow, 1952). Increasing the frequency further, to 30 Hz, produced a small decline in resistance in the cat. The hind
limb vascular bed in ducks may require a higher level of sympathetic activity than in cats, to generate the same degree of vasoconstriction, or the vasoconstrictor fibres to the hind limb may be more widely distributed in the sympathetic chain in birds, contrary to the opinion of Langley (1904). If Langley's view is correct, stimulation at a single site on the chain would not be sufficient to produce the degree of hind limb vasoconstriction of which the intact animal is capable, and a distorted frequency-response curve would result. This is likely the case in my experiments, since the conscious animal can increase hind limb vascular resistance by more than 10 fold during forced dives.

The lack of a difference between intact and chronically barodenervated animals in the sympathetic control of hind limb vasoconstriction is surprising, given the increase in resting resistance at two weeks after barodenervation (Figure 5). If the increase in wall thickness contributes significantly to resting resistance in barodenervates, a set amount of sympathetic drive should produce a greater increase in resistance in these animals, compared with the control group. The fact that the relationship between sympathetic activity in the vasoconstrictor fibres and hind limb resistance is unchanged after barodenervation may mean that, at the resistance sites, vascular neuroeffector function could actually be reduced in the denervates.
The act of sectioning the sympathetic trunk between lumbosacral segments 2 and 3 to apply the stimulating electrodes caused a fall in hind limb resistance in the barodenervated animals, but no change in baroreceptor-intact animals. It is apparent that, even in anaesthetized animals, the proportion of resting hind limb resistance generated by sympathetic vasoconstrictor tone is greater in barodenervates than in intacts. This finding suggests that neurogenic vasoconstriction arising from a lack of baroreceptor inhibition of the central vasomotor neurone pool is at least partly responsible for the chronically elevated peripheral resistance in barodenervates.

The increase in hind limb vascular resistance in barodenervated ducks at rest may be representative of changes in total peripheral resistance. From the results presented in this section, both neurogenic vasoconstriction and structural changes in the vascular tree appear to have a role in the development and maintenance of systemic hypertension in ducks after baroreceptor deafferentation.

Tonic baroreceptor control of the circulation in ducks at rest is expressed mainly through the cardiac limb of the baroreflex. This control works by inhibition of the sympathetic outflow to the heart, as indicated by the beta-blockade experiments in acutely barodenervated animals. That the baroreceptors do not exert the same degree of control over the periphery is evident from the lack of an immediate
change in peripheral resistance upon barodenervation (Figure 5), but the baroreceptors must have an indirect influence on resting resistance, for the long-term loss of these receptors did produce increased sympathetic drive to the hind limb.
SECTION III

Cardiovascular Responses to Diving
in the Transition From Acute
to Chronic Barodenervation

INTRODUCTION

The conclusions about the role of baroreceptor input in controlling the circulation during diving, drawn from various barodenervation studies in ducks (Section II; Jones, 1973; Lillo and Jones, 1982a; Jones et al., 1983 series I) differ, depending on the time after denervation the animals were dived. A systematic investigation of the dive responses at short time intervals from acute barodenervation to 16 days post-denervation was undertaken to resolve this controversy.

The reduced dive bradycardia in acute denervates may be due to an alteration in sympathetic or vagal control of the heart, related to the loss of baroreceptor input. The relative contributions of the sympathetic and parasympathetic cardiomotor pathways to the control of heart rate during diving are investigated by comparing dive heart rates in intact and denervated ducks before and after beta-adrenergic receptor blockade.
METHODS

1) **Diving Responses in Bilateral Barodenervates**

The animals used in the study of circulatory effects of barodenervation in Section II were dived on the same days that the resting measurements were taken. Dives were performed in the same way as in Section I, and lasted for 2 min 30 sec. Blood pressure, hind limb blood flow and heart rate were monitored by the methods outlined in Section II. Recording was begun 1-2 min before the dive, after the head was placed in the dive position, and continued until 3 min postdive, with a further 1 min record taken 15 min following the end of the dive. Arterial blood samples (0.6 ml) were withdrawn before and at 1 min 30 sec to 2 min into the dive for the analysis of blood gases in an IL 13 blood gas machine. If the same animal was dived several times in one day, these dives were made at least one to one and a half hours apart. Other repeated-dive studies have shown that, in intact ducks, at least 20-30 min is required for all cardiovascular, ventilatory and blood gas variables to return to normal following 2-4 min dives (Lillo and Jones, 1982a), so a recovery time of more than double this period was deemed adequate in the present experiments.

Diving experiments presented in this chapter were designed *a priori* to be analyzed using a blocked ANOVA model in which each animal was treated as one block. On any day
before or after barodenervation, the predive, dive and post-dive values of each cardiovascular variable were analyzed as successive treatments with time, and an F-value for "within-dive" on that day was obtained to determine if significant changes in the given variable occurred as the dive progressed. In the case of a significant within-dive F-value, pair-wise comparisons of means at different times in the dive were done with Tukey's multiple means comparison test. For the purposes of this analysis, the predive values were considered the control values for dives on any given day.

Longitudinal analysis of variations with time after denervation was performed using an expanded version of the ANOVA model employed in Section II. Values for the cardiovascular variables recorded on all days pre- and post-denervation were grouped for each dive time, and analyzed "within-days" for significant differences by day at any given dive time. If the within-days F-value was significant, a multiple means comparison test was done. All cardiovascular variables before, during and after diving in the transition from acute to chronic barodenervation have been presented as mean ± 1 standard deviation to give an estimate of the variability of the data. Other data have been presented as mean ± 1 standard error of the mean. The level of significance for all statistical tests in this section was set \textit{a priori} to 0.05.
2) **Effects of Beta-Receptor Blockade on the Cardiac Response to Diving Before and After Acute Barodenervation.**

Heart rates in intact and acutely barodenervated ducks were recorded during forced dives of 1 min 30 sec to 2 min 30 sec duration, before and after beta-receptor blockade with propranolol. These dives were made during the experiments for which resting observations are reported in Section II.
RESULTS

1) Diving Responses in Bilateral Barodenervates

The effects of barodenervation *per se* on resting cardiovascular variables in ducks have been described in Section II, Figure 5. These values are represented in Figure 11a, 11b and 11c by the Predive panels, and serve as control values for dives in each day group.

Forced submergence of ducks before barodenervation produced diving responses (for typical recordings, see Figure 10) which were similar to those in intact ducks in previous studies. Heart rate in controls was reduced by 85% and hind limb vascular resistance increased by 10.3 times in 2 min 30 sec dives (Figure 11b). Assuming that the stroke volume of the heart was little changed in these baroreceptor-intact animals in the dive, the degree of vasoconstriction in the hind limb (Figure 11c) must not have been matched throughout the body since blood pressure fell by 18% (Figure 11a). Both bradycardia and vasoconstriction in the control state were about 90% complete by 1 min in the dive.

The degree of bradycardia was considerably reduced in dives performed 1-5 days after barodenervation, heart rate dropping by less than 60% of the predive value. This response to submersion began to improve after 1 wk, however, and by the end of the experimental period the proportional
Figure 10. Arterial pressure (ABP) and hind limb blood flow (HLF) recordings from portions of 2 min 30 sec dives in the same duck before (a) and at different times after bilateral aortic nerve section (b, c). Times in the dive, and the surfacing time, are marked by arrows.
Figure 11. Cardiovascular responses during diving and recovery in the transition from acute to chronic barodenervation.

a) Arterial blood pressure (MAP), b) heart rate (HR) and c) hind limb vascular resistance (HLVR) before (Preden, shaded bars) and after (unshaded bars) bilateral barodenervation. Means ± 1 S. D. for 15 observations in 8 animals. The range of each day group is given under the bars. Asterisks(*) indicate significant differences from predive for that day group. Closed circles (•) represent significant differences between that day group and the predenervation value at the same dive time. Note the change in HLVR scale at the two dive times.
fall in heart rate was the same (81%) as that in controls, although absolute end-dive heart rate was significantly higher in chronic barodenervates than in controls (Figure 11b). The time course of the bradycardia in 1-7 day denervates was similar to that in control dives but, as the end-dive heart rate decreased, the time course of the bradycardia in chronics was delayed, the response being only 60% complete in chronic denervates by 1 min in the dive.

End-dive hind limb vascular resistance rose to the same absolute level, on all days following barodenervation, as it did in control dives (Figure 11c). However, due to the gradual increase of predive resistance with time after denervation, the proportional change in resistance during diving was gradually reduced until, by 11-16 days when predive resistance was double the control value, dive resistance increased by only half as much proportionally as it did in control dives. However, since resistance rose to the same absolute level in dives throughout the denervation period as it did in dives before denervation, the level of blood pressure was related to the prevailing dive heart rate at each stage after aortic nerve section.

Two to three days following barodenervation, the magnitude of the dive bradycardia was at a minimum and as a result, mean dive blood pressure rose significantly above the predive level. As the depth of the dive bradycardia improved with time post-denervation and the degree of vasoconstriction
remained constant, dive blood pressure began to fall. By 16 days post-denervation, when the bradycardia was fully developed, end-dive blood pressure decreased by 45% from the predive level in that day group.

Blood pressure and hind limb vascular resistance returned to predive levels in controls within 1 min of surfacing (Figure 11a and 11c), while heart rate recovered within 2 min 30 sec (Figure 11b). However, in barodenervates at all stages a post-dive tachycardia was maintained for more than 1 min following diving, but by 2 min 30 sec postdive, heart rates fell to predive levels. Blood pressure in denervates returned to predive levels more rapidly than heart rate at most stages of barodenervation.

Arterial blood oxygen and carbon dioxide tensions, and arterial pH levels in control and barodenervated animals before and during diving, are presented in Table 5. In both groups, resting blood gas and pH values were similar to those in intact animals, as were dive blood gases. The only significant difference in these variables after denervation was that dive pHa was lower in barodenervates than in controls.
TABLE 5

Effects of bilateral barodenervation on blood gases and pH during diving. Data are represented as means ± 1 S. E. M. for the number of observations in brackets, in 6 animals. Dive blood samples were taken at 90 to 120 sec after submergence. Asterisks (*) indicate dive values significantly different from predive in intact or barodenervated animals; the closed circle (•) means that pH in barodenervated ducks was significantly different from that in intact animals.

<table>
<thead>
<tr>
<th></th>
<th>PaO₂</th>
<th>PaCO₂</th>
<th>pHₐ</th>
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<tbody>
<tr>
<td><strong>PRE-DENERVATION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREDIVE</td>
<td>83.4 ±1.6</td>
<td>26.5 ±2.6</td>
<td>7.561 ±0.015</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>DIVE</td>
<td>44.1 ±2.0</td>
<td>34.6 ±4.3</td>
<td>7.409 ±0.032</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
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| **POST-DENERVATION**|            |            |           |
| PREDIVE            | 81.9 ±2.1  | 22.0 ±1.3  | 7.557 ±0.013 |
|                    | (12)       | (13)       | (13)      |
| DIVE               | 44.6 ±1.9  | 36.2 ±2.3  | 7.283 ±0.017 |
|                    | (12)       | (12)       | (12)      |
|                    | *          | *          | * •       |
2) Effects of Beta-Receptor Blockade on the Cardiac Response to Barodenervation and Diving

The effects of beta-adrenergic receptor blockade on resting and dive heart rates in ducks before and after acute barodenervation are presented in Table 6. Resting values (here labelled Predive) are repeated from Section II. Beta-blockade did not significantly affect heart rate during diving in intact animals. End-dive heart rate in beta-blocked barodenervates was also the same as before blockade, but the predive heart rate in these animals after blockade was significantly lower.
TABLE 6

Effects of beta-adrenergic blockade on predive and dive heart rate in intact (INT) and acutely barodenervated (DEN) ducks. Heart rates are given as mean \( \pm \) 1 S. E. M. for the number of observations in brackets, in 5 animals. Asterisks (*) indicate significant differences between predive and dive values in each group; closed circles (●) mean significant differences from the respective predenervation value before or during diving, and the closed triangle (▲) indicates a significant difference between the untreated and beta-blocked heart rates. All comparisons were made using a t-test.

<table>
<thead>
<tr>
<th></th>
<th>INT</th>
<th></th>
<th>DEN</th>
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<tbody>
<tr>
<td></td>
<td>PREDIVE</td>
<td>DIVE</td>
<td>PREDIVE</td>
<td>DIVE</td>
</tr>
<tr>
<td>UNTREATED</td>
<td>180.3</td>
<td>37.3</td>
<td>266.7</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td>( \pm 15.9 )</td>
<td>( \pm 11.6 )</td>
<td>( \pm 20.4 )</td>
<td>( \pm 10.4 )</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>●</td>
<td>*</td>
<td>●</td>
</tr>
<tr>
<td>BETA-RECEPTOR BLOCKADE</td>
<td>172.0</td>
<td>38.7</td>
<td>198.0</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>( \pm 8.2 )</td>
<td>( \pm 11.2 )</td>
<td>( \pm 12.6 )</td>
<td>( \pm 10.6 )</td>
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<td></td>
<td>(5)</td>
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DISCUSSION

The cardiovascular responses to dives before bilateral barodenervation were identical to the dive responses obtained in intact ducks (Butler and Jones, 1971; Jones, 1973) and in sham-operated ducks in chronic barodenervation studies (Jones, 1973). Thus, changes in the circulatory adjustments to diving subsequent to aortic nerve ablation were solely the result of deafferentation of the baroreceptors.

It is clear from the results of this study that the acutely and chronically barodenervated conditions do not represent discrete circulatory states, but rather are at the ends of a graded continuum of cardiovascular adaptations. The detailed changes in the diving responses during the transition from acute to chronic barodenervation (Figure 11) show that any assessment of the role of the baroreceptors in diving derived from barodenervation experiments alone must be qualified by considering the elapsed time from denervation to observation. The adaptation of the cardiovascular system itself to loss of the baroreceptors, examined in Section II, provides the background of change over which the diving responses are superimposed, and this adaptation, as well as any changes in circulatory control pathways within the brainstem due to the interruption of the afferent baroreceptor pathways, account for the differences in dive responses in acutely and chronically barodenervated animals.
The effects of baroreceptor loss on the cardiac and peripheral arms of the diving response were different: peripheral resistance responded to submersion in the same way in both acutely and chronically barodenervated animals, but the acute loss of baroreceptors impaired the ability of the animal to generate intense bradycardia (Figure 11).

