EFFECTS OF CIRCULATING CATECHOLAMINES ON DIVING IN DUCKS
(Anas platyrhynchos)

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
Department of Zoology.

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April, 1990
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Vancouver, Canada

Date **30 APRIL 1990**

DE-6 (2/88)
ABSTRACT.

Plasma catecholamines have been measured in chronically adrenalectomised (ADX) ducks, in chronically adrenal denervated ducks (DNX), in their respective sham-operated controls (SH-adx, SH-dnx) as well as in intact ducks after 3 minutes forced submergence. The results showed that 100% of the plasma Epinephrine (EP) and 40 to 80% of plasma Norepinephrine (NE) released during the dive came from the adrenal glands. 20 to 60% of plasma NE came from endings of the autonomic vascular sympathetic nerves which are strongly stimulated during diving. Adrenal catecholamines were released by nerve activation only; non neural mechanisms did not play any role in their release.

Maximum dive times (MDT) in chronically adrenalectomised ducks (ADX: 5 min. 19 ± 20 sec.) and in chronically adrenal denervated ducks (DNX: 7 min. 10 ± 13 sec.) were significantly lower than in sham-operated controls (respectively SH-adx: 9 min. 58 ± 45 sec., SH-dnx: 12 min. 10 ± 28 sec.). Venous infusion of catecholamines in ADX and DNX during the dive increased MDT: MDT of DNX ducks perfused with catecholamines (9 min. 46 ± 20 sec.) were significantly higher than in DNX perfused with saline (7 min. 21 ± 17 sec.), but did not reach the MDT observed in the SH-dnx: other adrenal products must be involved. Diving heart rates of ADX and DNX (at 4 min. dive respectively: 62 ± 16 and 31 ± 2 beats/min.) were significantly higher than in their sham-operated controls (23 ± 3 and 17 ± 2 beats/min.). Blood pressure during the dive was signifi-

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cantly lower in ADX and DNX (at 4 min. dive respectively: 93 ± 8 and 98 ± 4 mmHg) compared with their sham-operated controls (131 ± 12 and 118 ± 6 mmHg). Infusion of catecholamines in DNX raised blood pressure towards SH-dnx values, but there was no change in heart rate. PaO₂, CaO₂, pHa and lactate levels in DNX (respectively: 42 ± 2 mmHg, 4.5 ± 0.8 ml O₂ /100ml blood, 7.233 ± 0.016, 3.1 ± 0.3 mM) were significantly lower than in SH-dnx after 5 minutes submergence (53 ± 1 mmHg, 6.8 ± 0.4 ml O₂ /100 ml blood, 7.301 ± 0.007, 4.8 ± 0.4 mM). There was also a significant increase of plasma Na⁺ (+ 5.4 ± 1.7 mEq/L) in SH-dnx after 5 minutes submergence, but this was not the case in DNX where it was K⁺ (+ 1.1 ± 0.4 mEq/L) which increased. This suggested that adrenal catecholamines increase tolerance to underwater submersion by enhancing peripheral vasoconstriction, thus preserving the O₂ stores for the heart and brain. Moreover, they may affect the acid-base equilibrium during diving by increasing the activity of the Na⁺/K⁺ pump and may also have a direct effect on the rate of glycogenolysis.

Preventing the actions of catecholamines on the heart by injecting beta-blocker during forced submersion did not decrease MDT; however the cardiovascular response was markedly affected. During beta-blockade, diving heart rate rose steadily from 24 ± 6 beats/minute after 2 minutes to 52 ± 8 beats/minute after 6 minutes diving. In contrast, heart rates remained close to the levels reached at 2 minutes (17 ± 3 and 19 ± 4 beats/minute) throughout the control dives.
Perfusion pressure and blood flow have been recorded simultaneously in both hind limbs of ducks. One leg was perfused with different blood mixtures devoid of catecholamines (Test leg) and compared with the other, perfused with the ducks' own blood (autoperfused leg). This showed that hypercapnia has a depressant effect on the neural component of the peripheral vasoconstriction. Perfusion of test legs with hypoxic-hypercapnic blood to which catecholamines were added, showed that circulating catecholamines are needed to increase peripheral vasoconstriction during diving.

In summary, during forced submergence circulating catecholamines, released mainly by the adrenal glands, compensate for the depressant action of hypercapnia on the neural component of peripheral vasoconstriction. Maintenance of this peripheral vasoconstriction during forced diving ensures that $O_2$ stores are not wasted on peripheral tissues, and this explains how MDT is prolonged.
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ACKNOWLEDGEMENTS.

I thank my advisor, Professor David R. Jones, who provided me with the opportunity to pursue a Ph.D. in his research laboratory. I am most grateful for his advice and kindness. Dr. Jones' indomitable spirit was as much of an inspiration to me as was his invaluable scientific guidance. I also thank those in the Department of Zoology who helped me and allowed me to use their equipment: M. Hughes, D. Randall, W. Milsom, P. Hochachka, A. Perks and D. Mense. The pressure-flow controller in Chapter IV was designed with the help of R. Deane and I am grateful for his contribution. I especially thank my colleagues who helped me with my work, gave me advice and put up with my English. Among them are M. Hedrick, C. Kasserra, M. Lutcavage, P. Bushnell, R. Stephenson and last, but certainly not least, G. Gabbott, who made this thesis bearable for the reader. I thank my former colleague, F. Smith, who introduced me to the mysteries of electronics. The people working in the workshop, the administration, the Zoology stores and at South Campus were always very kind and helpful and I thank them very much.

For their unrelenting support and love, I thank my parents and my sister, who in spite of their worry, never demanded of me to follow another road. My thanks too, to Frederic, for being with them during the hard times. Lastly, I thank my husband Geoff for all his love, kindness and understanding during the completion of this degree.
GENERAL INTRODUCTION.
Paul Bert (1870) was the first to attempt an explanation for the remarkable ability of diving homeothermic vertebrates to survive periods of prolonged submersion. He suggested that ducks dive longer than chickens because they have a higher ratio of blood volume to body weight and thus a larger oxygen store. Even though this explanation of the phenomenon has proven to be insufficient, Bert pioneered the field of diving physiology by defining the problem clearly: air-breathing diving animals can tolerate underwater submersion far better than their non-diving counterparts (Table 1), and yet, although some diving animals may have evolved morphological adaptations to aquatic life, they do not possess any special respiratory organs which would allow them to extract oxygen from water. From that time on, the search has continued for the solution which permits such remarkable tolerance to underwater submergence. In 1899 Bert's explanation was challenged by Charles Richet who thought that the extra oxygen store in ducks was not sufficient to meet the requirements of a long dive. He found that oxygen consumption decreased markedly
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<table>
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<th>DIVERS</th>
<th>LONGEST DIVES</th>
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<tr>
<td>BIRDS</td>
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<td>(minute)</td>
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<tr>
<td>Pigeon (Columbia)</td>
<td>1 (Andersen 1966)</td>
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<td>12 (Andersen 1966)</td>
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<td>Guillemots (Uria grylle)</td>
<td>2 (Andersen 1966)</td>
<td>4 (Andersen 1966)</td>
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<tr>
<td>(Uria troile)</td>
<td>3 (Cross 1965)</td>
<td>4 (Andersen 1966)</td>
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<td>MAMMALS</td>
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<td>Man (Homo sapiens)</td>
<td>3 (Cross 1965)</td>
<td>5.4 (Kooyman et al. 1976)</td>
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<td>Muskrat (Ondatra zibethica)</td>
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<td>47.7 (Leboeuf et al. 1988)</td>
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<td>Northern fur seal (Callorhinus ursinus)</td>
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<td>16.53 (Evans 1974)</td>
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<td>Bottlenosed dolphin (Tursiops truncatus)</td>
<td>60 (Lackyer 1977)</td>
<td>30 (Andersen 1966)</td>
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<td>Sperm whale (Physeter catodon)</td>
<td>12 (Andersen 1966)</td>
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<td>Blue whale (Balaenoptera musculus)</td>
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<td>30 (Andersen 1966)</td>
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<tr>
<td>Fin whale (Balaenoptera physalus)</td>
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<tr>
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during diving which, he suggested, may be caused by physiological adjustments taking place during diving (Langlois and Richet 1898, a and b; Richet 1899). Bohr (1897) suggested that energy could be derived from an increase in anaerobic metabolism, and that this would account for the diminished oxygen consumption. Little more progress was made for the next few decades. Then in the 1930s a breakthrough came from the studies of Laurence Irving. The essence of his work remains to this day as the greatest contribution to diving physiology.

Irving (1934) postulated that the brain, and possibly the heart in homeothermic vertebrates, are not able to cope with oxygen deprivation. He contrasted this with the ability of muscle tissue to sustain ischemia for extended periods of time without suffering from irreversible damage. Neither physical nor chemical processes, he felt, could ever completely explain the ability of the diver to cope with prolonged submergence and he advocated the study of cardiovascular reflexes which adjust blood flow during diving. He proposed that during diving, oxygen stores would be conserved exclusively for the brain and heart. This could be achieved by redirecting blood flow away from the peripheral organs and tissues toward the heart and brain. He supported this hypothesis by demonstrating that during respiratory arrest an increase in blood flow to the brain does indeed occur and that flow to the muscles decreases (Irving 1938). Scholander (1940) corroborated Irving's theory by showing that although plasma lactate doubled, or tripled, during diving, it increased even more (10 times the pre-dive values) after emersion. These results suggested
that blood flow does not perfuse muscles during diving. Not only do the muscles undergo anaerobic metabolism but also the end product of this metabolism, lactate, cannot be removed from the muscles during diving. Only upon emersion, when blood flow is reestablished, is lactate flushed out of these peripheral tissues. It then appears in the circulation, causing the huge post-dive peak in plasma lactate levels (Irving et al. 1942; Scholander et al. 1942).

In an attempt to understand mechanisms allowing such an adaptation to underwater submersion, research has focused on the following factors:

1) the oxygen stores available during dives,

2) the ability of blood to load and unload oxygen at the lungs and tissues respectively, and to buffer the excess CO₂ and lactic acid,

3) the ability to conserve, as efficiently as possible, oxygen stores for the heart and brain,

4) special metabolic adaptations of tissues to oxygen deprivation,

5) the ability to control the respiratory drive during diving in order to maintain apnoea.
1- OXYGEN STORES AND BLOOD PROPERTIES IN DIVING BIRDS AND MAMMALS:

Gas exchange is not possible during diving and consequently, animals must rely on their oxygen stores and on anaerobic metabolism to survive during submergence. Oxygen is stored in the blood, in the muscles, and in the lungs (and air sacs in birds). Oxygen stores and blood properties of diving mammals and birds have been extensively reviewed by Lenfant (1969); Butler and Jones (1982); Snyder (1983); Jones and Furilla (1987); Stephenson and Jones (1989) and Kooyman (1989).

It is not easy to establish the importance of the lungs (and air sacs) as oxygen stores during diving, because it is not known if dives occur on inspiration or expiration. Diving on inspiration increases oxygen stores, but also increases buoyancy and for deep divers, raises the risk of nitrogen related accidents (such as narcosis and bends). Snyder (1983) suggested that the lungs are not a site of oxygen storage in long duration divers, such as seals and whales: the small lungs of whales and the lungs of seals are likely to collapse during diving. Short duration divers such as rodents, dolphins and porpoises are thought to dive on inspiration and to use lung oxygen during diving (Snyder 1983). Some diving birds such as tufted ducks and pochards dive on expiration (Woakes and Butler 1975; Butler and Woakes 1976; 1979), while others, such as emperor penguins dive on inspiration (Kooyman et al. 1971a). In Pekin ducks, the oxygen stores of the lung and air sacs make up half of the oxygen available during forced-diving (Hudson and Jones 1986).
The size of the blood oxygen store depends on the blood volume per unit of body mass and on the hemoglobin concentration. A wide range of values for blood oxygen stores have been recorded for a variety of diving mammals, from the same to over 3 times the levels found in terrestrial mammals. The larger oxygen stores are due to higher concentrations of hemoglobin and larger blood volumes (Table 2). The highest values recorded are those of the pinnipeds which are known to be long-duration divers (Table 2; Butler and Jones 1982; Snyder 1983; Kooyman et al. 1989). Aquatic birds tend to have blood volumes and hemoglobin concentrations in the upper end of the range for all birds (Jones and Furilla 1987; Stephenson and Jones 1989; Kooyman 1989). The recent observation by Qvist et al. (1986) of an increase in hemoglobin concentration of a seal during diving, suggests that the spleen may be responsible for releasing stored red blood cells during dives and thus adding another component to blood oxygen stores.

Skeletal muscle myoglobin concentrations in diving mammals and in some diving birds, such as adult penguins, can be as much as one order of magnitude higher than terrestrial animals (Table 2; Castellini and Somero 1981; Kooyman 1989). In ducks and geese, the concentrations of myoglobin are in the upper range of values usually observed for birds (Keijer and Butler 1982; Snyder et al. 1984).
TABLE 2: Survey of the blood oxygen stores (Hematocrit in %; hemoglobin in g/100ml; O₂ capacity in ml/100ml), of the blood volume (% of body mass), of the muscle oxygen stores (g/100g) and of the respiratory properties of whole blood ($P_{50}$ in mmHg; Bohr effect; Hill coefficient; Haldane effect in mM/L; and buffer capacity in mM HCO₃⁻/L.pH unit) of diving (Table 2-A) and non-diving (Table 2-B) mammals.

The respiratory properties of the blood were measured at a pH of 7.4 and at temperatures of 37-38°C.