The results of the beta-receptor blockade experiments give some insight into cardiac control during diving and the consequences of barodenervation on this control. By comparing heart rates in beta-blocked and untreated animals from the present study with the heart rates obtained by Furilla and Jones (1987) during sympathetic and parasympathetic cardiac nerve stimulation, an estimation of the approximate contributions of these efferent pathways to heart rate can be made in intact and barodenervated animals during diving.

Resting heart rate in animals before barodenervation is set by vagal activity alone, as pointed out in Section II. The estimated level of vagal drive to produce the resting levels of heart rate in the intact animals in these experiments is about 25% of the maximum available vagal drive, based on the data of Furilla and Jones (1987). Dive heart rate in baroreceptor-intact animals was unchanged by beta-receptor blockade, so the bradycardia resulted from an increase in vagal drive from 25% to 95% of maximum vagal drive, with no sympathetic contribution. As in intact
animals, the cardiac sympathetic component made no contribution to heart rate in the dive (no change in end-dive rate after beta-blockade in barodenervates), but dive heart rate fell to a level corresponding to a vagal output of only 62 % of maximum. This means that removal of the baroreceptors resulted in an acute deficit of approximately 1/3 of the total available vagal drive during diving.

The lack of a sympathetic component in setting dive heart rate in my experiments confirms the observations of Butler and Jones (1971) in forced dives and Furilla and Jones (1987) in both free and forced dives in diving and dabbling ducks. The present findings do not support the contention by Hollenberg and Uvnas (1963) and Folkow et al. (1967) that the cardiac sympathetic innervation plays a role in the forced dive response in ducks.

The differential effects of baroreceptor loss on the cardiac and peripheral arms of the diving response in ducks have a parallel in studies on barodenervated rabbits. The cardiovascular responses to smoke inhalation, initiated by trigeminal input, include a decrease in heart rate and an increase in peripheral resistance (White and McRitchie, 1973). The rise in resistance is unaffected in barodenervated animals, but the cardiac response, as in acutely barodenervated ducks during diving, is reduced (McRitchie and White, 1974). In ducks, however, the cardiac response returned with time, and chronically barodenervated
animals were able to develop a normal dive bradycardia. This would suggest that, if the central neural integration of the cardiovascular responses in dives in acutely barodenervated ducks is similar to that of the smoke response in rabbits after loss of the arterial baroreceptors, then that integration undergoes a change in the transition from acute to chronic barodenervation in ducks.

Dive blood pressure in barodenervated ducks followed the course of heart rate behaviour, rising from the predive level in acute animals as the limited bradycardia could not compensate for the maintained peripheral resistance response, and falling to nearly half the predive value in chronic animals as full bradycardia returned.

Blood pressure in pressure-regulated and unregulated circulatory systems is a function of the relative levels of cardiac output and the hydraulic resistance against which the heart is pumping. In a pressure-regulated system, these effector limbs are neurally adjusted in a coordinated fashion by negative-feedback control loops using information from arterial baroreceptors which signal to the brain an integrated mean arterial pressure level (Arndt et al., 1977). Pressure is maintained within the limits required for adequate perfusion of the organs in the body during the current activity of the animal (see Sagawa (1983) for a detailed discussion of barostatic control theory). In a forced dive, this means that changes in peripheral resistance
are approximately balanced by changes in cardiac output, in the opposite direction, so that the heart and brain are adequately perfused while blood flow to most of the rest of the circulatory system is reduced.

In baroreceptor-deafferented animals there is no functioning pressure-regulation system because there is no control signal to the regulator mechanism, and blood pressure is not defended against disturbances. Thus, when baroreceptor-independent cardiac output and peripheral resistance changes occur in the dive, pressure is simply a hemodynamic consequence of the changing levels of these cardiovascular variables. The fact that these adjustments still occur in dives after acute barodenervation, and continue to occur in chronically denervated animals, should lay to rest the argument by Andersen and Blix (1974), and Blix and Folkow (1984), that the baroreceptors have a primary role in generating the dive responses in ducks. However the large swings in dive blood pressure, both above and below the predive level, in barodenervated animals, demonstrate the importance of these receptors in balancing the circulatory adjustments to maintain dive pressure within reasonable limits in intact animals.

Angell James et al. (1978) have suggested that the full dive bradycardia in the chronically barodenervated ducks in the study of Jones (1973), and by virtue of a similar protocol, in the chronically denervated animals in the
present study, could be the result of enhanced chemoreceptor drive. Their suggestion was that, when chronically denervated ducks are dived, greater changes in blood gases would occur than those in intact animals because of incomplete peripheral vasoconstriction in the denervates, and any deficit in the cardiac response due to the loss of the baroreceptors would be compensated for by greater chemoreceptor input. A systematic analysis of dive blood gas levels with time after barodenervation was not done in the present experiments, but the measurements presented in Table 5 showed no significant differences in blood gases between intact and denervated animals during diving. It is apparent, from these experiments, that the baroreflex in an intact animal does little to aid the conservation of oxygen during diving in ducks.

In ducks up to 16 days after barodenervation, peripheral resistance rose to the same level as in intact animals during diving (Figure 11). Jones (1973) has shown, however, that in 30-50 day barodenervates, end-dive peripheral resistance rises to only half the predenervation level. This suggests that further changes in the integration of the peripheral resistance response to submersion occurs between 16 and 30-50 days after barodenervation. This possibility is examined in the next section.

The baroreceptors contribute to the control of blood pressure during forced diving by operating through the
cardiac limb of the baroreflex in ducks. However, dive blood pressure is only maintained within fairly broad limits in the intact animal, and so pressure maintenance by the baroreflex appears to be secondary to the development of the oxygen-conserving cardiovascular adjustments, which are unimpaired by barodenervation. The integration of the diving responses changes within the first two weeks after barodenervation, as cardiac control recovers from the effects of baroreceptor loss.
SECTION IV

Cardiovascular Responses to Aortic Nerve Stimulation During Diving in the Transition from Acute to Chronic Barodenervation

INTRODUCTION

Chronic barodenervation experiments of the type presented in Section III are limited in the information they can provide about the function of the baroreflex in cardiovascular control during diving. Activation of the baroreflex by aortic nerve stimulation provides information on the dynamic role of the baroreceptors in diving, and a modification of the stimulation technique used in Section I is employed in this section to extend the study of acutely barodenervated ducks to animals in the chronically barodenervated state. The changes in resting cardiovascular variables and in diving responses with time after barodenervation, reported in Section III, may be accompanied by alterations in the way that input from the baroreceptor nerve is processed.

In trial attempts to stimulate the aortic nerve chronically, it was found that the enclosed electrode configuration used by Jones and West (1978), Jones et al. (1983) and in the experiments in the first section of this
thesis did not work for more than a few days after implantation. A technique for chronic stimulation of the aortic and carotid sinus nerves with implanted electrodes in rabbits has been reported by Karemaker et al. (1980), and various electrode configurations have been described for long-term stimulation of peripheral nerves in several other systems. Electrodes have been implanted chronically in humans for phrenic stimulation to augment a failing respiratory pump (Glenn et al., 1977), for pain control (Picaza et al., 1975) and on the carotid sinus nerve for the treatment of angina pectoris (Borst et al., 1974). Chronically implanted electrodes have been used in animal preparations for diaphragm pacing (Kaneyuki et al., 1977; deVilliers et al., 1964) and for vagal stimulation (Bourde et al., 1970; Slaughter and Hahn, 1975; Barone et al., 1979). All of these electrodes were of the type which enclosed the nerve, and the studies were done on nerves which were otherwise intact, with healthy blood supplies.

The above experiments were not hindered by the requirement of the present study that normal afferent traffic in the stimulated nerve be blocked. After deafferentation, reinnervation of the baroreceptors had to be prevented, yet destruction of tissues surrounding the nerve during implantation and deafferentation had to be minimized so that local blood supply to the nerve would not be compromised. The electrode configuration used in acute stimulation
experiments in barodenervated ducks (Section I) required severing the nerve so that a length of the central stump could be pulled through the electrode body. This procedure deprived the nerve of its blood supply where it entered the electrode body, involved excessive handling with attendant risk of fibre damage, and traction on the nerve resulted from the normal movement of surrounding structures during the cardiac cycle. These problems were solved by the use of a "patch" type of surface electrode, implanted over the nerve in situ, with a minimum of trauma, and by a nerve ligation and crushing procedure which left the blood supply at the electrode site unimpaired. One nerve was sacrificed at the time of surgery for the sake of implanting the electrodes. The contralateral aortic nerve was left intact, and control responses for these unilaterally denervated animals prior to complete barodenervation are compared with the control responses of the bilaterally baroreceptor-intact animals presented in the last section, to determine the effects of partial barodenervation.

In mammals, many recent studies have shown that the baroreflex is influenced by arterial chemoreceptor input (Bristow et al., 1974; Heistad et al., 1974; Attinger et al., 1976; Wennergren et al., 1976; Iriki and Korner, 1979; Wennergren and Oberg, 1980; Chruscielewski et al., 1981; Kidd et al., 1981; Marshall, 1981; Pisarri and Kendrick, 1984). A wide range of inputs from other receptor groups, such as
cardiac and low-pressure pulmonary mechanoreceptors (Kidd et al., 1981; Holmberg et al., 1983), somatic muscle and joint receptors (Kalial et al., 1981), and exteroceptors of the face and upper respiratory tract innervated by the trigeminal nerve (for summary, see Abboud and Thames, 1983) affect heart rate and blood pressure directly, and interact with the baroreflex as well. Respiration also influences the baroreflex (Davidson et al., 1976; Gandevia et al., 1978), and baroreceptor control of blood pressure is affected in the altered cardiovascular states produced by exercise or stress (Cunningham et al., 1972). Interactions among several receptor groups are involved in circulatory control during forced diving in ducks (Jones et al., 1982), and the influence of these inputs on the baroreflex may be at least partly responsible for the attenuation of blood pressure control by the baroreceptors during diving in intact ducks (Millard, 1980) and in acutely barodenervated ducks (Section I, Jones et al., 1983).

In dabbling ducks the chemoreceptors are of primary importance in the circulatory responses to forced submersion (Jones and Purves, 1970). Increased chemoreceptor drive during diving may attenuate the baroreflex by over-riding it centrally, in the same manner as chemoreceptor-baroreceptor interactions alter baroreflex behaviour in non-diving situations in mammals (Korner, 1979). This possibility was investigated here, using stimulation of the aortic nerve to
invoke the baroreflex, in ducks under varying conditions of chemoreceptor loading and unloading in both diving and non-diving situations.
METHODS

1) Aortic Nerve Stimulation During Diving

There were technical difficulties associated with having a viable stimulating electrode on one of the aortic nerves, and also having both of these nerves intact for the pre-denervation control measurements. Several different approaches to this problem were tried, including implanting a miniature heated-wire electrocautery unit on the intact left aortic nerve distal to the electrodes, but all attempts failed and these trials were not pursued. Since the cardiovascular effects of placing snares on both aortic nerves have been established (Section II) and shown to be negligible, a compromise was made in the present experiments. Electrodes were implanted on one aortic nerve, which was then interrupted distal to the electrode site, and a snare was placed around the otherwise intact contralateral aortic nerve. In this way, after recovery from surgery, the effects of the loss of one nerve could be assessed in both resting and diving conditions before complete barodenervation, and these observations served as the controls for this set of experiments.

12 female white Pekin ducks were used in stimulation experiments during diving. Mean body mass was 3.2 ± 0.2 (S.E.M.) kg. Before and after surgery, and when not being observed, the animals were held in the same holding
facilities and under the same conditions as described in Section I.

The stimulating electrodes developed for this study were patterned after those designed for long-term recordings of surface electrical activity in the mammalian spinal cord (A. Hoffer, University of Calgary, personal communication, 1985). These electrodes consisted of two 40 Ga. stranded stainless steel wires insulated with Teflon (AS631; Cooner Wire Co., Chatsworth, California), the bare ends of which were sewn into a 3-4 mm square by 0.5 mm thick silicone-rubber patch, reinforced internally with fine-mesh silk. The leads to these electrodes were left about 15 cm long and attached to a skin connector. Construction and testing of electrodes is given in more detail in the Appendix. Implantation began with the opening of the clavicular air sac to expose the central circulatory region in the thorax, under general anaesthesia, as described for snare implantation in Section II, but differed from the earlier procedure after the left aortic nerve was located. No attempt was made to free the nerve from surrounding tissue, except to remove any overlying fat, if present. Two 30-50 cm long sutures of 6-0 Vicryl (Ethicon) with 8 mm atraumatic curved needles swaged onto both ends of each suture were used to anchor the electrode patch over the nerve on the medial side of the fascial sheet
(see Figure 12). The fascia was pierced from the medial to

Figure 12. Medial view of the left aortic nerve and surrounding tissue in a duck. The method of attaching the stimulating electrodes to the fascia over the nerve is shown, as well as the site of nerve ligation and crushing.
common carotid artery

left vagus

left aortic nerve

nodose ganglion

electrode patch

vicryl suture

nerve crushed
the lateral side with one of the suture needles, about 1-2 mm to one side of, and midway along the course of the nerve in the fascia. The needle was then manoeuvered until the tip emerged again on the medial side of the sheet, about 2 mm away from its original entry site. The whole suture was then threaded through the two holes, to the centre of its length. A second suture was then placed in a matching position across the nerve from the first, leaving a total of four threads emerging from the medial side of the sheet of fascia, down which the electrode patch could be slid to its position over the nerve.

Each of the four suture needles was pushed through a hole in one of the four corners of the electrode patch, with each thread through a particular hole matching the corresponding position of that thread in the fascia. All free suture ends were then clamped together and held with slight tension while the patch was carefully slid down the four threads to lie centred in its final position over the nerve and flat against the fascia, with the two exposed wires oriented across the nerve, as in Figure 12. Matching free suture ends were then knotted at the back of the patch to secure it, and cut short. A strain relief loop was taken in the electrode leads inside the chest, after which the leads were anchored to the dorsal body wall by a single loose stitch. In some animals the angle of the lead wires to the patch required resetting to prevent traction on the nerve.
When thus implanted, the entire electrode assembly was light and flexible enough that it did not pull on the fascia or the nerve during movement of the nearby vessels during the cardiac cycle.

After the electrodes were secured, about 2 mm of the aortic nerve was dissected free of surrounding tissue at a point 6-10 mm distal to the electrode site, where the nerve runs close to the brachiocephalic arch on the left side. Two ligatures of 5-0 silk, 2mm apart, were tied around the exposed nerve segment, then this segment was macerated thoroughly several times with the jaws of a sharp pair of jeweller’s forceps. As soon as the ligatures were tied, the heart stopped for the equivalent of 2-3 beats, and heart rate was slowed for up to 1-2 min afterwards. Then heart rate usually increased to a level above that before ligation, and the great vessels visibly distended, indicating an acute rise in blood pressure. The efficacy of the electrodes was tested at this point, electrical stimulation producing an immediate drop in heart rate and a partial collapse of the great vessels. When the success of the electrode implantation had been verified, a snare was placed around the right aortic nerve as described in Section II.

In three of these animals, total peripheral resistance was to be estimated along with hind limb vascular resistance. These were fitted with flow probes on the central systemic vessels to measure cardiac output. In the experiments in
Section I, pulmonary artery flow was measured to obtain cardiac output, but this site was not suitable in the present experiments because of the risk of interference with aortic nerve function, since the right nerve would have to be dissected free from the pulmonary artery to place the probe. One ultrasonic probe was placed on the descending aorta away from the course of the right aortic nerve, and another on the left brachiocephalic trunk where it posed no threat to the already ligated left aortic nerve. Probe size was chosen for a non-constrictive fit on the vessels, and a plastic cuff of the same internal diameter as the probe on the left brachiocephalic trunk was placed in a corresponding position on the right to equalize resistances in the two trunks. This was necessary because of the enlargement of these vessels after barodenervation. Neither the probe bodies nor the leads were anchored to nearby tissue, and thus the implants moved freely with the vessels during the cardiac cycle.

After ensuring that no residual bleeding was evident at any site within the chest, all leads and the snare were brought out of the chest, the air sac was repaired and the skin incision closed. The free end of the snare was anchored to the skin, as were the connectors for the electrodes and probes. Animals were observed on the operating table during the first hour following surgery, then were transferred back to their cages for the remainder of the recovery period. During the next two days, blood flow probes were implanted on
the ischiatic arteries, and the animals were cannulated as
described previously. The recovery period in these
unilateral denervates was the same as in the previous
barodenervation experiments, about 1 wk.

Aortic nerve stimulation was done using a biphasic
waveform generated with the system previously described for
stimulating the sympathetic outflow tract to the hind limb.
The principle of "biocalibration", described by Swett and
Bourassa (1981), was used in setting the stimulus parameters.
This principle involves the use of a relative biological
index for setting the intensity of stimulation in each
preparation, and on different days in the same preparation in
the present experiments, so that results could be compared in
different animals and across time. In a preparation in which
the relationship between the stimulating electrodes and the
nerve could be changing on a daily basis over a period of
weeks, it was not practical to use an absolute standard of
calibration, such as stimulus current or voltage. Instead,
at the beginning of each stimulus run on each day, the pulse
frequency was set to a level (20-30 Hz) which was known to be
on the plateau of the stimulus-response curve (i. e. produced
maximal response), the duration of each of the paired pulses
set at 0.5 ms, the interpulse delay to 0.5 ms, and the
current increased until a maximal decrease in blood
pressure (the biological index of effectiveness of
stimulation) was obtained. Frequency was reduced until blood
pressure fell by an estimated 45-50% of pre-stim for the duration of a 20 sec pulse train. This frequency was usually between 10 and 20 Hz.

The experimental protocol for diving the ducks in these experiments was similar to that used to force-dive animals in the earlier barodenervation experiments. To obtain control responses before barodenervation, the animals were placed on the operating table, baroreflex function was checked by pressor tests, and the animals were dived two or three times over the course of three hours. The electrodes were also tested with short pulse trains at this point. If a deep bradycardia in the dive, and a cardiac response similar to that of intact animals during the pressor tests were obtained, and if heart rate dropped in response to aortic nerve stimulation, the animals were assumed to have recovered from the operation and the single snare was withdrawn to complete barodenervation. The head of the animal was then clamped in the diving position, the stimulus was repeated, and the cardiovascular variables before and during stimulation were recorded as the control levels for that dive. The animal was then dived for a total of 2 min 30 sec, and identical 20 sec stimulus trains were applied at 1 min and at 2 min 10 sec in the dive, the end of the last pulse train corresponding to the end of the dive. Stimulation was repeated at 1 min, 2 min 10 sec and 15 min postdive.

Arterial blood samples were taken before, at 2 min in
the dive, and at 1, 2 and 15 min after the dive in both control and barodenervated animals for blood gas analysis in an IL13 blood gas machine. The sample volumes (0.6 ml) were replaced immediately with equivalent volumes of avian saline. Withdrawing multiple blood samples during a dive-recovery routine of 20 min did not significantly affect haematocrit.

At the end of each experiment, flow probe calibration was carried out as described in Section II and the Appendix. Animals were anaesthetized for probe calibration, and during this period the left vagus nerve was exposed and sectioned high in the neck. When the aortic nerve was then stimulated, using the same parameters as in the last dive, no cardiac response to stimulation occurred, indicating that the electrodes were stimulating vagal afferent and not efferent fibres. There was no leakage of stimulus current to nearby neural structures. Barodenervation and the relation of the electrodes to the aortic nerve were confirmed by microdissection during post mortem examination.

The changes in cardiovascular variables during diving, and the effects of stimulation on these variables, were analyzed using a variation of the ANOVA model described in Section III. If a significant (P ≤ 0.05) absolute change occurred either during diving or as a result of stimulation, the proportional change was also assumed to be significant. Statistical analysis of the proportional differences themselves was not done. In order to analyze differences
between means of proportions, Zar (1984) and other authors recommend the transformation of each of the proportions comprising a mean to the arcsine of the square root of that proportion. When this was done for a part of the present data, the distribution approached normality more closely than the untransformed proportions but there was still some doubt that this transformed data fulfilled the assumptions of normally distributed data required for the ANOVA.
2) **Chemoreflex-Baroreflex Interactions**

a) Aortic Nerve Stimulation During Dives After Exposure to Oxygen.

Four of the barodenervated ducks with implanted electrodes were exposed to 100% oxygen by clamping the head in the diving funnel and surrounding the funnel with a plastic sheet. Oxygen gas was delivered to the animal through the funnel spout at a rate of 2 l. min$^{-1}$. The aortic nerve was stimulated to obtain a predive response after 5 min of oxygen breathing, then water was quickly poured into the funnel after removing the gas flow and closing the spout. The dive and stimulation protocols and blood gas analysis previously described for normoxic dives were used and the results were analyzed in a similar fashion.

b) Blood Pressure Response to Graded Aortic Nerve Stimulation in Normoxia, and Effects of Hypoxic Hypercapnea.

Three of the barodenervated ducks fitted with stimulating electrodes were used in experiments to establish a frequency-response curve for baroreceptor nerve activation in animals at rest, breathing air (normoxia). Stimulus parameters were set as described previously, then 20 sec pulse trains at different frequencies in the range of 0.2-30
Hz were given in random order. In two animals, these runs were repeated on the next observation day, while one animal was only given one run (total of 5 complete stimulus - response curves). Once these responses had been established, the animals were given a hypoxic-hypercapnic gas mixture (PO2 = 79 mmHg; PCO2 = 48 mmHg) to breathe for 2 min and the nerve was stimulated for 20 sec at 15 Hz. Blood pressure responses were normalized by converting to proportional pressure changes for plotting against frequency. Arterial samples for blood gas analysis were taken in normoxic animals and just before aortic nerve stimulation in hypoxic hypercapnea. Neither hypoxic nor hypercapnic gases were given independently to the animals.

c) Aortic Nerve Stimulation During Carotid Body Perfusion.

6 female ducks (mean body mass 3.0 ± 0.3(S.E.M.) kg) were used in carotid body perfusion experiments to test the effects of different levels of arterial chemoreceptor loading on the operation of the baroreflex. The preparatory surgery was similar to that described by Jones et al. (1982) for vascular isolation and perfusion of the carotid body. Animals were placed on their backs, anaesthetized with sodium pentobarbital (40 mg kg⁻¹) and the clavicular air sac opened. All arteries on the left side in the area of the carotid body were ligated except for the common carotid artery, on which a
balloon occluder was placed upstream of the carotid body (see Figure 13). The carotid artery was then cannulated downstream of the carotid body, with the cannula tip facing towards the chemoreceptors and the heart. Cannulation was facilitated by inflating the carotid occluder, and this also showed whether any side branches from the carotid artery had been missed during the ligation procedure. Stimulating electrodes were placed on the left aortic nerve as described previously. The right vagus was located low in the neck, a short section of the nerve was soaked in local anaesthetic, and then cut to denervate both baroreceptors and chemoreceptors on the right side. The air sac and skin incision were repaired after passing the electrode leads, the carotid cannula and the occluder tube out of the chest. The right ulnar artery and vein, exposed under local anaesthetic in the wing, were occlusively cannulated and this incision was closed. The animal was turned to an upright position on the operating table and lightly restrained.

The venous and carotid artery cannulas were led to a 50 ml syringe in a Harvard infusion pump (Harvard Apparatus, South Natick, Massachusetts), via an arrangement of stopcocks to which a pressure transducer (Narco Biosystems, Houston, Texas) and a saline flushing syringe were also attached (Figure 14). The pump syringe was kept at body temperature by a heating coil which was connected to a recirculating constant-temperature water bath. The arterial cannula was
Figure 13. Schematic ventral view of the region of the left common carotid artery in a duck. The relationship of the carotid artery cannula, pneumatic occluder and artery ligation sites to the carotid body are illustrated, along with the left aortic nerve and stimulating electrodes.
Figure 14. Arrangement of the experimental apparatus for pressure recording and blood perfusion of the vascu larly isolated left carotid body in a duck.
pneumatic occluder syringe

stimulation

carotid artery cannula

infusion syringe

constant temperature water bath

arterial blood pressure
connected to a second pressure transducer for recording arterial pressure. The animal was allowed to recover for 3-4 hours before the experiment began. All wound sites were periodically infiltrated with local anaesthetic throughout the recovery and experiment, and cannualae were flushed with heparinized (100 I. U. ml\(^{-1}\)) avian saline to prevent blood clotting.

Stimulus parameters for aortic nerve activation were adjusted according to the criteria set out in the previous section, while the animal perfused the innervated left carotid body with normoxic arterial blood from the heart (autoperfusion; carotid occluder deflated). The blood pressure response to stimulation during autoperfusion was the control response for these experiments. About 10 ml of venous blood was withdrawn into the infusion pump (no change in blood pressure resulted from this procedure), and the carotid occluder was then inflated to vascularly isolate the carotid body. This isolation was verified by the immediate loss of pulsatile pressure recorded from the carotid cannula, and the subsequent gradual decrease in mean pressure as the small volume of blood trapped in the carotid region drained through the venous outflow. Perfusion of the carotid body with venous blood from the syringe pump was begun, and the infusion rate was set to produce the same mean pressure in the isolated carotid region as during the previous autoperfusion. The aortic nerve was stimulated again after
1-3 minutes of perfusion with venous blood, and the blood pressure responses recorded. Several similar runs were performed in each animal at 15-20 min intervals, then the carotid body was perfused with arterial blood obtained from the carotid artery. Aortic nerve stimulation given during carotid body perfusion with arterial blood from the syringe served to evaluate the effects of the perfusion process itself on the blood pressure response. Samples were withdrawn from the pump syringe during perfusion of the carotid body with both arterial and venous blood, for analysis of blood gases in an IL 13 blood gas machine.
1) **Aortic Nerve Stimulation During Diving**

a) Blood Pressure, Heart Rate and Hind Limb Vascular Resistance Responses to Stimulation.

The circulatory state of unilaterally barodenervated ducks was midway between that of intact and completely barodenervated animals. Resting blood pressure, heart rate and hind limb vascular resistance were all higher 5 days to 1 wk after removal of one aortic nerve (Figure 17a, 17b and 17c) than in the control animals in Section II which had both aortic nerves intact (Figure 11), but the baroreceptor-heart rate reflex during pressor tests in unilateral barodenervates was just as effective (-1.0 beats min\(^{-1}\) mmHg\(^{-1}\)) as in animals with both aortic nerves intact. Section of the remaining aortic nerve eliminated the baroreflex effects on the heart but there was no further rise in resting blood pressure. Resting heart rate and hind limb resistance were not significantly affected by complete barodenervation except for a transient rise in heart rate on days 3 and 4 post-denervation.

During control dives, heart rate fell by 78 % of the predive value at 1 min, and by 2 min 30 sec of diving had dropped by 83 % (Figure 17b). Over the first minute of these
Figure 15. Arterial pressure (ABP) and hind limb blood flow (HLF) recordings from portions of 2 min 30 sec dives without (a) and with (b) periods of aortic nerve stimulation. Dives were recorded 1 day after barodenervation. Times in the dive and the time of surfacing are indicated by the arrows. The bars under the traces at (b) indicate the duration of the stimulation periods.
DEN.
1 day

a) Dive without stim.

b) Dive with stim.

Predive stim
1:00 Dive
2:00 Dive

10 sec
Figure 16. Arterial pressures and hind limb blood flows recorded during dives without and with aortic nerve stimulation in the same animal as in Figure 15, at 21 days after barodenervation. Labels and events are the same as in Figure 15.
a) Dive without stim.

DEN.
21 day

Dive without stim.

b) Dive with stim.

Predive stim

1:00
Dive
stim

2:00
Dive
2:10
stim
up
Figure 17. Cardiovascular responses to diving and to aortic nerve stimulation. a) Arterial blood pressure (MAP), b) heart rate (HR) and c) hind limb vascular resistance (HLVR) are presented as mean ± 1 S. D. for 14 observations in 12 unilaterally barodenervated animals before (Preden, shaded bars) and after complete barodenervation. The range included in each denervate day group is indicated under the bars. For each day group, values are presented just prior to stimulation (unshaded bars) and during stimulation (solid bars). Asterisks (*) indicate prestimulation values significantly different from predive for that day group. Closed circles (•) indicate values in denervates significantly different from the predenervation value at that dive time. The (+) symbols represent significant effects of stimulation in each day group at each dive time.
dives hind limb vascular resistance increased by 5.5 times and by end-dive was 6.5 times the predive value (Figure 17c). This degree of vasoconstriction did not occur in all vascular beds, however, since blood pressure was not maintained even at 1 min in the dive, in the face of the large fall in heart rate. By the end of the control dive, blood pressure had decreased by 24% from the predive value (Figure 17a). During recovery, all cardiovascular variables returned to their predive levels within 1 min of surfacing, and did not change significantly over the next 15 min (Figure 17a, 17b and 17c).