A₁, Andersen (1966);
B₁, Binkley 1980; B₂, Bryden and Lim (1969); B₃, Blessing (1972); B₄, Blessing and Hartschen-Niemeyer (1969); B₅, Biological Handbook 1971);
C₁, Clausen and Ersland (1968); C₂, Castellini and Somero (1981); C₃, Clausen and Ersland (1969);
H₁, Horvath et al. (1968);
K₁, Kooyman (1968);
L₁, Lenfant et al. (1970); L₂, Lenfant (1969); L₃, Lenfant et al. (1969); L₄, Lane et al. (1972); L₅, Lenfant et al. (1968); L₆, Lechner (1976);
P₁, Parer and Metcalfe (1967);
R₁, Ridgway and Johnson (1966);
S₁, Scholander (1940); S₂, Simpson et al. (1970); S₃, Sleet et al. (1981).
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<td>MAN (Homo sapiens)</td>
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Blood oxygen loading and unloading properties, at the lungs and tissues respectively, are important for underwater survival: Blood with a high affinity for oxygen would favour full utilization of oxygen in the lung, and blood with a low affinity would allow a more complete oxygen unloading at the tissues. However, there is no clear relationship between $P_{50}$ and diving ability (Table 2). Snyder (1983) has suggested that the blood oxygen affinity of diving mammals may be correlated with their lung oxygen stores. For instance, large cetaceans and pinnipeds whose lungs collapse during diving have a lower blood oxygen affinity than terrestrial mammals of equivalent weight, and this would tend to promote the diffusion of oxygen into the tissues. However, rodents and small cetaceans have a higher oxygen affinity than their terrestrial equivalents, which may promote oxygen loading at the lungs. Another factor which promotes the unloading of blood oxygen in the tissues of diving mammals such as pinnipeds and cetaceans is their enhanced Bohr effect (Table 2). Hill coefficients of marine mammals are not different from those of terrestrial mammals (Table 2). Aquatic mammals cannot expel the end products of metabolism which accumulate in the body during diving. In pinnipeds and cetaceans, the higher buffering capacity of their blood as well as the larger Haldane effect are able to reduce pH and $CO_2$ fluctuations (Table 2). Less data are available for diving birds. Blood oxygen affinity and the Bohr effect in birds do not seem to be correlated with diving ability (Jones and Furilla 1987; Stephenson and Jones 1989). The buffering capacity of Adelie penguin blood (a good diving bird) is higher than that of terrestrial birds (Lenfant et al.)
and the capacity of duck blood is no different from chicken blood (Nightingale and Fedde 1972; Scheiper et al. 1975).

The stored oxygen available for dives by Weddell seals is 60 ml oxygen Kg\(^{-1}\) (Kooyman 1989) or 1339 mmoles for a 500 Kg animal. The metabolic rate of a 500 Kg Weddell seal is 83 mmole.min\(^{-1}\) (Hochachka 1981). Therefore, if we suppose that dives performed by seals are strictly aerobic, a 500 Kg individual could stay underwater for 16 minutes. Most of the observed dives range between 5 and 25 minutes, and thus, they are likely to have been performed aerobically. This is confirmed by the lack of plasma lactate during and after these dives. However, some of the dives recorded have reached 50 minutes and even 1.2 hours (Kooyman et al. 1980). These dives could not have been performed purely aerobically, and it is clear that other mechanisms are involved in these feats of submergence.

By using a similar calculation for aerobic metabolism in Pekin ducks, it can be estimated that these ducks cannot perform dives of more than 2.5 minutes duration (O\(_2\) stores = 57 ml for 2.5 Kg ducks, Hudson and Jones 1986; and whole body metabolism = 11.3 x 2.5\(^{0.723}\) = 21.9 ml.min\(^{-1}\), Schmidt-Nielsen 1979). It was, therefore, astonishing to discover that the tolerance of a Pekin duck to forced submersion is considerably longer: a 2.5 Pekin duck can withstand 22 minutes of submer- sion without sustaining physiological damage (Personal data).

These simple calculations confirm, beyond any doubt, that
in addition to an increase of oxygen stores, other special adaptations are necessary for aquatic animals to tolerate prolonged underwater submergence.

2- **DIVING RESPONSE: APNOEA AND CARDIOVASCULAR ADJUSTMENTS:**

As Irving and Scholander proposed, in addition to the apnoea exhibited by aquatic mammals and birds during forced submersion, an increase of vascular resistance in many peripheral tissues and organs must occur (Figure 1; Andersen 1959; Johansen 1964; Butler and Jones 1971; Daly 1972; Jones et al. 1979; Zapol et al. 1979; McKeen 1982; Heieis and Jones 1988). This peripheral vasoconstriction is mediated by the sympathetic branch of the autonomic nervous system (Kobinger and Oda 1969; Butler and Jones 1971; Andersen and Blix 1974). As Irving suspected, cerebral blood flow is unchanged (Kerem and Elsner 1973; Zapol et al. 1979) or may even increase during diving (Jones et al. 1979; Heieis and Jones 1988). Likewise, coronary blood flow remains unchanged in ducks (Jones et al. 1979) and in seals, it appears to decrease proportionately with the reduction in cardiac output (Blix et al. 1976; Zapol et al. 1979). Resistance of the pulmonary vascular bed increases during diving; however, the increase is considerably less than in the peripheral organs (Jones and Holeton 1972; Sinnett et al. 1978). A profound decrease of cardiac output accompanies these vascular changes, thus preventing any undue rise of arterial blood pressure. This decrease of cardiac output is caused primarily by an extreme reduction in heart rate (Figure 1) mediated by the vagus nerves (Butler and Jones 1968; 1971). Measurement of stroke volume in diving mammals
FIGURE 1: Cardiovascular responses to forced submersion of a Pekin duck (*Anas platyrhynchos*). Peripheral resistance refers to the resistance to blood flow in one leg. Blood oxygen tension was measured in the brachiocephalic artery. (from Gabbott 1985).
air flow (L/min) •

heart rate (beats/min) •

peripheral resistance (pru) •

arterial blood pressure (mm Hg) •

blood oxygen tension (mm Hg) •

dive •
surface •
and birds has proven to be difficult and the data are confusing. Some studies have recorded that stroke volume decreases (Sinnett et al. 1978; Zapol et al. 1979; Blix and Folkow 1983), while others concluded that it remains unchanged (Elsner et al. 1964; Blix et al. 1976; Folkow et al. 1967; Jones and Holeton 1972). The overall consequence of these cardiovascular changes during diving is, as Irving suggested, a preferential redistribution of the circulating blood flow, so that oxygen supplies are confined almost exclusively to the central nervous system and the heart.

3- INITIATION AND MAINTENANCE OF THE DIVING RESPONSE:

In order to survive long periods of submersion, cardiovascular adjustments have to be set in place quickly, as soon as the dive begins, in order to prevent oxygen stores from being prematurely depleted by the peripheral tissues. Moreover, once these cardiovascular adjustments are in place, they must be maintained throughout the dive. It has been difficult to define the precise mechanisms by which submersion initiates and maintains the cardiovascular adjustments described above. This is mainly because of the many different sensory inputs which may be involved, and the fact that interspecific differences exist.

Diving mammals, as well as some diving birds, respond to face immersion with an instantaneous bradycardia which suggests a reflex activity triggered by facial or nasal receptors (Angell-James and Daly 1969; Folkow et al. 1971; Dykes 1974; Drummond and Jones 1979; Catlett and Johnston 1974; Mangalam
and Jones 1984). Furilla and Jones (1986) dived redhead ducks (*Aythya americana*) after having sprayed xylocaine (a local anesthetic) into their nares. They observed that the immediate bradycardia associated with submersion was virtually eliminated, which confirmed the role of the nasal receptors as an initiating mechanism.