After complete barodenervation, forced diving resulted in variable degrees of bradycardia and hind limb vasoconstriction, depending on the time after denervation at which the dives were performed. The pattern of the cardiovascular responses to diving can be seen in the pressure and flow recordings from the same animal at two times after barodenervation (acute, Figure 15a; chronic, Figure 16a). The reduction in dive bradycardia can be seen clearly in the acutely denervated state (Figure 15a). For the first 4 days after nerve section, dive heart rate was more than double that in control dives at both 1 min and 2 min 10 sec after submergence (Figure 17b), and the proportional decrease in heart rate from predive was reduced (Figure 17b). After 5-6 days without baroreceptors the dive bradycardia deepened in both absolute and proportionate
terms: heart rate dropped to the same level (Figure 16a, Figure 17b), as well as by the same percentage of the predive rate, as in control dives. The time course of the bradycardia in barodenervates throughout the observation period was similar to that in control dives.

The hind limb vascular resistance response to diving in barodenervates was the same as that in controls, up to 14 days following nerve section (Figure 17c). However, after this time the response was reduced significantly, resistance rising only to 4.2 times the predive value, compared with rises of 5.5 times or more in control and acutely denervated animals.

Dive blood pressure in barodenervates fell by 25-50 %, the proportional reduction depending of the length of time without baroreceptors (Figures 15a, 16a, 17a). This progressive trend towards dive hypotension followed the trend toward increasing bradycardia: while the cardiac response to submersion was reduced in acute barodenervates, blood pressure was significantly higher than in chronic barodenervates, in which the heart rate fell to the same level as in control dives.

Blood gas values from predive, dive and postdive arterial blood samples taken from unilaterally and completely barodenervated ducks are presented in Table 7. Resting \( \text{PaO}_2 \) in animals with one aortic nerve intact was the same as in animals with both nerves intact (Table 5). When unilateral
TABLE 7

Arterial blood gases and pH during diving and recovery in unilateral (a) and bilateral (b) barodenervates. Means ± 1 S. E. M. (mmHg) are given for the number of observations in brackets, in 6 animals. All dive blood samples were taken at 2 min. Asterisks (*) indicate dive and recovery values significantly different from the predive value within each dive group.
### a) PRE-DENERVATION

<table>
<thead>
<tr>
<th>Time</th>
<th>PaO2</th>
<th>PaCO2</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PREDIVE</strong></td>
<td>85.3</td>
<td>26.9</td>
<td>7.488</td>
</tr>
<tr>
<td></td>
<td>±1.6</td>
<td>±2.2</td>
<td>±0.040</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td><strong>2:00 DIVE</strong></td>
<td>53.4</td>
<td>38.3</td>
<td>7.383</td>
</tr>
<tr>
<td></td>
<td>±3.1</td>
<td>±2.7</td>
<td>±0.041</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td><strong>1:00 REC</strong></td>
<td>99.7</td>
<td>21.5</td>
<td>7.451</td>
</tr>
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<td></td>
<td>±3.2</td>
<td>±2.1</td>
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<tr>
<td><strong>2:10 REC</strong></td>
<td>105.2</td>
<td>21.9</td>
<td>7.490</td>
</tr>
<tr>
<td></td>
<td>±3.3</td>
<td>±1.9</td>
<td>±0.039</td>
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<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
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<td>86.6</td>
<td>24.5</td>
<td>7.522</td>
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<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
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</tbody>
</table>
### b) POST-DENERVATION

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<th>PaCO2</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
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<td>7.469</td>
</tr>
<tr>
<td></td>
<td>±0.8</td>
<td>±1.0</td>
<td>±0.010</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td><strong>2:00 DIVE</strong></td>
<td>59.0</td>
<td>36.2</td>
<td>7.333</td>
</tr>
<tr>
<td></td>
<td>±1.5</td>
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<td></td>
<td>(9)</td>
<td>(10)</td>
<td>(9)</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1:00 REC</strong></td>
<td>102.9</td>
<td>17.9</td>
<td>7.436</td>
</tr>
<tr>
<td></td>
<td>±2.4</td>
<td>±0.6</td>
<td>±0.020</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2:00 REC</strong></td>
<td>97.8</td>
<td>20.1</td>
<td>7.437</td>
</tr>
<tr>
<td></td>
<td>±1.9</td>
<td>±0.5</td>
<td>±0.012</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>15:00 REC</strong></td>
<td>92.8</td>
<td>21.1</td>
<td>7.430</td>
</tr>
<tr>
<td></td>
<td>±3.3</td>
<td>±1.3</td>
<td>±0.015</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>
denervates were dived, however, $PaO_2$ did not fall as much as in baroreceptor-intact animals. $PaCO_2$ and pH in unilateral denervates at rest were the same as in resting intact animals, and showed the same changes in the dive. Total barodenervation did not alter the resting blood gas variables, and these animals, when dived, had the same changes in blood gases as did unilaterally denervated ducks. Dive $PaO_2$ in this group of barodenervates continued to be above that in the bilateral barodenervates in part 1 of this section (Table 5). After resumption of breathing at the end of the dive, $PaO_2$, $PaCO_2$ and pH recovered quickly in both unilateral and total barodenervates.

Responses to stimulation of the aortic nerve before and during diving are illustrated in Figure 15b (acutely barodenervated) and in Figure 16b (same animal, chronically barodenervated). Stimulation of the aortic nerve induced mean heart rate reductions of 35-42% at all times post-denervation in animals before diving (solid dark bars, Figure 17b). Hind limb vascular resistance, however, responded to predive nerve stimulation only at 2 and 9-10 days post-denervation (Figure 17c). The reduction in blood pressure (Figure 17a) produced by stimulation at rest, therefore, was almost entirely due to the cardiac effects of aortic nerve stimulation. A uniform 46% drop in blood pressure was observed during stimulations performed up to 14 days after nerve section, but beyond this time only a 32% pressure drop
occurred despite an unchanged effect of stimulation on heart rate.

When the aortic nerve was stimulated early in the dive in barodenervated animals, a greater proportional heart rate response (40-60% reduction) was obtained than could be produced by the same stimulus given predive (35-42% reduction) (also see Figures 15b and 16b). The decrease in heart rate during stimulation at this dive time was not dependent on the prestimulus rate: stimulation was no more effective proportionally when the dive heart rate was high, as at 1-4 days post-denervation, than in chronic barodenervates, in which dive heart rate was low. Hind limb vascular resistance was not significantly affected by stimulation early in the dive, and so did not contribute to the baroreflex-induced circulatory changes. Despite the enhanced cardiac response to stimulation, blood pressure dropped by only 32% during stimulation at 1 min in the dive, representing a significant reduction from the predive response.

Aortic nerve stimulation late in the dive affected heart rate inconsistently, producing significant effects only on some days after denervation. When end-dive stimulation was effective on the heart, the proportional reduction in rate was less than that occurring earlier in the dive or at predive (Figure 16b, 17b). Stimulation had no significant effects on hind limb vascular resistance at any time after
barodenervation. As a result of the reduction in cardiac response and the lack of a peripheral response, stimulation had no significant effect on end-dive blood pressure in ducks after denervation.

Heart rate, hind limb vascular resistance and, consequently, blood pressure recovered quickly in control animals after diving, returning to predive levels within 1 min of emergence. Blood pressure in barodenervates, however, remained below predive levels for more than 2 min following return to the surface, even in acute barodenervates in which post-dive heart rates were above their respective predive levels. By contrast, at all times after barodenervation, hind limb vascular resistance returned to predive levels within 1 min of surfacing. Aortic nerve stimulation was effective in altering heart rate and blood pressure throughout the recovery period, but the full response to stimulation did not return until 15 min after the dive. As at predive and during the dive, hind limb vascular resistance did not change during stimulation during the recovery period.

b) Cardiac Output and Total Peripheral Resistance Responses to Stimulation.

Measurements of cardiac output were obtained along with blood pressure, heart rate and hind limb blood flow, in 3 of the unilateral barodenervates with electrodes implanted on
Figure 18. Cardiac and peripheral resistance responses to aortic nerve stimulation in barodenervated ducks before and during diving. a) Cardiac output (CO) and stroke volume (SV); and b) total peripheral resistance (TPR) and hind limb vascular resistance (HLVR) are presented as mean ± 1 S. D. for 6 observations in 3 animals. The range of each day group in denervates is indicated under the bars. Notation, bar shading and symbols indicating significant differences are the same as in Figure 17.
the aortic nerve. Stroke volume, total peripheral resistance and hind limb vascular resistance were estimated before and during diving, then the remaining aortic nerve was destroyed and the animals were dived for 16 days post-denervation (Figure 18a, 18b).

Prior to complete barodenervation, cardiac output decreased by 84% in a 2 min 30 sec dive while stroke volume was unchanged, and the cardiac response to diving was virtually complete by 1 min in the dive (Figure 18a). Total peripheral resistance increased by 4.9 times at end-dive, while hind limb resistance in these animals rose by 5.8 times (Figure 18b). Hind limb and total peripheral resistance responses followed the same time course during the dive, rising to within 10% of their end-dive value by 1 min of diving.

Complete barodenervation did not significantly affect the absolute level of any of the resting cardiovascular variables presented in Figure 18, and neither total peripheral resistance nor hind limb vascular resistance rose with time after denervation. Stimulation of the aortic nerve prior to diving affected only cardiac output, reducing this by 25-35% (Figure 18a). During diving in these 3 animals the cardiac output decreased by the same proportion as did heart rate in the whole group of denervates, and with the same time course. The effects of stimulation on cardiac output in the dive (Figure 18a) also followed the same
pattern as on heart rate (Figure 18b). Stimuli delivered at 1 min in the dive had the same proportional effect on cardiac output as at predive, for most stages of barodenervation, but at end-dive the same stimulation produced no change in cardiac output at any time after denervation. Dive stroke volume was not altered significantly from the predive level, and thus all reductions in cardiac output during diving were solely the result of decreases in heart rate. Stroke volume was also not influenced by nerve stimulation in the dive.

The data presented in Figure 18b show that while the change in total peripheral resistance in the dive was less in the first 4 days following barodenervation than before denervation, baroreceptor removal did not alter either the magnitude or the time course of hind limb vasoconstriction over a 16 day period. Neither total peripheral nor hind limb vascular resistance responded to aortic nerve stimulation during diving in these 3 animals.
2) **Chemoreflex-Baroreflex Interactions**

a) **Aortic Nerve Stimulation During Dives After Exposure to Oxygen.**

Ducks with implanted stimulating electrodes were given 100% oxygen to breathe after having been barodenervated for 1-2 wk. 5 min of hyperoxic exposure raised blood oxygen tension significantly to 4 times the normoxic level but did not significantly affect PaCO₂ or pHₐ (Table 8). Heart rate, hind limb vascular resistance and blood pressure in hyperoxic animals (Figure 19) were unchanged from their respective levels in normoxia before diving (cf. Figure 17). Analysis of blood samples taken at 90 sec in hyperoxic dives showed that PaO₂ had decreased significantly but was still well above the resting normoxic value. PaCO₂ at 90 sec in hyperoxic dives was significantly higher than during predive hyperoxic exposure and above that recorded during dives in normoxic animals (Table 7). Arterial pH fell significantly in hyperoxic dives, to the same level as in normoxic dives.

Stimulation of the aortic nerve in hyperoxic animals before diving provoked a 42% fall in heart rate and a 54% decrease in mean arterial pressure, with no contribution to the pressure response from changes in hind limb vascular resistance (Figure 19). Hyperoxic animals showed a 43% decrease in heart rate by 1 min in the dive, while hind limb resistance rose by a factor of 3.3, and blood pressure was
**TABLE 8**

Arterial blood gases and pH before and during oxygen dives in barodenervated ducks. Means ± 1 S. E. M. for 6 observations in 4 animals. Predive blood samples were taken after 5 min of breathing 100 % oxygen, and dive samples were taken 2 min after submergence. All dive values are significantly different from predive.

<table>
<thead>
<tr>
<th></th>
<th>PaO2 (mmHg)</th>
<th>PaCO2 (mmHg)</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PREDIVE</strong></td>
<td>359.6 ± 7.1</td>
<td>24.7 ± 2.4</td>
<td>7.447 ± 0.038</td>
</tr>
<tr>
<td><strong>DIVE</strong></td>
<td>264.0 ± 29.1</td>
<td>48.2 ± 5.3</td>
<td>7.218 ± 0.044</td>
</tr>
</tbody>
</table>
Figure 19. Cardiovascular responses to aortic nerve stimulation during oxygen dives. Arterial blood pressure (MAP), heart rate (HR) and hind limb vascular resistance (HLVR) means ± 1 S. E. M. are given for 7 observations in 4 barodenervated ducks. Asterisks (*) on dive values indicate significant changes from predive and (+) symbols mean that, at a given time, the stimulation values (solid bars) were significantly different from the prestimulation values (unshaded bars).
maintained. Nerve stimulation at this time produced no change in hind limb vascular resistance but induced a 61% drop in heart rate, leading to the same proportional decline in blood pressure as that obtained during predive stimulation. By the end of the dive, heart rate had fallen by a total of 66% with no further increase in hind limb vascular resistance, and end-dive pressure was not significantly different from either predive or 1 min dive values. End-dive stimulation, however, reduced heart rate by half, with no effect on hind limb vascular resistance, and the net effect of stimulation on blood pressure was a 42% decrease.

b) Blood Pressure and Aortic Nerve Stimulation Frequency in Normoxia and Hypoxic Hypercapnea

The stimulus-response curve for the blood pressure response to aortic nerve activation is presented in Figure 20. Increasing stimulus frequency at a maximal intensity produced a smoothly graded proportional fall in pressure until a maximum depression was obtained at 20 Hz. At this frequency, the blood pressure fell to 38% of the prestimulation value. Further increases in frequency do not produce significantly greater hypotension.
Figure 20. Normalized arterial blood pressure responses to aortic nerve stimulation in normoxia and hypoxic hypercapnea. Pressure at each stimulation frequency is expressed as percent change relative to the pressure just before stimulation:

\[ \text{MAP change} = \frac{1 - \text{MAP change}}{\text{prestim MAP}} \times 100 \]

Closed circles (•) represent mean values during 5 complete stimulation runs in 3 normoxic animals, and the closed triangle (▲) is mean pressure change during 3 stimulations in 3 animals at 15 Hz, after breathing a hypoxic, hypercapnic gas mixture. Error bars represent ± 1 S. E. M., expressed as a percentage of the prestimulation value.
Table 9 shows blood gas values and pH during air-breathing and hypoxic hypercapnea in the three animals used to erect the frequency-response curve. Reduction of the inspired O\textsubscript{2} by approximately half, combined with an inspired CO\textsubscript{2} level of 6.4 %, produced PaO\textsubscript{2} and PaCO\textsubscript{2} levels close to those recorded in the same animals during dives (Table 7). When the aortic nerve was stimulated during hypoxic hypercapnea, blood pressure decreased by only half as much, proportionally, as during normoxia for the same stimulus (triangle symbol at 15 Hz in Figure 20).

c) Aortic Nerve Stimulation During Carotid Body Perfusion.