When heart rate is plotted against time, the profile of diving bradycardia in domestic ducks (*Anas platyrhynchos*) shows a gradual fall in heart rate several seconds after the start of the dive. This differs from the instantaneous bradycardia observed in redhead ducks, and argues against a reflex triggered by a water stimulus. Denervation of the carotid body chemoreceptors in Pekin ducks suppresses the diving bradycardia almost completely (Jones and Purves 1970). This work supports the hypothesis that the progressive hypoxia and hypercapnia, which develops as a consequence of diving apnoea, are the stimuli which trigger the cardiovascular adjustments. Jones et al. (1982) estimated that approximately 85% of the total bradycardia and 67% of the hind limb vasoconstriction were due to stimulation of peripheral chemoreceptors; 30% of the hind limb vasoconstriction was due to stimulation of central chemoreceptors and the rest was due to stimulation of baroceptors and other unidentified inputs. In diving ducks, where bradycardia is initiated by the contact of water on nasal receptors, carotid body chemoreceptors may also be partially involved in the maintenance of the cardiovascular adjustments during long dives (Stephenson and Jones 1989).
In spite of the huge changes in heart rate and peripheral vascular resistance during diving, there is little change in mean arterial blood pressure. This could imply a role for arterial baroreceptors in the initiation and maintenance of the cardiovascular adjustments. Anderson and Blix (1974) suggested that diving bradycardia is merely the expression of a baroreflex which responds to an incipient rise of mean arterial blood pressure, caused by a chemoreceptor driven vasoconstriction. However, Jones (1973) did not observe any changes in the diving bradycardia of chronically baroreceptor denervated ducks compared with their sham-operated controls. After noticing a fall in mean arterial blood pressure and a reduced peripheral vasoconstriction in barodenervated ducks, he advanced the idea that it is peripheral vasoconstriction which is the expression of a baroreflex. The increase in peripheral resistance was caused by the drop of blood pressure triggered by a chemoreceptor driven bradycardia. However, recent work (Jones et al. 1982; 1983; Smith 1987) has shown that during diving, bradycardia and peripheral vasoconstriction are both mediated by the chemoreceptors. Within the first minute of submersion, arterial baroreceptor activity helps to balance cardiac output against vasoconstriction. As submersion continues, there is a progressive attenuation of the baroreflex caused by a negative feedback of the chemoreflex on the baroreflex.

Several other sensory inputs have been thought to play a role in the initiation of the diving response. For instance, postural changes, such as those executed by voluntarily diving
animals, may also contribute to the development of diving responses (Huxley 1913; Paton 1913). Furthermore, there is no doubt that the activity from diverse receptor groups must interact; for example, hypoxia and hypercapnia have to be associated with a cessation of respiratory activity in order for bradycardia and vasoconstriction to develop (Butler and Taylor 1973).

In Pekin ducks apnoea is initiated by contact of water on receptors in the nares, the glottis and around the base of the beak (Bamford and Jones 1974). The maintenance of this apnoea is crucial to the maintenance of the dive. Curiously, trigeminal and glossopharyngeal inputs arising from these receptors are ineffective after 100 seconds of diving, and yet, despite this, apnoea continues (Bamford and Jones 1974). Moreover, the progressive hypoxia and hypercapnia developing during the dive should steadily increase the respiratory drive. Since it has been shown that a 3 kg Pekin duck, for example, can endure submersion for at least 13 minutes (Hudson and Jones 1986), this increasing respiratory drive must be suppressed. The mechanisms causing this suppression are not known but they involve a marked change in respiratory sensitivity to CO2 linked to the withdrawal of rhythmic pulmonary afferent informations during asphyxia (Cohen 1964; Bamford and Jones 1976 a and b).

It has been suggested that the cardiovascular response to forced diving is nothing more than a modification of the Type II fear reaction, or freezing response, which is typically
characterized by bradycardia, hypotension and a decrease of ventilation (Gabrielsen et al. 1977; Smith et al. 1981 a and b). This proposal finds some support in the discovery of a huge increase in plasma catecholamine levels during forced diving (Hudson and Jones 1982). The hypothesis was put forward that the fear response is triggered by the manipulation of the animal for the dive itself (Gaunt and Gans 1969; Kanwisher et al. 1981). Although there may be certain apparent similarities in behavior, there are fundamental differences between the diving response and the fear reaction. The following two examples alone provide a strong argument for the rejection of the fear hypothesis: 1) if Pekin ducks breathe pure oxygen before forced dives, the bradycardia and plasma catecholamine levels are considerably reduced (Furilla and Jones 1986; Mangalam et al. 1987) even though the handling of the animal is the same; and 2) when all brain tissue above the mesencephalon is removed, including the hypothalamus (which mediates the fear response: Evans 1976), the response to forced submersion is exactly the same as that in intact ducks (Gabbott 1985).

4- **METABOLISM**:

When compared with non-divers, diving mammals and birds do not exhibit any exceptional adaptations for anaerobic metabolism which would explain their remarkable underwater tolerance.

Although the brains of seals have been shown to be slightly more tolerant to hypoxia than those of terrestrial mammals, this could be explained by a higher cerebral capil-
lary density, a larger glycogen store, and a higher level of lactate dehydrogenase activity (Elsner et al. 1970; Kerem and Elsner 1973; Kerem et al. 1973; Murphy et al. 1980; Castellini et al. 1981). In the case of mallard ducks, however, their brain tissue appears to be no more resistant to hypoxia than that of chickens (Bryan and Jones 1980). Weddell seal hearts also possess unusually high glycogen stores, which may be used as fuel during long dives (Kerem et al. 1973). The activity of lactate dehydrogenase in seal hearts is higher than in terrestrial mammals, and this presumably provides them with the ability to use lactate as a fuel during dives (Murphy et al. 1980; Castellini et al. 1981; Hochachka 1981), preserving blood glucose for the brain. Lactate is also used as fuel by the lungs during diving in seals (Murphy et al. 1980). Unlike other body tissues, brain and heart metabolism in seals remains aerobic during prolonged dives or forced submersions.

With the exception of higher buffering capacity, the skeletal muscle of diving birds and mammals as a general rule does not seem to be any more adapted to hypoxia than the muscle of terrestrial animals (Kerem et al. 1973; Storey and Hochachka 1974; Castellini et al. 1981; Castellini and Somero 1981).

It should be possible to calculate the theoretical maximum dive time (MDT) of ducks from measures of the oxygen store and the rate of oxygen consumption by the central nervous system (CNS), the heart and the lungs. Thus, for a 1 Kg duck:

\[
\text{MDT} = \frac{\text{Oxygen stores}}{\text{O}_2 \text{ uptake by the CNS, heart and lungs}}
\]
The only oxygen available to heart and brain is stored in
the respiratory system and the blood (Hudson and Jones 1986):

\[ \text{O}_2 \text{ stores } = 19.2 \text{ ml} \]

If brain weight is 4.9 g (Hudson and Jones 1986) and if
the brain metabolic rate estimated for the chicken is used
(Mink et al. 1981), then:

\[
\text{oxygen uptake by the brain } = 4.9 \text{ g x } 0.06 \text{ ml.g}^{-1} \\
= 0.29 \text{ ml.min}^{-1}
\]

If the spinal cord is approximately half of the weight of
the brain, and if its weight specific metabolic rate is half
that of the brain (Mink et al. 1981), then:

\[
\text{oxygen uptake by the spinal cord } = 2.45 \text{ g x } 0.03 \text{ ml.g}^{-1} \\
= 0.07 \text{ ml.min}^{-1}
\]

The weight of the lungs is about 12 g for a 1 Kg duck
(Lasiewski and Calder 1971). As estimates for the metabolic
rate of avian lung tissue are unavailable, values obtained for
mammals can be used. Metabolic rates for lung tissue range
from 0.004 to 0.02 ml.g\(^{-1}.\)min\(^{-1}\) (Krebs 1950; Wallace et al.
1974). Hence the lung could use anywhere from 0.048 to 0.24
ml.g\(^{-1}.\)min\(^{-1}\). However, since blood flow to the lungs is
strongly reduced during diving (Jones et al 1979), perhaps the
lowest metabolic rate should be chosen.
Power output of the heart = cardiac output x arterial blood pressure, as defined by Jones and Johansen (1972), and cardiac output = heart rate x stroke volume

Thus if stroke volume is estimated at 1.5 ml during the dive (Jones and Holeton 1972), and mean arterial blood pressure and heart rate are 118 mmHg and 29 beats.min\(^{-1}\) respectively (Hudson and Jones 1986), then:

\[
power \text{ output (left ventricle)} = 118 \times 1333 \times 1.5 \times 29
= 6.8 \times 10^6 \text{ erg.min}^{-1}
\]

If mean blood pressure in the pulmonary artery is 60 mmHg (Jones personal communication), then:

\[
power \text{ output (right ventricle)} = 60 \times 1333 \times 1.5 \times 29
= 3.5 \times 10^6 \text{ erg.min}^{-1}
\]

Therefore, the total power output of the heart is:

\[
cardiac \text{ power output} = (6.8 + 3.5) \times 10^6
= 10.3 \times 10^6 \text{ erg.min}^{-1}
\]

Assuming that the utilization of 1 ml of oxygen releases the calorific equivalent of \(2.2 \times 10^8\) ergs, and that the efficiency of the heart is 10%, the total uptake of oxygen by the heart is:

\[
O_2 \text{ uptake} = 10.3 \times 10^6 \times (100/10) \times 10^{-8}/2.2
= 0.47 \text{ ml O}_2 \cdot \text{min}^{-1}
\]

Given the above calculations, the oxygen consumed during the dive by the brain, spinal cord, lungs and heart equals 0.88 ml.min\(^{-1}\), thus:
MDT = 19.2 / 0.88 = 22 minutes.

However the MDT determined for a 1 kg duck (using EEG to indicate the dive limit) was only 6.6 minutes (Hudson and Jones 1986). It takes 1 minute for a 1 Kg duck to establish the cardiovascular adjustments to diving. The increase in peripheral vasoconstriction is indirectly reflected by the drop in heart rate in the first minute of a dive. From this, it can be estimated that half as much blood flows to the peripheral organs during the first minute of the dive. Therefore, the oxygen consumption during the first minute would be:

\[ \text{O}_2 \text{ consumption} = 11.3 M^{0.723} \times 1/2 \]
\[ = 5.7 \text{ ml.min}^{-1} \]

and MDT, thus corrected, would be:

\[ \text{MDT} = \frac{(19.2 - 5.7)}{0.88} + 1 \]
\[ = 16.3 \text{ minutes} \]

This calculated value is still much greater than the measured value of 6.6 minutes. For the MDT to be only 6.6 minutes, the oxygen uptake in a 1 Kg duck during diving would have to be:

\[ \text{MDT} = \frac{(19.2 - 5.7)}{\text{VO}_2 \text{ dive}} + 1 \]

(\text{VO}_2 \text{ dive} is oxygen consumed after the first minute of the dive)

\[ 6.6 = \frac{13.5}{\text{VO}_2 \text{ dive}} + 1 \]
\[ \text{VO}_2 \text{ dive} = 2.4 \text{ ml.min}^{-1} \]
The value of 2.4 ml.min\(^{-1}\) represents the oxygen consumption rate of the duck after the first minute of diving. However, since only 0.88 ml.min\(^{-1}\) is consumed by the CNS, lung and heart, what accounts for this extra 1.52 ml.min\(^{-1}\) of O\(_2\) uptake?

It has been shown that blood flow decreases considerably in peripheral tissues during diving; however, it does not stop completely. Thus, the remaining flow could account for some of the extra oxygen (1.52 ml.min\(^{-1}\)) consumed during the dive. For example, blood flow dropped to 7-9% of the pre-dive value in the kidney, to 11-13% in the leg and to 4% and 21% in the gizzard and the intestines respectively. Furthermore, when blood flow is maintained to the brain, as described above, flow also continues to other tissues of the head, such as the cranial muscles and skin (Johansen et al. 1964; Butler and Jones 1971; Jones et al. 1979).

Hochachka (1981) calculated that if the MDT in Weddell seals was 1.2 hours (Kooyman et al. 1980) and if oxygen stores were consumed exclusively by the brain, heart, and lungs, then the oxygen store would be depleted by only 25%, and thus, could not be the factor limiting the length of the dive. The blood glucose level drops during diving in Weddell seals (Murphy et al. 1980) and so Hochachka suggested instead, that the factor limiting the dive length was the rate of blood glucose utilization. However, the above calculation determines the maximum underwater tolerance and it is not certain that the longest free dive observed (1.2 hours) represents the maximal dive time of Weddell seals. Again, although blood flow
in peripheral tissues is greatly decreased, it is not stopped entirely: approximately 42% of the cardiac output during diving perfuses the peripheral tissues (Zapol et al. 1979). This factor should be taken into consideration in the calculation of oxygen store depletion and could also contribute to a replenishment of blood glucose by the liver during diving. In order to discover the factors limiting the length of dives, the end-dive values of the blood gases (PaO₂, PaCO₂, pHₐ) and metabolites (glucose, lactate) should be correlated with the end of a maximum forced dive, as defined by Hudson and Jones (1986). By following such a procedure it has been shown that blood oxygen stores, not blood glucose levels, seem to be the limiting factor of MDT in forced dived Pekin ducks (Hudson and Jones 1986).