The effects of carotid body chemoreceptor stimulation on the baroreflex in conscious animals are shown in Figure 21. PaO\textsubscript{2} was 92.1 ± 2.1 (S.E.M.) mmHg, PaCO\textsubscript{2} was 19.2 ± 2.9 mmHg and pH\textsubscript{a} was 7.536 ± 0.009 pH units. The spontaneous ventilation rate during autoperfusion of the carotid body was 18.7 ± 1.5 breaths min\textsuperscript{-1}. Stimulation parameters for aortic nerve activation were set to bring blood pressure down by 47 % during autoperfusion. When the carotid body was perfused with venous blood (PvO\textsubscript{2} 45.5 ± 3.2 mmHg, PvCO\textsubscript{2} 48.5 ± 2.9 mmHg, pHv 7.347 ± 0.015), the same stimulation of the aortic nerve reduced blood pressure by only 33 %. That the carotid
### TABLE 9

Arterial blood gases and pH in barodenervates during air breathing, and during breathing a hypoxic, hypercapnic gas mixture. Means ± 1 S. E. M. are presented for the number of observations in brackets, in 3 animals.

<table>
<thead>
<tr>
<th></th>
<th>PO2 mmHg</th>
<th>PCO2 mmHg</th>
<th>pH</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AIR</td>
<td>87.9</td>
<td>25.5</td>
<td>7.532</td>
<td>+1.6</td>
<td>+1.8</td>
</tr>
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<td></td>
<td>(10)</td>
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<tr>
<td>HYPOXIC</td>
<td>62.3</td>
<td>43.8</td>
<td>7.349</td>
<td>+1.9</td>
<td>+4.3</td>
</tr>
<tr>
<td>HYPERCAPNEA</td>
<td>+0.021</td>
<td>(3)</td>
<td>(3)</td>
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</tr>
</tbody>
</table>
Figure 21. Effects of carotid body perfusion with venous blood on the arterial pressure response to aortic nerve stimulation. Normalized blood pressure changes (MAP % prestim) during stimulation were calculated as for the data in Figure 20. Means ± 1 S. E. M. are given for 15 observations when the animal was perfusing its own carotid body with blood from the heart (auto perf), 15 observations during perfusion of the vascularly isolated carotid body with venous blood from an extracorporeal pump (ven perf) and 6 observations during isolated carotid body perfusion with arterial blood (art perf) in 6 animals. The asterisk (*) indicates a significant difference between "ven perf" and "auto perf".
body chemoreceptors were in fact stimulated during venous perfusion was indicated by an increase in ventilation frequency to $25.5 \pm 1.1$ breaths $\text{min}^{-1}$. When the carotid body was perfused with arterial blood from the syringe, ventilation rate was the same as during autoperfusion and aortic nerve stimulation produced the same decrease in pressure as did stimulation during autoperfusion.
DISCUSSION

The sacrifice of one aortic nerve for electrode implantation produced resting levels of cardiovascular variables which were higher than those in intact ducks (cf. Figure 11, predenervate values), and the circulatory changes occurring when the unilaterally barodenervated animals were completely barodenervated (Figure 17) were much smaller than those occurring at simultaneous bilateral denervation in baroreceptor-intact ducks. It is clear that loss of a baroreceptor nerve one week prior to removing the other had put in train a substantial portion of the cardiovascular adjustments which take at least a week to develop when intact animals are barodenervated. Therefore, when the other aortic nerve was sectioned in unilateral denervates, these animals represented a more advanced stage of circulatory adaptation. This was also evident in the diving responses of the animals in this section: dive blood pressure decreased in the acutely barodenervated animals in Figure 17, whereas in Figure 11 blood pressure rose during diving at the same stage of barodenervation.

By 14-23 days of barodenervation, the peripheral resistance response to diving was reduced to the point where it resembled the response obtained by Jones (1973) in 30-50 day barodenervated ducks. The degree of vasoconstriction at end-dive decreased with time after denervation in the animals
of Figure 17 and, by 14-23 days, resistance had only risen by half the amount seen before complete barodenervation. This is a similar result (at 3 weeks post-denervation) to that obtained by Jones (1973) (at 5-8 weeks post-denervation), and bears out the suggestion at the end of Section III that the circulatory adaptations to loss of the baroreceptors in the animals of that Section were not complete by 16 days post-denervation.

Stimulation of the aortic nerve at rest in the acutely barodenervated animals of the present study produced a different response pattern from that during intermittent stimulation in the acutely barodenervated ducks of Section I. In the latter experiments, blood pressure decreased by 54%, as stimulation parameters were set to produce substantial changes in the dive, while in the experiments in the present section, the stimulation parameters were set to give a uniform 46% pressure drop at predive, and stimulation intensity was less than in the Section I experiments. Baroreflex activation by aortic nerve stimulation at rest in the present experiments did not engage the peripheral resistance limb. Thus the blood pressure response was due to a fall in heart rate alone, whereas in the earlier stimulation experiments both cardiac and peripheral limbs of the baroreflex were engaged.

In the present experiments, proportional cardiovascular responses to stimulation were consistent over time after
barodenervation even though stimulus intensity was not constant, which implies that even though the baroreceptor inputs to the brainstem were not tonically active, there was little change in the functioning of the baroreflex arc during this time, not only at rest but in the dive as well. The change in intensity required to obtain the same blood pressure responses on different days was probably the product of a changing relationship between the aortic nerve and the electrodes.

The proportional fall in heart rate during stimulation early in the dive was not dependent on the prestimulus heart rate: in acute denervates a stimulus frequency which reduces predive heart rate by 35% produces a fall in rate from 102 to 62 beats min⁻¹ at 1 min in the dive; in chronic barodenervates a stimulus frequency which reduces predive heart rate by 34% produces a heart rate decline from 41 to 22 beats min⁻¹ (46%) after 1 min of diving (Figure 17b). The non-linear relationship between vagal outflow and heart rate in ducks, described by Furilla and Jones (1987), means that in order to depress heart rate from 41 to 22 beats min⁻¹, a proportionally greater vagal drive would be required than that to reduce rate from 102 to 62 beats min⁻¹ in the dive. Therefore, given the same proportional decrease in heart rate by baroreflex stimulation for the two conditions before the dive, the baroreflex may have been more effective when heart rate was low in the dive (chronic) than when it
was high (acute).

The results from the baroreceptor nerve stimulation experiments in this Section bear out the suggestions in Sections I and II that the baroreflex operates primarily via heart rate during diving. Early in the dive, as discussed above, substantial drops in heart rate occurred when the aortic nerve was stimulated (see Figure 15b and 16b), and this response pattern suggests that the baroreceptors may contribute to the initiation of the bradycardia in intact animals, if pressure rises at the beginning of the dive.

The changes in cardiac output during diving before and after barodenervation in the subgroup of animals fitted with central flow probes followed a similar pattern to heart rate changes in the whole group. Blood pressure responses under these circumstances were therefore due to heart rate changes and not to altered stroke volumes. The lack of significant stroke volume change during diving throughout the denervation period in these experiments supports the results in acute barodenervates presented in Section I, and confirms earlier reports of unchanged end-dive stroke volume in intact ducks (Jones and Holeton, 1972; Jones et al., 1979). Cardiac output responses to stimulation before and during diving were also similar to those of heart rate in the whole group.

Total peripheral resistance was different in barodenervates compared with hind limb resistance in the same animals, in dives up to 4 days post-denervation. Hind limb
resistance rose in the dive by the same factor in denervation, but the total peripheral resistance response was significantly reduced in dives after denervation. This means that, in dives at this stage of barodenervation, not all vascular beds responded with the same degree of vasoconstriction as the hind limb. The present results concur with work in intact animals by Butler and Jones (1971), who reported that resistance to blood flow in the hind limb increased more than that in the vascular beds supplied by the carotid artery in intact ducks during diving, and by Jones et al. (1979), who recorded non-uniform flow redistribution throughout the body in force-dived ducks, indicating the differential nature of regional vasoconstriction during diving in these animals. From 5-16 days post-denervation, the total peripheral resistance response to diving was the same as the predenervation response, while hind limb vascular resistance was the same throughout the denervation period as before denervation. These results show that, in barodenervated animals at least, changes in hind limb vascular resistance during diving are not reliable as an index of the changes in total peripheral resistance, which is unfortunate since the hind limb vascular bed is more easily accessible than the central circulation.

In mammals, the chemoreflex interacts occlusively with (i.e. attenuates) sinoaortic baroreflexes (Abboud and Thames, 1983) to the extent that strong chemoreceptor drive can
reverse pre-existing baroreflex effects on the circulation. There is certainly strong chemoreceptor drive in force-dived ducks (Jones et al., 1982). I made an attempt to simulate this situation by loading the arterial chemoreceptors in non-diving conditions. Setting blood gases to levels similar to those during diving, by presenting spontaneously breathing ducks with low-oxygen, high-CO2 gas mixtures to breathe, reduced the baroreflex effect on blood pressure (Figure 20). In these experiments, heart rate and peripheral resistance were similar to those in normoxic animals, but baroreflex attenuation still occurred. That the chemoreceptors were stimulated in hypoxic hypercapnea was confirmed by the marked increase in breathing rate. The results of these experiments in ducks would appear to be an analogue of the situation in rabbits during arterial hypoxia, in which the gain of the baroreflex-heart period and baroreflex-blood pressure relationships were reduced (Iriki and Korner, 1979). The authors reached this conclusion only after they had done an elegant analysis of the baroreflex-independent resetting of cardiovascular variables during hypoxia. These authors also found, however, that the gain of the baroreflex-sympathetic nerve discharge relationship in some vascular beds was enhanced during hypoxia, implying that the cardiac but not the peripheral limb of the baroreflex is over-ridden by the chemoreflex in rabbits. This was not the case for the hind limb vascular bed in ducks: stimulating the aortic nerve at
an intensity which produced substantial changes in blood pressure at rest produced no significant changes in hind limb resistance either at rest or during diving.

An attempt to reduce the chemoreceptor drive during diving was made by giving animals 100% O₂ to breathe before the dive. This was partially successful, since end-dive PaO₂ remained above the level in non-diving normoxic animals, but PaCO₂ built up to a higher level in oxygen dives (Table 8) than in animals dived after breathing air (Table 7). Jones et al. (1982) have shown that a greater proportion of the chemoreceptor-induced cardiac and peripheral adjustments in forced dives are due to hypoxia than to hypercapnea, and so the reduction in dive responses in hyperoxic dives (Figure 19) compared with those in normoxic dives in the same animals (Figure 17) may be ascribed to reduced chemoreceptor drive. Under these conditions, then, the fact that end-dive stimulation of the aortic nerve was nearly as effective in reducing blood pressure as stimulation before the dive may indicate less central inhibition of the baroreflex by chemoreceptor input in hyperoxic dives than in dives in normoxic animals.

In a normoxic, non-diving animal, the effects of aortic nerve stimulation on blood pressure are substantially reduced when the chemoreceptors in one carotid body are perfused with venous blood. This was not an artefact of the perfusion procedure, since carotid body perfusion with arterial blood
had no such effect on the baroreflex. In the experiments in which chemoreceptor drive was elevated in spontaneously breathing animals, ventilation frequency also increased, and this may have been a factor in reducing the effectiveness of baroreceptor nerve stimulation on blood pressure. However, the animals were apneic during diving, and reduced chemoreceptor drive in hyperoxic dives was accompanied by an enhanced blood pressure and heart rate response to aortic nerve stimulation, while these responses were attenuated in dives after breathing air. This suggests that the attenuation of the baroreflex during chemoreceptor stimulation is independent of ventilation.

The results in this section support the conclusions in previous sections that the baroreceptors act primarily via heart rate to control blood pressure in the dive. The responses to aortic nerve stimulation before and during diving in chronically barodenervated ducks were similar to those in acutely denervated animals, which indicates that the central pathways of the baroreflex remained relatively unchanged for up to three weeks after baroreceptor loss. The responses of hind limb vascular resistance and peripheral resistance during diving differ, up to 5 days after barodenervation, and over this time, hind limb resistance cannot be used as an index of resistance changes throughout the body.
GENERAL DISCUSSION

1) Baroreceptor control of the circulation at rest.

In the intact duck the barostatic control system appears to function in regulating mean pressure, rather than adjusting pressure on a beat-by-beat basis, as in mammals. This may be partly due to the fact that birds have higher resting heart rates than mammals of equivalent body size (West et al., 1981) which would make beat-by-beat pressure regulation impractical, but birds may also have central integration mechanisms for the processing of baroreceptor information which are different than those in mammals.

Ducks have only one set of arterial baroreceptor nerves, instead of the two sets in mammals, and the sensitivity of the baroreceptor-cardiac reflex is lower in ducks (less change in heart rate for a given pressure rise in ducks than in mammals).

Smyth et al. (1969) were the first workers to propose that the sensitivity of the baroreflex-cardiac relationship could be quantified by plotting the systolic pressure of each pulse during drug-induced pressure rises against the pulse interval between that beat and the next successive beat. The slope of this linear relationship gives the sensitivity of the reflex. This analysis presupposes that the reflex can operate rapidly enough that the cardiac interval will begin to lengthen as pressure is still ramping upwards, and in fact
this feature of the mammalian baroreflex was used by Pickering and Davies (1973) to estimate a conduction time of about 400 ms for the baroreceptor-cardiac reflex in man. In their study, the latency of the first lengthening of the interbeat interval from the beginning of the pressure ramp, at heart rates below 75 beats min\(^{-1}\), was taken as the reflex latency.

In ducks, which commonly have heart rates of 150 beats min\(^{-1}\) or greater at rest, the latency for the baroreceptor-cardiac reflex is double that in humans, so this reflex is better suited to mean pressure regulation than to adjusting the pulse pressure of the next heartbeat.