In 1899, Richet observed that 3 and 8 day old ducks did not dive as long as adults. Recently, Hudson and Jones (1986) also observed a great variability in MDT among ducks, and they established that MDT is related to body mass (M), thus:

\[ \text{MDT} = 6.6 \, M^{0.64}. \]

As it is demonstrated above, MDT can be obtained by dividing O₂ stores available for the CNS, lung and heart by the O₂ consumption of these organs during diving. In ducks, these oxygen stores are proportional to M^{1.19} (Hudson and Jones 1986). The spinal cord and the lungs consume very little of the oxygen during diving, and they were consequently neglected in Hudson and Jones' (1986) calculations. The combined mass of
the heart and brain in ducks, over 0.5 Kg, is proportional to \( M^{0.85} \) (Hudson and Jones 1986), and the metabolic rate can be obtained by multiplying the mass exponent by the metabolic exponent (0.723) (Schmidt-Nielsen 1979).

Therefore, MDT is proportional to

\[
M^{1.19} / (M^{0.85})^{0.723} = M^{0.61}
\]

This calculated value of \( M^{0.61} \) is quite close to the measured value of \( M^{0.64} \), and suggests that MDT increases with body mass because the \( O_2 \) stores increase with body mass faster than oxygen uptake by the heart and brain.

During forced diving, aerobic metabolism in a 1 kg duck drops to 2.4 ml.min\(^{-1}\), about 20% of the pre-dive value (see calculation above), and anaerobic metabolism occurs in peripheral tissues (Scholander 1940). Moreover, not only does aerobic metabolism decrease during forced dives, but a drop of total metabolic rate in diving birds, as well as diving mammals, seems to occur. Pickwell (1968), using direct calorimetry, calculated that forced dived Pekin ducks may experience a reduction in total energy metabolism by up to 90% of pre-dive resting levels. Moreover, drops in temperature of the brain (by 2.5°C), abdomen and muscles (by 1°C) of the bladdernose seal were observed by Scholander and coworkers (1942) during 15 minute forced dives. A decline of 1-2 °C in rectal temperature was observed during a forced dive in the harbor seal, but this was not accompanied by any changes in brain temperature (Elsner et al. 1975). Decreases in core temperature have also been recorded in free diving Weddell seals. Most free dives
by seals are less than 25 minutes long and are aerobic; however, occasional exploratory dives may be considerably longer, ranging from 25 minutes to 1.2 hours (Kooyman et al. 1980). During these longer dives, lactate production (indicative of anaerobic metabolism) increased and body temperature dropped. In a 53 minute dive, body temperature dropped from 38°C to 34.9°C (Kooyman et al. 1980). Qvist et al. (1986) observed that the central blood temperature in Weddell seals varied from a maximum of 38.6°C at rest to 35.1°C during free diving. Also the oxygen debt measured after a forced dive in ducks was less than the predicted deficit (Scholander 1940).

These results are striking because they suggest that these animals may be able to lower their basal metabolism in order to achieve maximum underwater tolerance. This could obviously contribute to the ability of diving animals to sustain long dives by decreasing the rate of oxygen and glucose consumption. A decrease in metabolic rate has a further advantage in that it would reduce the accumulation of end products, such as CO₂ and lactate, during diving. This would decrease the recovery period (the time which has to be spent on the surface) after long dives, such as the exploratory free dives observed in Weddell seals (Kooyman et al. 1980). The mechanisms allowing such a metabolic feat during diving are still unknown.
Even though they have been observed to dive for food (Furilla and Jones 1987b) and for escaping capture (personal observations), Pekin ducks (*Anas platyrhynchos*) are mainly dabblers. Their strategy, when foraging underwater for food, consists mainly of immersing only the upper part of their body for 9 seconds at most (Jones and Furilla 1987). In such short dives, ducks are essentially aerobic and have little need for any defense against asphyxia. Thus, it is all the more surprising that these animals are able to execute such profound cardiovascular changes and can survive for more than 20 minutes underwater (Figure 2) without any physiological damage (Hudson and Jones 1986). This duality of diving behavior (aerobic free dives with little cardiovascular change versus anaerobic forced dives with strong bradycardia) observed in Pekin ducks (Figure 2), is also found in true diving birds (*Aythya americana*) and in diving mammals (Weddell seals) (Kooymen and Campbell 1972; Kooymen et al. 1980; Liggins et al. 1980; Butler and Jones 1982; Furilla and Jones 1986; Hill et al. 1987).

Although the pattern of response exhibited by forced dived animals is not normally seen in the short dives of voluntarily diving ducks, this pattern has been observed in ducks that have been suddenly trapped underwater (Stephenson et al. 1986; Furilla and Jones 1987a). In free diving mammals also, the forced dive pattern of response has sometimes been observed during long exploratory dives (i.e. exceeding 20 minutes: Kooymen and Campbell 1972; Kooymen et al. 1980). It
FIGURE 2: Heart rate during

A: Forced dive in Pekin duck (personal data),

B: Free dabble in Pekin duck (from Furilla & Jones 1986).

Head in (↓) and out of (↑) water.
is likely that divers have evolved an important corticolimbic control of their cardiovascular bulbar reflex responses (Gabbott 1985; Furilla and Jones 1987a; McCulloch 1989) which could explain the great difference in magnitude of the diving responses observed. This enables animals to adjust their asphyxic defense according to the anticipated challenge of the dive, and allows them to cope with unexpected events happening during the dive (Blix 1987). In forced dives, the full potential of their defense against asphyxia is activated and thus the most extreme cardiovascular responses are observed.

Speculation aside, the diving responses of forced dived Pekin ducks are similar to those observed in diving mammals and birds during long exploratory free dives and during forced dives. Furthermore, unlike mammals, ducks possess only one set of arterial baroreceptors (located in the wall of the ascending aorta) which is anatomically separated from the peripheral chemoreceptors (located in the carotid bodies) (Jones and Purves 1970; Jones 1973). This anatomical peculiarity has permitted study of the differentiation between the roles of peripheral chemoreceptors and the roles of baroreceptors in the initiation and maintenance of the dive response (Jones and Purvis 1970; Jones 1973; Lillo and Jones 1982; Jones et al. 1983). The many and extensive studies of the diving responses of Pekin ducks have provided invaluable insights into the full potential of one of the most efficient defenses against apneic asphyxia which has evolved in diving mammals and birds.
The mechanisms underlying survival for such long periods of apnoeic asphyxia are still not very well known. For example, the question of whether or not adrenal catecholamines have a significant role in forced diving of birds and mammals has been debated without having been directly investigated (Hance et al. 1982; Hudson & Jones 1982; Mangalam et al. 1987). This study has been undertaken in this thesis.

A certain amount of evidence is beginning to accumulate which suggests that catecholamines may play a vital role in the diving response. For instance, in contrast to the situation in peripheral tissues, the blood supply to the adrenal glands continues during forced submersion. In fact, it may even increase in ducks (Johansen 1964; Jones et al. 1979), although it is unchanged in nutria (McKean 1982) and decreases by 40% in Weddell seals (Zapol et al. 1979). Another observation is that forced diving is associated with a significant increase in circulating catecholamines (epinephrine, EP; and norepinephrine, NE). In ducks, plasma catecholamine concentrations double after a dive of one minute and increase 1000 fold after 10 to 16 minutes of diving (Huang et al. 1974; Hudson & Jones 1982; Figure 3). Four to 30 fold increases in catecholamine levels have also been observed in the Harbor seal after 4 to 6 minutes of submergence (Hance et al. 1982). It has been recently noted that catecholamine secretion may be triggered by stimuli known to be crucial for invoking diving responses, such as a variation in blood gas tensions (Jones et al. 1982; Mangalam et al. 1987; Rose et al. 1983) or stimulation of
FIGURE 3: Increase in Norepinephrine (open symbols) and Epinephrine (closed symbols) during forced dives in ducks. All dives started at dive time 0 and ended when the last sample was taken. Recovery time 0 represents when each animal was allowed to surface. (from Hudson and Jones 1982).
nasal receptors by water (Allison & Powis 1971; Drummond & Jones 1979). Kobinger and Oda (1969), Andersen and Blix (1974) and Butler and Jones (1968; 1971) have demonstrated the importance of the sympathetic branch of the autonomic nervous system in the initiation and maintenance of peripheral vasoconstriction during forced-diving in ducks. This was accomplished with the use of adrenergic blockade or catecholamine depleting agents; however, none of these studies differentiated between the role of sympathetic nerves and the role of adrenal catecholamines.

As sympathetic nerve activity and plasma catecholamines are known to engender similar physiological responses, the question raised is: What are the relative roles of the sympathetic nerves and of these enormous levels of circulating catecholamines during diving? It is reasonable to consider that this remarkable increase of circulating catecholamines plays an important role in the diving response because catecholamines are known to influence a variety of physiological processes indispensable to diving. Gooden (1980) suggested that circulating catecholamines could enhance peripheral vasoconstriction and could counteract the depressant action of hypoxia on neural vasoconstriction. Hypoxia also has a depressant action on myocardial contractility (McKean 1984; Nakhjavani et al. 1971; Tyberg et al. 1970), and this could be compensated for by circulating catecholamines, which have a well known positive inotropic effect on the myocardium. Circulating catecholamines have been shown to protect the brain against
ischemic damage (Koide et al. 1986). They may also enhance the excitation of chemoreceptors (Milsom and Sadig 1983), and consequently increase the intensity of the dive response. Circulating catecholamines could compensate for the drop of blood oxygen affinity caused by acidosis (Nikinmaa 1983) during dives and thus, ducks could maximize the utilization of pulmonary oxygen stores. During diving, anaerobic metabolism increases, and by promoting glycogenolysis, circulating catecholamines could also help satisfy the substrate requirements.

Throughout the history of diving physiology, there has been little effort made to study the humoral control of the diving response (Robin et al. 1981; Mangalam et al. 1987). As has been shown in this review, the primary emphasis has been the neural control of the diving response. This work will show that in addition to the autonomic nervous system, hormones, such as adrenal catecholamines, play an important role in the defense against apnoeic asphyxia.

In the first part of this thesis, I determined the relative importance of the adrenal glands in the release of circulating catecholamines during forced diving of Pekin ducks. Then I explored the influence of adrenal catecholamines on the maximum tolerance to forced submersion. Ducks in which catecholamine release from adrenal glands had been prevented, were forced dived and maximum dive times were compared with times measured with control ducks. To understand the mechanisms involved in the process, heart rate, blood pressure, blood gas...
values as well as blood metabolites and ions were measured. In order to estimate the role of circulating catecholamines on peripheral vasoconstriction and on cardiac function during diving, two sets of experiments were conducted on Pekin ducks. One set involved hind limb perfusion while the second set involved beta-blockade.
CHAPTER 1:

THE SOURCE OF CIRCULATING CATECHOLAMINES
IN FORCED DIVED PEKIN DUCKS.
INTRODUCTION.

Activation of the sympathetic branch of the autonomic nervous system can be triggered (Figure 4) by the contact of water on the nasal receptors (Allison and Powis 1971), by variations of blood gas tension at the chemoreceptors (Rose et al. 1983), by a drop of blood pressure (Ito et al. 1984; Engeland et al. 1981), by hypothermia and hypoglycemia (Young et al. 1984) and by stress (Archer 1979). Circulating catecholamines can be released by the sympathetic nerve endings and by the adrenal glands (Figure 4). Mangalam et al. (1987) showed that catecholamine release was triggered mostly by hypoxia during diving. Acidosis, hypercapnia and even a stress component related to the handling of the animal for the dive itself, had little effect on catecholamine release. By comparing adrenal denervated ducks with sham operated ducks, Mangalam et al. (1987) showed that half of the plasma norepinephrine (NE) and 90% of plasma epinephrine (EP) released during dives came from neural activation of the adrenal glands. They thought that non-neural releasing mechanisms might also have triggered catecholamine release from the adrenals during
FIGURE 4: Schematic view of catecholamines release (for explanations see text).
STRESS
HYPOGLYCEMIA
HYPOTHERMIA

HYPO O\textsubscript{2}, HYPER CO\textsubscript{2}

chemoreceptors

nasal receptors

baro-receptors

BLOOD PRESSURE

CNS

ACTH

SYMPATHETIC NERVES

† plasma renin

† angiotensin II

CORTEX (corticosteroids)
MEDULLA (catecholamines)

ADRENAL GLANDS

HYPOXIA ACIDOSIS

release of catecholamine from nerve endings
forced dives. Non-neural releasing mechanisms could involve inherent chemosensitivity of the adrenal gland itself (Figure 4: Comline and Silver 1961; Nahas et al. 1967 Steinsland et al., 1970; Jones and Robinson 1975) or humoral mechanisms secondary to the effects of asphyxia (Figure 4). For example, hypoxia causes a release of adrenocorticotropic hormone which mediates catecholamine release (Critchley et al., 1982). Also, during forced diving, vasoconstriction of the renal bed (Jones et al., 1979) associated with hypoxia and hypercapnia (Drummond and Lindheimer 1982; Rose et al., 1984) would stimulate the renin-angiotensin system causing release of catecholamines from the adrenals (Felderg and Lewis 1965; Wilson and Butler 1983a; Corwin et al., 1985). Hence, in the presence of non-neural mechanisms, the importance of the adrenal glands compared with contributions from autonomic sympathetic vascular nerve endings, to the increase in circulating catecholamines during a dive remains a matter of speculation.

In order to obtain an estimate of the relative importance of the adrenal glands and autonomic vascular sympathetic nerves to the catecholamine increase observed during a forced dive, I compared the plasma levels of free catecholamines released in 3 minute dives by adrenalectomised ducks (ADX) with their sham-operated controls (SH-adx). Further, to estimate the importance of non-neural mechanisms stimulating the release of catecholamines from the adrenal glands, I also compared these values with those obtained in adrenal denervated ducks (DNX) and their sham operated controls (SH-dnx).
MATERIALS AND METHODS.