Loss of baroreceptor input to the brainstem removes the short-term capability of the cardiovascular system to buffer blood pressure. The primary evidence for this is the lack of correction of induced pressure changes in barodenervates (Table 4, Figure 3). In a closed circulatory system in which pressure is regulated partly by adjustments in output of the pump, it would be expected that, over a wide pressure range, higher pressures would correlate with lower heart rates and lower pressures with higher rates as the baroreceptor-cardiac reflex operates in an intact animal to correct pressure disturbances (assuming, of course, that the system is operating at the same "set point" for pressure during the time the measurements are being taken). In Figure 22, blood pressures over a wide range are plotted against their
Figure 22. Effect of barodenervation on the relationship between mean arterial blood pressure (MAP) and heart rate (HR) at rest. In (a), spontaneously occurring HR are presented for 81 MAP measurements in 8 intact animals. In (b), spontaneous HR observations corresponding with 189 MAP observations are presented for the same animals after barodenervation. Regression lines were fitted by the least squares method.
a) Intact

\[ y = -0.502x + 223 \]

\[ r^2 = 0.39 \]

b) Den
corresponding heart rates for intact ducks. The regression line for this data, produced by the least-squares method, shows that an inverse relationship exists between pressure and heart rate, as predicted, although with a low but still significant r-squared value of 0.386. The slope of this line, and thus the sensitivity of the pressure-heart rate relationship, was -0.50 beats min\(^{-1}\) mmHg\(^{-1}\). The goodness of fit of the regression line was significant (F-test) and there was a significant correlation between the two variables (t-test). The r-squared value indicates that blood pressure is about 40% responsible for determining any particular heart rate, with pressure-independent factors being responsible for the rest. This relationship disintegrates after section of the baroreceptor nerves: there is no significant correlation between heart rate and pressure in barodenervates (Figure 22b). The slope of the regression line is not significantly different from zero, and heart rate at rest is no longer a controlled variable, at least by the baroreflex.

The slope of the spontaneous pressure-heart rate relationship in Figure 22 is about half that derived from pressor tests in intact ducks (calculated from the data in Table 4). Millard (1980) presented data in *Anas boscas* to show that the slope of the baroreflex-heart rate response during pharmacologically induced pressure changes was -1.17 beats min\(^{-1}\) mmHg\(^{-1}\), but he found that when the pressure-
cardiac relationship was plotted at spontaneously occurring pressures, the slope over a much narrower pressure range was 3.3 times that obtained pharmacologically, whereas the reverse was true in the experiments shown in Figure 22. Heart rate and pressure appear to be much more tightly linked in *A. boscas* than in *A. platyrhynchos*. The difference in cardiac sensitivity to spontaneous and pharmacologically induced pressure changes in both studies illustrates the point that manipulation of one arm of the baroreflex to obtain the relationship of pressure with the other arm may give a misleading picture of baroreflex function.

In the present experiments there appear to be different threshold levels of baroreceptor input required to alter cardiac output and peripheral resistance. That is, for a given amount of stimulation of the aortic nerve in ducks, heart rate changes make a greater contribution than changes in peripheral resistance to the new level of blood pressure. In mammals, the relative engagement of these effector limbs by the baroreflex is controversial, varying with species, with experimental technique, and, within one species, with the state of barodenervation. Ninomiya *et al* (1971) in anaesthetized cats found that sympathetic nerve discharge to spleen and kidney vascular beds had lower thresholds and higher sensitivities than cardiac sympathetic nerve activity, in response to a given change in pressure in one isolated carotid sinus, with the rest of the baroreceptor sites
denervated. In addition, these authors reported that, at a fixed carotid sinus pressure, there was less sympathetic activity to the heart than to the peripheral beds. These results would seem to indicate that peripheral resistance plays a greater role in carotid sinus adjustments of blood pressure than does cardiac output in cats.

Schmidt et al (1971) also found that the carotid sinus baroreflex employed peripheral resistance preferentially to cardiac output for blood pressure control, but these results were obtained in anaesthetized dogs with the aortic baroreceptors intact. When they denervated the aortic baroreceptors and repeated the experiments, they found that cardiac output and peripheral resistance participated equally in the systemic pressure responses to carotid baroreceptor input. Allison et al (1969), in carotid sinus denervated dogs, reported a greater contribution from total peripheral resistance than cardiac output to the baroreflex responses evoked by changing pressure in the isolated aortic arch.

The above experiments were done in anaesthetised animals, and the application of these results to the understanding of baroreflex function in awake animals is by no means certain. In conscious rabbits in which all baroreceptor nerves but one, to a carotid sinus, had been cut, a capsule was placed around the innervated sinus. The intra-capsule pressure could be adjusted to alter transmural pressure across the wall of the sinus and thus stimulate the
baroreceptors without opening the vascular system (Faris et al., 1981). Most of the arterial pressure change resulting from such baroreceptor stimulation was due to changes in peripheral resistance. In awake toads, however, Van Vliet and West (1986) reported that bilateral stimulation of the recurrent laryngeal nerve, which carries fibres from baroreceptors in the pulmocutaneous arch, produced equal decreases in heart rate and aortic resistance to reduce arterial pressure.

It is clear that a common effector pattern for the control of blood pressure in the various vertebrate groups does not exist. The differences in the relative contributions of both limbs of the baroreflex to pressure control in the studies cited above are due to the diversity of methods and preparations used, as well as to genuine species differences. The present experiments, in spontaneously breathing, conscious animals, showed that the degree of engagement of peripheral resistance in the response to aortic nerve stimulation in ducks depends on the intensity of stimulation. The higher threshold for resistance changes than for the cardiac response implies that heart rate adjustments, which are much more rapid than peripheral responses, serve to correct small pressure disturbances without needing to engage the periphery. Presumably larger pressure disturbances would also involve the peripheral resistance in baroreflex control.
If this is true, then tonic input from the baroreceptors probably does not contribute to the resting level of peripheral resistance in an intact animal. Evidence from the barodenervation studies in Section II supports this view: acutely barodenervated ducks do not show elevated hind limb resistance, and when the sympathetic innervation of the hind limb was interrupted in baroreceptor-intact animals, no change in resistance occurred. However, there seemed to be peripheral effects of baroreceptor deafferentation which did not become apparent for some time, since there was an elevated sympathetic vasoconstrictor outflow to the hind limb in ducks at two weeks after barodenervation.

Little work has been done on the specific efferent pathways of sympathetic vascular innervation in birds. Langley (1904) determined that skin vasoconstriction and movement of the feathers over various portions of the body could be produced by stimulating the sympathetic trunks in ducks, chickens and pigeons. He concluded that the distribution of sympathetic outflow to the periphery was much more limited in birds than in mammals, stating that the post-ganglionic fibres of each ganglion appeared to run almost exclusively in the spinal nerve with which that ganglion was associated. Bamford and Eccles (1983) were able to influence brain temperature by stimulating the cervical sympathetic trunk to alter blood flow in heat exchange retes in the heads of ducks and mammals, although no details of the stimulation
sites are given. Folkow et al (1968), in the course of experiments designed to examine the responses of hind limb skeletal muscle and vasculature to forced submersion in the duck, stated that: "It was technically impossible, unfortunately, to expose the sympathetic chain for direct, graded stimulations in the birds..." (p. 329-330). This somewhat pessimistic view was belied by the experiments in Section II.

The details of sympathetic neuroeffector outflow to the vasculature in mammals have been well documented. In an early study, Langley (1893) used the pilo-motor response to electrical stimulation of the paravertebral ganglion chain to determine the segmental distribution of sympathetic outflow by working through the length of the chain segment by segment. From his observations on skin vasoconstriction during these procedures, he concluded that the post-ganglionic fibres of a given ganglion contributed not only to the spinal nerve with which the ganglion was associated, but also to spinal nerves arising both anterior and posterior to that ganglion. This general scheme has held up well in subsequent investigations. Studies in the dog (Clonninger and Green, 1955; Donald and Ferguson, 1970), in cats (Kjellmer, 1965; Lundvall and Jarhult, 1976; Sonnenschein and Weissman, 1978) and in a comparative study in cats and humans (Kuntz, 1951) have shown that there is sympathetic innervation from multiple vertebral segments to one vascular
Some authors, however, have claimed that stimulation of the lumbar sympathetic cord in cats (Folkow, 1952) and in rabbits (Humphreys and Joels, 1982) between lumbar segments 4 and 5 gave total control of vasomotion in the hind limb. I do not believe this to be true for the lumbosacral stimulation site used in my experiments in ducks, since intact animals were able to generate greater increases in hind limb vascular resistance than were obtained in the hind limb sympathetic stimulation experiments in Section II.
2) **The Role of the Baroreflex in Control of Blood Pressure During Diving.**

The baroreflex is effective in regulating pressure in intact ducks at rest, but the performance of the barostatic control system during diving is less than optimum from the standpoint of blood pressure regulation. End-dive blood pressure drops by 18-20 % in intact animals (Section III; and Butler and Jones, 1971). When the baroreceptors are removed, the level of blood pressure is the hydraulic consequence of the prevailing cardiac output and peripheral resistance, and without these receptors "in-circuit", blood pressure in the dive fluctuates much more widely than in animals with a functional barostatic reflex.

The aortic nerve experiments showed that the cardiac limb of the baroreflex was active at 1 min in the dive, but whether or not peripheral resistance responded to stimulation predive, the baroreceptor-peripheral resistance reflex did not contribute to the operation of the baroreflex during diving. Therefore, pressure regulation early in the dive was accomplished by changes in cardiac output. In terms of barostasis, then, the function of the baroreceptors during diving is to balance the degree of bradycardia against the change in peripheral resistance to maintain adequate pressure for perfusion of the vascular beds which remain open in the dive.

McRitchie and White (1974) have suggested that the
arterial baroreceptors contribute to the bradycardia, but not to the rise in peripheral resistance, in response to nasopharyngeal stimulation in awake rabbits. These authors propose that input from the trigeminal nerve, which innervates nasopharyngeal receptors, initiates apnea. The cessation of lung afferent input along with continued trigeminal influence on cardiac and vasomotor neurons were responsible for generating both the bradycardia and peripheral vasoconstriction. The trigeminal reflex-heart rate relationship was facilitated by baroreceptor input, so the baroreceptors contributed to the bradycardia as blood pressure rose due to vasoconstriction. In the ducks in the present study, the baroreflex also acted through heart rate and not peripheral resistance during diving, suggesting that the integrative control of the diving responses is at least qualitatively similar to that in rabbits during nasopharyngeal stimulation. However, in ducks the baroreceptor control of the circulation is reduced after the first minute of diving, yet the cardiovascular adjustments are maintained. The experiments of White and McRitchie (1973) were not extended beyond about 30 sec, so their data cannot be compared to end-dive results in the present study.

The results of the chemoreceptor-baroreceptor interaction studies in Section IV suggest that some degree of central nervous attenuation of the baroreflex results from strengthened chemoreceptor drive in ducks. Chemoreceptor
stimulation in the dive should increase gradually as graded blood oxygen and CO2 changes occur (Jones and Purves, 1970; Butler and Jones, 1971), and this input may over-ride baroreflex function centrally. The study of central nervous interactions between even two sensory inputs which project to the same final common output pathways is a very complex problem, however, and rigid controls are needed to ensure that only a single effect is being observed during the experiments (Abboud and Thames, 1983). This situation is made even more difficult in diving studies by the fact that circulatory adjustments are the result of the integration of input from many different receptor groups. The attempt to analyze the interaction of chemoreceptor and baroreceptor inputs in diving and non-diving situations in the present study has, however, begun a possibly fruitful line of investigation which could lead to a clearer understanding of the integrative control of the circulation during diving.

There are two other possible mechanisms for the reduction of baroreflex effectiveness during diving. In intact animals, both heart rate and peripheral resistance are pushed close to their limits for adjustment during forced dives. If it is assumed that the relative changes in sympathetic outflow to the periphery, and in vagal drive to the heart, are the same during diving as before the dive, for the same degree of baroreceptor input, then these changes in outflow would produce less effector response because of the
more limited scope for effector adjustment. This would be
difficult to verify directly: some index of vagal and
sympathetic outflow to several different organs (integrated
whole-nerve discharge would be a good candidate) would be
needed during baroreflex activation before and during diving
in conscious animals. Nevertheless, the non-linear
relationship between hind limb sympathetic activity and
resistance in that bed has been established (Figure 4), and
it is obvious from this data that a given proportional change
in sympathetic drive when the resistance is high would not
alter resistance as much as the same change in drive when the
resistance is low. The same holds true for the non-linear
heart rate-vagal drive relationship reported by Furilla and

The second possibility is that central nervous
inhibition of the baroreflex pathway in diving is an indirect
effect of increased afferent input to the brainstem as
arterial hypoxia develops. Millhorn et al (1980a) proposed
that the long-lasting tachypnea in cats after exposure to
hypoxia was the result of a buildup of some ponto-medullary
neurotransmitter during the hypoxic period. This tachypnea
was found to be dependent on intact carotid body
chemoreceptor input, and the neurotransmitter involved was
later shown to be serotonin (Millhorn et al, 1980b). This
transmitter is implicated in the central autonomic control of
the circulation (Antonaccio, 1984), and Franz et al (1982)
have suggested that serotonin may be involved in the central depression of circulatory reflexes. There is a post-dive tachypnea in ducks, the duration of which varies in proportion to the length of the preceding dive (Lillo and Jones, 1982a), and a portion of this post-dive tachypnea is dependent on the integrity of carotid body chemoreceptor input during the dive (Lillo and Jones, 1982a; M. Shimizu, unpublished (1987)). The time for post-dive ventilation to return to the predive level after a 2 min dive (Lillo and Jones, 1982a) is about the same time required for the return of the full blood pressure response to aortic nerve stimulation after diving (Figure 9). The possibility therefore exists that indirect central neural effects from increased afferent input during the dive may linger long enough to inhibit the baroreflex during recovery from diving in ducks. Certainly the blood gases have returned to normal by 1 min post-dive (Table 6), so continued direct carotid chemoreceptor inhibition of the baroreflex could not be present at this time.

Investigation of this problem would require the measurement of neurotransmitter concentrations within the brainstem cardiovascular control areas, during submersion with and without arterial chemoreceptor stimulation. Neurotransmitter measurements would also be needed during chemoreceptor stimulation alone, and an attempt would have to be made to duplicate the possible effects of diving on
transmitter secretion by the application of various known transmitter substances to those regions of the brainstem involved in the control of the cardiovascular system during diving.
3) **Role of the Baroreceptors in Generating and Maintaining the Cardiovascular Adjustments to Diving.**

I have presented evidence in this thesis that the development of the diving responses in ducks does not depend on input from the baroreceptors. However, I have shown that the baroreflex did contribute to the decrease in heart rate in the first minute of forced dives, and also that the degree of peripheral vasoconstriction in the dive was independent of baroreceptor input. In the overall integration of the cardiovascular responses to forced diving in these animals, apnea must occur at the time of submersion for the cardiovascular adjustments to be set in train (Bamford and Jones, 1974). Receptors in the facial and oral area, on contacting water, initiate apnea, blood oxygen begins to fall and CO₂ to rise, and input from the arterial chemoreceptors increases. Baroreceptor-independent changes in heart rate and peripheral resistance occur, intensifying as chemoreceptor drive continues to rise with time after submersion. Up to about the first minute of diving, if the balance between peripheral resistance and cardiac output changes so that arterial pressure rises, the baroreceptor-heart rate reflex will help to intensify the bradycardia. Pressure changes in the opposite direction (that is, unloading the baroreceptors) probably would not produce reflex increases in heart rate because of the strong chemoreceptor drive to the vagal motor neurones. Input from
the baroreceptors later in the dive will have little effect on the level of cardiovascular variables. The role of the baroreceptors in the integration of the cardiovascular adjustments to diving is to contribute to the vagally mediated fall in heart rate, as the peripheral resistance rises. The reduction of the bradycardia in acute barodenervates is due to a deficit in vagal control of the heart, since the sympathetic component makes no contribution to heart rate during diving in these animals, even though increased cardiac sympathetic drive resets the resting heart rate to a higher level than in intact animals (Table 6).

Dive blood gases are similar in intact animals and barodenervates, but in acutely denervated animals the input from the arterial chemoreceptors could be reduced, perhaps due to altered blood flow patterns through the carotid body resulting from circulatory changes after denervation. If, however, chemoreceptor input is not altered, the possibility remains that there is a change in the effectiveness of the chemoreflex on cardioinhibitory motor neurones, this change resulting from the sudden loss of baroreceptor input. In dogs, Heistad et al (1974) have shown that a reduction in baroreceptor input produces a central nervous facilitation of the chemoreflex. This mechanism would not explain the abrupt reduction in diving bradycardia in the acutely denervated animal, however, for this is precisely the situation in which such an effect should be most evident.
With time after barodenervation, the bradycardia returns, but this is not correlated with greater changes in dive blood gases in chronic barodenervates than in acutely denervated animals (see discussion in Section III), and the mechanism behind this recovery remains unclear. One possible explanation is that the relationship of chemoreceptor input to cardiomotor output changes with time after barodenervation, causing the depth of the bradycardia to increase as the central nervous pathways adapt to baroreceptor loss.

The gradual rise in resting hind limb vascular resistance in the transition from acute to chronic barodenervation involves both neurogenic and structural adjustments. Given these changes, it might be expected that a given amount of sympathetic outflow to the hind limb in chronic barodenervates during diving would produce a greater rise in hind limb resistance than the same outflow in intact animals. Instead, as the cardiac response waxes with time after barodenervation, the peripheral response wanes. The changes in the peripheral vasculature documented in resting animals (Section II) do not account for this, and the possibility is left that the degree of neurogenic vasoconstriction during diving decreases with time after barodenervation. If this is true, a change in the relationship between chemoreceptor input and vasomotor output, in the opposite direction to that suggested for the
chemoreceptor-cardiomotor system, may occur centrally. Such a "central reorganization" after the loss of an afferent pathway has not been reported for the cardiovascular control system, but this mechanism has been proposed to explain the return of ventilatory responses to hypoxia, mediated by aortic receptors, after carotid chemoreceptor denervation in mammals (Smith and Mills, 1980; Majumdar et al., 1983). However, pressure-related control of the systemic circulation in ducks did not return over the course of the denervation experiments in Section IV. Yet, stimulation of the aortic nerve in chronically barodenervated ducks showed a strong pressure response, so this pathway had not degenerated centrally.
4) Consequences of Different Denervation Methods on Conclusions About the Effects of Acute Barodenervation

The method used to deafferent the arterial baroreceptors is an important consideration when interpreting the results of these experiments. Some procedures will produce artefactual cardiovascular effects which are not due to the loss of the baroreceptors, and these must be taken into account. To illustrate this point, a comparison of the results from various experiments in which ducks were acutely barodenervated is presented in Figure 23, based on data drawn from experiments in this thesis and from the Series 1 experiments of Jones et al (1983).

The denervation protocols employed were: 1) simultaneous bilateral aortic nerve section, 1 week after recovery from preparatory surgery; 2) bilateral section of aortic nerves during surgery; 3) unilateral aortic nerve section during surgery, followed by section of the contralateral aortic nerve 1 week later; and 4) unilateral aortic nerve section during surgery, then cooling or section of the contralateral vagus nerve 24-48 hours after surgery. All observations in total barodenervates were made within 24-48 hours of denervation. The significant differences indicated in Figure 23, if not derived from within the cited study, were obtained by an unbalanced t-test (P < 0.05).

Cardiovascular variables measured at rest and during
Figure 23. Comparison of the effects of various methods of barodenervation on resting and diving cardiovascular variables. Arterial blood pressure (MAP), heart rate (HR) and hind limb vascular resistance (HLVR) means ± 1 S. E. M. are presented from different studies. A: Section III, Figure 11 (Preden, snares on intact aortic nerves after recovery from surgery); B: Section III, Figure 11 (den, both aortic nerves sectioned by pulling snares); C: Section I, Table 1 (DEN, both aortic nerves cut during surgery; resistance is total peripheral resistance; divide HLVR scale by 10); D: Section IV, Figure 17 (Preden, one aortic nerve cut during surgery, snare on intact aortic nerve); E: Section III, Figure 17 (snare on aortic nerve pulled to complete denervation); F: Jones et al. (1983) Series 1 (left aortic nerve cut during surgery); G: Jones et al. (1983) Series 1 (right vagus cut to complete barodenervation). Predive values are indicated by unshaded bars, dive values by solid bars. Asterisks (*) indicate significant differences from the predive values in A, while plus signs (+) mean that the dive value within any group is significantly different from that group's predive value.
diving in the intact ducks in Group A (control measurements from Figure 11, Section III) are the reference for comparison with the other groups presented here. During forced submersion the large fall in cardiac rate was not completely offset by increased peripheral resistance and end-dive blood pressure fell by about 20%.

Group A animals became Group B animals after withdrawal of both aortic nerve snares 1 wk after surgery. Blood pressure increased after barodenervation owing to a sympathetically mediated heart rate increase. When barodenervation was performed during surgery (Group C), however, no acute hypertension ensued even though the resting heart rate doubled. Stroke volume was not significantly altered by denervation, and the lack of hypertension in animals denervated during surgery was due to a decrease in total peripheral resistance. The protocol used in these experiments did not include either sham operations or measurements in intact animals prior to denervation, so no control data are available for this group. For comparison, total peripheral resistance at rest in intact ducks of similar body mass (Lillo and Jones, 1982a) was 0.34 P. R. U. for the same stroke volume as in the barodenervated Group C ducks, but in the latter animals total peripheral resistance was only 0.22 P. R. U., 65% less than that in intact animals. The peripheral resistance drop after barodenervation in the Group C animals may have been the
result of incomplete recovery from either the anaesthesia or the surgical trauma of the operation, and the lack of a pressure rise following denervation could be an artefact of the experimental protocol.

Comparison of the diving responses of the two groups of bilateral denervates in B and C shows differences in heart rate but not in blood pressure. In Group B, a similar fall in pressure to that in intacts occurred in the dive but in the former group this fall was not significant owing to the increase in pressure variability after denervation. Group C also showed no significant change in dive blood pressure, but the proportional drop in heart rate in Group C (to 30% of predive) was greater than that in Group B (to 44% of predive); this must mean that the change in peripheral resistance in Group C was also greater than that in B, since in neither case did dive blood pressure change. If the change in hind limb vascular resistance in Group B animals can be taken as an index of the behaviour of total peripheral resistance during diving, this proposal appears valid: Group C total peripheral resistance increased by 9.3 times while Group B hind limb resistance only rose by 6 times.

The above comparison shows that the effects of barodenervation alone were revealed only after the compounding effects of surgery and anaesthesia were eliminated. The absence of an acute pressure rise after barodenervation in animals denervated during surgery is
misleading, since a significant pressure rise occurred in animals deafferented after recovery from surgery. The cumulative effects of the operation seem to be primarily on peripheral resistance, since the same degree of post-denervation tachycardia was present in both experimental groups. Differences in the relative intensities of cardiac and vasomotor responses to head submersion between B and C also show that the diving responses are not comparable in animals denervated by these procedures.

Partial deafferentation of the baroreceptors (Group D animals, one aortic nerve removed) resulted in increases in both heart rate and total and hind limb resistance, and blood pressure was significantly above the intact level. Even though the baroreflex, mediated by baroreceptors in the remaining aortic nerve, was still fully functional, bilateral baroreceptor input is evidently required to maintain resting blood pressure in the normal range. It is clear, as outlined in Section IV, that the cardiovascular system in this state has already begun to adapt to the partial baroreceptor deficit.

When these unilateral barodenervates were dived, blood pressure fell more, proportionally, than in intact animals during diving. The absolute dive heart rate in Group D animals was the same as that in intacts, as was the proportional drop in heart rate, but the proportional change in hind limb vascular resistance in Group D animals (9.1
times) was less than that in intacts (10.3 times). Total peripheral resistance in Group D increased by 4.9 times in the dive, a similar increase to that reported in intact animals by Lillo and Jones (1982a). The importance of these results is that animals with one baroreceptor nerve do not possess the same facility as baroreceptor-intact animals for maintaining dive blood pressure, although the cardiac and peripheral responses are qualitatively similar.

After section of the remaining aortic nerve (Group E) there was no further change in peripheral resistance, while the blood pressure rose due to an acute tachycardia. In the dive, however, the proportional cardiac and peripheral responses were reduced, with more relative reduction in vasoconstriction than in bradycardia, resulting in a greater decrease in dive blood pressure in Group E than in D. The cardiovascular state in partially barodenervated animals cannot be considered analogous to that in intact animals, but is midway between the intact and totally barodenervated states. Comparison of the total denervates of Group E with those of Group B, however, show that the differences resulting from the two denervation protocols are of magnitude and not of kind. Having established the nature of these differences, to use the animals in Group E can then be used for studying the effects of baroreflex activation by stimulating one aortic nerve.

Group F also represents animals unilaterally
barodenervated during surgery (Jones et al., 1983; Series 1), but observations were made in this group after only 1-2 days of recovery, in contrast to the Group D animals which had 1 week to recover. Resting blood pressure in F was the same as in unoperated animals (A), in contrast to the other group of unilateral denervates, D, which developed hypertension. However, in both F and D, heart rate and hind limb vascular resistance were higher than in intacts. If, despite an increase in hind limb vascular resistance, total peripheral resistance was depressed in Group F animals just after surgery, as has been suggested for Group C, then this may explain the lack of resting hypertension in Group F despite the loss of one baroreceptor nerve. The diving responses of this group of unilateral denervates also contrast with those in Group D, in that end-dive blood pressure was maintained in F despite a bradycardia of the same proportion as that in D. The major difference between the two groups, accounting for the maintained dive blood pressure in F, was a greater increase in peripheral resistance in the latter group.

The animals in Group F were completely barodenervated by cooling or cutting the contralateral vagus nerve (Group G). This resulted in the highest resting heart rate of any group, and this tachycardia was responsible for the acute hypertension, since vagotomy did not affect hind limb vascular resistance. Unilateral vagotomy under these conditions would probably enhance the barodenervation-induced
increase in sympathetic drive to the heart by withdrawing a portion of vagal cardiac restraint, as well as by disrupting the central interplay of inputs from the other vagally mediated receptor groups with cardiovascular effects. The lack of one vagus also affected the diving bradycardia independently of, and in addition to, the loss of the baroreceptors, since the absolute level of dive heart rate after unilateral vagotomy in baroreceptor-intact animals was not as low as in unoperated animals (Jones et al., 1983).

The technique developed in Section II for studying the effects of barodenervation without complications allows deafferentation of the baroreceptors in animals returned to a normal condition following surgery. Control responses were obtained in these animals before denervation, and the cardiovascular adaptations after barodenervation represented only the effects of baroreceptor loss. It is apparent, on comparing the results of these studies, that the interpretation of results from acute barodenervation experiments must take the methodology of denervation into account.
SUMMARY

1) Arterial baroreceptors in dabbling ducks contribute to the cardiovascular responses to forced diving by modulating heart rate to help balance cardiac output against vasoconstriction as these adjustments develop in the first minute after submersion.

2) The sensitivity of the arterial baroreceptor-heart rate response in intact ducks was $-1.16 \text{ beats min}^{-1} \text{ mmHg}^{-1}$, in response to pharmacologically induced changes in blood pressure. This response was eliminated in acutely (1 day) and chronically (3 wk) baroreceptor-deafferented ducks.

3) In ducks barodenervated by bilateral aortic nerve section 1 week after implantation of snares around these nerves, heart rate rose due to an increase in cardiac sympathetic activity. This acute rise was moderated over the next three weeks following barodenervation, but heart rate did not return to the pre-denervation level. Hind limb vascular resistance was unaffected immediately after barodenervation but rose gradually with time, partly because of wall hypertrophy in small muscular leg arteries (wall thickness to lumen diameter ratio increases), and partly because of increased neurogenic vasoconstriction. The initial rise in heart rate after barodenervation elevated arterial blood...
pressure and this hypertension was maintained over the three week observation period.

4) During forced diving in intact ducks, the proportional increase in total peripheral resistance was not balanced by the proportional decrease in heart rate so dive blood pressure fell. However, in acutely barodenervated ducks, the degree of vasoconstriction was unchanged but the cardiac response to diving was reduced, so dive blood pressure remained near the predive level. There was no sympathetic component in the cardiac response to diving in acute barodenervates, and the reduction in the degree of bradycardia after denervation was due to a deficit in vagal drive to the heart. This deficit represented a loss of about one-third of the vagal outflow required to produce the bradycardia seen in intact animals, and was a result of the loss of baroreceptor input.

5) In chronically barodenervated animals the heart rate response recovered so that the degree of bradycardia was the same as in intact animals, while the proportional change in peripheral resistance was reduced. The net effect of these modifications was to produce a greater fall in end-dive blood pressure than in intact animals. Without baroreceptors, blood pressure during diving was primarily a function of the degree of cardiac slowing, so dive
hypotension became more profound as the cardiac response returned with time after barodenervation. Why the diving bradycardia recovered in chronic barodenervates is not clear.

6) Implantable electrodes were developed for electrical stimulation of the aortic nerve in barodenervated ducks, to activate the baroreflex by simulating a change in baroreceptor discharge. Stimulation at rest produced low-threshold cardiac depression, but a higher intensity was required to invoke changes in peripheral resistance. Correction of small pressure disturbances by the barostatic reflex in intact ducks was accomplished by cardiac adjustments, with the peripheral vasculature participating only during larger pressure changes. The relative effects of aortic nerve stimulation on the efferent limbs of the baroreflex did not change with time after denervation.

7) Stimulation of the aortic nerve during diving produced blood pressure responses which were entirely the result of changes in heart rate, since neither total peripheral nor hind limb vascular resistance responded to stimulation. The effects of stimulation on arterial pressure at 1 min in the dive were reduced, compared with the pressure response before diving, and stimulation effects were eliminated by the end of a 2 min 30 sec dive. In animals given 100 % oxygen to breathe before diving, to reduce input from carotid body
chemoreceptors during the dive, the arterial pressure response to aortic nerve stimulation at end-dive was nearly the same as before the dive. The chemoreflex was shown to inhibit the baroreflex in non-diving situations in ducks, and may therefore have been partly responsible for the progressive attenuation of the baroreflex during diving.

8) The results of barodenervation experiments depend on the methodology of denervation. Cardiovascular changes in animals barodenervated by instantaneous aortic nerve section, performed one week after preparatory surgery under general anaesthesia, represent the effects of barodenervation alone. Results obtained from animals denervated by nerve section during surgery are compromised by complications arising from the surgical procedures. The time after barodenervation that observations are made must be taken into account, since acutely and chronically denervated ducks in the present experiments represented two ends of a continuum of progressive circulatory adaptation.
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APPENDIX

1) STIMULATING ELECTRODE CONSTRUCTION AND STIMULUS PARAMETERS

a) Electrode construction.

The electrodes used for chronic aortic nerve stimulation were made of 40 gauge Teflon-insulated stranded stainless steel wire (AS 631; Cooner Wire Co., Chatsworth, California) threaded through a patch of silicone rubber reinforced with silk mesh (Figure A1a and A1b).

The patch was cut from a larger sheet of silicone rubber which was molded from Dow Corning RTV 732 bathtub caulk. About 2-3 ml of 732 was thinned with xylene (toluene can also be used) until the mixture flowed slowly when the mixing vessel was tipped. The inclusion of air bubbles in the mixture was inevitable during this process, and these were removed by degassing in a vacuum pump-evacuated dessicator. The mold was made from sheets of 0.5 mm thick Teflon. The centre section of a 5 cm square piece of this material was removed to make an inner hollow square 3 cm on a side. This frame was placed flat on another sheet of Teflon and together these pieces defined the edges and the bottom surface of the mold. The mixture of 732 and xylene was then poured slowly into the mold, then a swatch of
Figure A1. Electrodes for stimulating left aortic nerve in ducks. a) Nerve side, showing bare strands of stainless steel wire. b) Insulated side, showing position of Silastic tubing and the manner of securing the free ends of the wires by wrapping. The silicone rubber insulation has been left off at b) for clarity.
fine-mesh silk 2.5 cm on a side was soaked in xylene to remove air bubbles, and laid on the surface of the poured mixture. When this swatch was prodded gently it subsided into the mixture. A third sheet of Teflon was placed over the top of the mold and the whole was then sandwiched between two sheets of plate glass. This procedure squeezed excess mixture and trapped air out of the mold. The entire assembly was then placed into a 60 degree C oven for 24 hours to cure. The Teflon sheets were then peeled off the frame to reveal a sheet of silicone rubber with silk mesh reinforcing, 0.5 mm thick and 3 cm on a side. This was cut with a new razor blade into 3-4 mm square patches.

Holes were punched near the 4 corners of each patch for the anchoring sutures. These holes were made with a 20 gauge hypodermic needle, cut off blunt and with the rim sharpened to act in the same manner as a conventional cork borer. Towards the centre of the patch another group of 4 holes was punched using a 23 gauge needle, prepared as above. These holes also formed a square pattern (Figure A1a), about 2 mm on a side. A 1.5 mm length of 0.8 mm diameter Silastic (Dow Corning) tubing was attached with a dab of Type A adhesive in the centre of one side of the patch, with one end of the tubing projecting over the edge (Figure A1a), and put in the oven to set for 1 hour. This tubing formed the support for the electrode leads.

15 cm lengths of wire were cut and about 1 cm of Teflon
insulation was removed from one end of each wire. This is difficult to do without damaging or nicking the wires, which in a chronic implant will eventually break at any weak spot, and a stripper for these wires was invented to solve this problem. The stripper consisted of a plastic block with a hole drilled from one side to the other, into which the wire fitted snugly. A fine slot, for a single-edged razor blade, was cut into the block at a right angle to the axis of the hole, the blade being attached to a swinging arm so that the edge could be swung into the slot to partially occlude the hole. The depth of the blade in the hole was set by an adjustable stop on the arm. The wire was then inserted into the hole, the blade swung into the slot, and the depth the blade was allowed to enter the hole was adjusted so that it cut almost all the way through the outer layer of insulation but not far enough to nick any of the wire strands. The wire was then spun in place, between finger and thumb, so that the blade made a circumferential cut in the insulation, and the wire was withdrawn sharply from the block. If the depth of the blade was set correctly the insulation was stripped cleanly from the end of the wire, with no fraying.

The first 2 mm of the exposed stainless steel strands were tinned with 4% silver-bearing tin solder containing an acid-core flux (sold commercially for soldering stainless steel), then the tip of the tinned portion was cut off square. The process of tinning the wires was done simply to
bind the strands together during the next stage of assembly, and it was important to prevent solder from flowing down the strands for more than 2 mm because this tinned section was later removed (tin is highly toxic to biological tissue). The tinned end of the wire was inserted through the piece of tubing on the patch, and then threaded through one of the nearest centre holes to the other side of the patch with fine forceps. This end was brought back through the patch via the other nearby centre hole and the whole bared end was then pulled through the patch until the end of the Teflon jacket approached the hole through which the bare portion of the wire was first threaded. The free end of the wire was wrapped several times around the section of bare wire close to where the insulation ended, and cut off short, as shown in Figure Alb. This completed one of the electrodes. The other lead was inserted, threaded through the patch and anchored in a similar manner in the centre holes furthest away from the tubing. The tubing side of the patch was then insulated with Type A adhesive. After curing the adhesive for 1 hour in the oven, the exposed wires on the nerve side of the patch were teased apart and fanned out slightly to increase the active surface area.

The other ends of the leads were stripped for 5 mm, tinned and soldered to miniature terminals which were inserted into the body of a modified Molex Model 2695 crimp terminal connector housing, originally meant for circuit
board connections. This housing was modified to form flanges with suture holes so that the connector housing could be anchored to the skin, and the entry points of the leads into the housing were sealed with RTV 732. The leads were not twisted together, since twisting reduced the flexibility of the whole assembly.
b) Stimulus waveform and parameter selection.

Monopolar stimulation of peripheral nerves is adequate in acute experiments but in chronic experiments this waveform results in a substantial net charge transfer from the electrodes to the surrounding electrolyte and tissue. This charge transfer results in electrolytic and toxic damage to the nerve, and can eventually lead to failure of the implanted electrodes (Brummer and McHardy, 1977; Swett and Bourassa, 1981). The use of a bipolar stimulating waveform (the so-called "Lilly" waveform, after Lilly, 1961), which consists of a charge-balanced positive and negative pulse pair separated by a short delay, prevents much of the chronic damage to peripheral nerves resulting from monopolar stimulation. If a pulse of one polarity is followed within a short time by a second pulse carrying an equivalent charge but of opposite polarity, any electrochemical reactions occurring at the nerve-electrode interface from the first pulse will be reversed by the second pulse, cancelling any potentially harmful effects on the nerve. When the two pulses are exactly balanced, there is no net transfer of charge to the nerve and long-term electrolytic damage is prevented. Use of this waveform also means that greater pulse currents can be employed than when monopolar pulses are used, to offset the slow decline of stimulation effectiveness which occurs with chronically implanted stimulation.
electrodes.

The area of electrode exposed to the nerve was maximized within the size requirements of the electrodes developed in this study (Section IV). This was done as described in Part (a) above, by using stranded stainless steel wire and slightly fanning out the strands as they lay flat on the surface of the silicone rubber patch. The area of each electrode was 0.0075 cm², and for pulses of 0.5-2.0 mA, the current density (quotient of pulse current and area) was 67-267 mA/cm², well below the value of 500 mA/cm² listed by Weinman (1965) as a reasonable upper limit for normal physiological stimulation. Brummer and McHardy (1977) set a safe operating limit for charge transfer without damage during stimulation of 40 microcoulombs(uC)/cm² for single monopolar pulses, but stated that this could be increased by a factor of two or more for biphasic waveforms. The electrodes developed here delivered between 33 and 133 uC/cm² during each pulse at the current (0.5-2.0 mA) and pulse width (0.5 ms) used during aortic nerve stimulation. A pulse width of 0.5 ms was chosen for the present study on the basis of theoretical recommendations by Swett and Bourassa (1981). The above calculations for area were based on the assumption that the electrode wires have smooth surfaces. In reality, the surfaces are microscopically rough, and this could multiply the actual surface area several times (Dymond, 1976), which means that current and charge density
could have been even lower than I had calculated.

In tests of the patch electrodes, no electrolysis at the electrode-saline interface was observed in vitro at 50 times magnification when the electrodes were driven for 10 min with a biphasic waveform at 5 mA, 0.5 ms duration for both pulses, a 0.5 ms delay between the pulse pairs, and 100 Hz repetition rate. This current and repetition frequency were well above the levels actually used during stimulation experiments. If the biphasic waveform was unbalanced by 25%, however, the formation of very fine bubbles of gas at the electrodes indicated that electrolysis was beginning in the last few minutes of the tests, and therefore the use of the biphasic waveform represented a distinct advantage.

Bergveld (1976) has analyzed the effects of electrolysis in electrical stimulation, and devised simple tests to detect when the limits of voltage and current for any particular electrode arrangement have been exceeded. His results show that, for constant-current pulses of finite duration delivered to a pair of electrodes in an electrolyte, the voltage rises continuously from the leading edge to the trailing edge of the pulse due to the charging of the inter-electrode capacitance. If the current is too high or the pulse duration too long for the particular electrode in use, the voltage will reach a plateau before the pulse is ended, and this behaviour is easily seen on an oscilloscope during stimulation. When this plateau is reached, electrolytic
breakdown will begin, producing local damage at the electrodes. It follows that, if the pulse parameters are manipulated so that during a pulse the voltage developed between the electrodes never reaches steady state during the constant-current pulse, the limits for neural damage will not be exceeded. These criteria were adhered to in the present study, in which voltage, current and pulse-width were monitored on a differential oscilloscope at all times during stimulation.

When current is held constant, the voltage varies as the electrode impedance varies and so, despite changes in electrode impedance which are common during bouts of stimulation (Dymond, 1976), the actual charge delivered to the nerve will not vary with impedance. Therefore, stimulus intensity, which is set by the biocalibration technique, will not vary within one experimental run, an assurance which the constant-voltage mode of stimulation does not give.

It is clear from this simplistic analysis that the use of a constant-current, biphasic waveform is justified in chronic stimulation of the aortic nerve. The stimulus parameters must, however, be monitored continuously throughout the experiment and readjusted if safe limits are exceeded, in order not to damage the nerve. The methods outlined here have permitted a greater degree of repeatability in my experiments, both among animals in the same study, and between studies, than would otherwise have
been possible.
2) ULTRASONIC FLOW PROBE MODIFICATION AND CALIBRATION

a) Flow probe modifications.

Parks Electronics Laboratory (Beaverton, Oregon) supplied ultrasonic probes which, in their stock form, had several disadvantages for the present study. The styrofoam bodies of the probes were large, relative to the lumen size, and had sharp corners and a large flat braided surgical tie for apposing the two body halves around a vessel. In addition, the leads attached to the probes were 22 gauge copper wires with thick and stiff PVC insulation. In order to adapt these probes for implantation in the limited space available in the hind limb, the probe bodies were disassembled and the stock tie and leads cut off. As much styrofoam as possible was removed by filing off the body, and the lumens were enlarged, smoothed and chamfered internally to remove sharp edges. The leads were replaced with 10 cm lengths of 36 gauge Teflon-insulated stranded copper wire, and were insulated at the probe body with RTV 732 or Silastic Type A medical adhesive, which also anchored a new tie of 3-0 surgical silk. The free ends of the leads were attached to modified Molex connectors, as described for the stimulating electrodes, and the leads were not twisted together. The very light weight inherent in this probe design, coupled with
the thin flexible leads and the practice of not anchoring any parts of the probe to the tissues except to tie the two halves of the probe body around the vessel, promoted rapid healing. The animals therefore regained full use of the leg within a day or two of the implantation procedure. No vessel occlusion problems were encountered after proper placement of the ultrasonic probes, which was not the case for the electromagnetic probes previously used.
b) Calibration Procedures.

Flow probes were calibrated in vitro, in situ or in vivo. For in vitro calibration at post mortem, a vessel segment with the probe at the midpoint was removed entirely from the leg and the cut ends tied over fixed wide-bore cannulae. Whole blood obtained from the animal after heparinization was channeled from a rotary perfusion pump through the vessel segment and past the probe at known rates. In situ calibration was performed by exposing and sectioning the artery upstream and downstream of the probe and cannulating both ends of the arterial segment with tubing from the perfusion pump. Heparinized blood obtained from the animal was run through the probe as outlined for the in vitro method.

For in vivo calibration the animal was anaesthetized with sodium pentobarbital at the end of the experiment. The probe site was exposed, the artery clamped upstream and divided about 10 mm downstream from the probe, and 1000 I. U. of heparin were administered to the animal. The cut ends of the vessel were cannulated with PE 190 tubing, one cannula facing the heart, the other facing into the vascular bed of the leg, and both cannulae were connected to a syringe-type infusion pump (Model 901, Harvard Apparatus, Millis, Massachusetts). Blood could be withdrawn at arterial pressure into the pump through the probe at known rates,
after unclamping the artery, and returned from the pump to the vascular bed of the leg after each calibration run. The pump thus acted as a sink, into which the blood would be driven by the heart at in vivo pressures. This procedure was the method of choice for calibration, because the relationship of the probe to the vessel wall remained undisturbed and the arterial pressure maintained near-normal flow conditions through the probe. This was sometimes difficult to do with the other calibration methods. In vivo calibration was tried first in most animals, and if this did not work, the in situ method was used. In vitro calibration was the last resort, and was also used in early experiments to compare ultrasonic flow probe characteristics with those of electromagnetic probes. All of the calibration methods gave similar results, and the relationship between flow and pen displacement was linear over a range of 5-100 ml/min.