1-ANIMALS:

The 35 ducks used in this study were 2-3 month old, male, white Pekins (*Anas platyrhynchos*) ranging in mass from 2 to 3.5 kg. Males were used because adrenalectomy and adrenal denervation are easier to perform than on females. In female birds, ovaries prevent access to adrenal glands. Stock birds were grouped in an outdoor field with free access to water and food (Buckerfield’s 16% layer pellets, Abbotsford B.C.). During the experiments, they were kept indoors in individual cages (55 X 55 X 60 cm) at 22°C on a 12 hour light/dark cycle with food and water supplied *ad libitum*. Seven ducks were adrenalectomised (ADX) and 6 were sham operated following the procedure used for adrenalectomy (SH-adx). In 8 ducks adrenal glands were denervated (DNX) while in 7 others sham operation was performed following the procedure for adrenal denervation (SH-dnx). Seven ducks were left intact: they were not submitted to surgery.

2-MAJOR SURGICAL PROCEDURES:

The surgical techniques used for adrenalectomy and sham operation were essentially those described by Thomas and Phillips (1975). A major difference was that the interval between the two laparotomies was one week instead of 1 or 2 days. One hour before the second laparotomy, 2 mg/kg body weight of prednisone was given orally to the ducks. Also, a
deeper plane of anaesthesia was used (10 mg/kg body weight i.v. of sodium pentobarbital (Somnotol, MTC) followed by 8 mg/kg every 1/2 hour). Finally, the bird was tidally ventilated with pure O₂ as surgery violated the air sacs and may cause a decrease in respiratory efficiency. When the ducks were surgically anaesthetised, they were secured on one side, with their upper leg extended backward to draw the sartorius muscle away from the site of incision which was plucked and disinfected with alcohol. An incision was made between the sixth and seventh vertebral ribs. The ribs were retracted very carefully in order not to tear the lung tissue present at the dorsal border of the incision, and a small hole was made in the anterodorsal portion of the abdominal air sac to gain access to the adrenal glands which were surrounded by the vena cava, the aorta, lungs, kidney and testis (Figure 5). Adrenalectomy itself was performed under a dissecting microscope (X 5; Carl Zeiss, Germany). The adrenal glands had to be removed as far as possible in one piece. Extensive use of ligatures prevented bleeding and kept the operative field clear so that small fragments of gland could be seen and removed. At this time, only one gland was removed. After the gland was removed, the area was carefully inspected for residual adrenal tissue, and the ligatures on the adrenal blood vessels were consolidated with tissue cement (Histoacryl; B. Braun Melsungen AG, Melsungen, W., Germany). The abdominal air sac was then closed with surgical silk. The ribs were approximated with ligatures, and the skin wound sutured with surgical silk. One week later, a second laparotomy was performed on the other side following the same procedure, to remove the other adrenal gland. Sham-
FIGURE 5: Ventral view of the right adrenal gland (in situ X15) of the Pekin duck (*Anas platyrhynchos*).
Operation consisted of all the foregoing procedures, except that the glands were left undisturbed with the exception of freeing the lumbar vein as though it was going to be ligatured. Sham-operated ducks were left open 4 hours on the surgical table, matching the time needed to perform the removal of the adrenal glands. Recovery after the end of the second surgery was at least two weeks, which allowed the bird to regain pre-surgical body weight. The average body masses of adrenalectomised (ADX) and sham-adrenalectomised (SH-adx) ducks were 2.9 ± 0.2 kg and 2.8 ± 0.3 kg before surgery respectively, and 2.6 ± 0.3 kg and 2.7 ± 0.2 kg at the end of the recovery period. Haematocrits were 37.2 ± 0.5% for SH-adx, and 35.8 ± 0.8% for ADX at the end of the recovery period. Because cortical tissue was also removed, ADX ducks were given prednisone orally (2 mg/kg body weight daily), and salt water (0.8% NaCl) to drink after the second laparotomy.

The innervation of the adrenal glands has been described in the fowl by Freedman (1968). The surgical technique used for adrenal gland denervation and sham-operations was that described above for adrenalectomy. In order to facilitate access to the nerves, the lumbar vein was ligated and cut in both adrenal denervated (DNX) and Sham-operated (SH-dnx) ducks. To make certain that all nerves had been sectioned, the adrenal glands were freed from the surrounding tissue (connective tissue, vena cava, aorta and testes), and only maintained in place by the adrenal veins and arteries. Sham-operation consisted of all the foregoing procedure, but the adrenal nerves were not sectioned. The time for recovery after
surgery was 2 to 3 weeks. The average body weight of DNX and SH-dnx ducks were, 2.6 ± 0.1 kg and 2.7 ± 0.2 kg before surgery respectively, and 2.6 ± 0.1 kg and 2.6 ± 0.1 kg at the end of the recovery period. At this time, the haematocrit was 37.2 ± 0.9% for DNX, and 36.3 ± 1.9% for SH-dnx.

All the ducks were killed with an overdose of anaesthetic at the end of the series of experiments except for one ADX bird which was maintained on food and salt water only and killed 2 years later. The successful removal of the adrenal glands in ADX and section of adrenal nerves in 6 DNX was checked post mortem.

3-DIVING PROTOCOL:

Cannulation of a brachial artery was done under local anaesthesia (Lidocaine hydrochloride; Xylocaine 2% Astra) at least one day before any dives. The tip of the arterial cannula (P.E. 90; Intramedic Polyethylene tubing, Clay Adams) was advanced until it lay near the junction of the brachiocephalic artery and aorta.

The bird was placed in the sitting position and secured with tape to an operating table. The electrocardiogram leads were inserted subcutaneously, one above the left thigh, the other below the right shoulder, while a ground lead was attached to the right foot. The arterial cannula was used to measure blood pressure and to take blood samples before and at 3 minutes submergence, which interrupted blood pressure recording. The duck was left undisturbed behind a screen for 15
minutes before an experiment started. Ducks were dived by lowering their head gently into a beaker of water (16-20°C). Samples of arterial blood were taken anaerobically in 1 ml chilled heparinized plastic syringes, and immediately put in ice. Plasma was separated within 5 minutes after blood sampling, and was stored at -80°C.

4-MEASUREMENT OF PHYSIOLOGICAL VARIABLES:

Free catecholamines in the plasma were measured by HPLC, following the technique described by Mangalam et al (1987). In a 1.5 ml polypropylene vial, 0.5 ml plasma, 0.5 ml iced 3.5 M Tris buffer (pH 8.6), 50μl of dihydroxybenzylamine (DHBA, a synthetic catecholamine used as an internal standard, from Sigma), and 14 mg of chromatographic grade activated alumina (BDH) were combined. Catecholamine standards were prepared using 0.1 M Na₂PO₄ buffer (pH 7.0) (0.5 ml), the solution standard of norepinephrine (NE) and epinephrine (EP) (50 μl), the tris buffer (0.5 ml), DHBA (50 μg) and also alumina (14 mg). Standards and plasma were processed together and kept on ice when not being manipulated. The vials were shaken for 5 minutes and after the alumina settled, the supernatant plasma was aspirated. About 1 ml of deionized water was added, the vials were shaken for 2 minutes, and the supernatant aspirated again; this was repeated. After the final shaking, the supernatant was aspirated, until near dryness of the alumina. 50 μl of 0.1 M HClO₄ were added to the vials to elute the catecholamines from the alumina. The vials were vortexed, left standing for 5 minutes on ice, then vortexed again. Brief centrifugation concentrated the alumina and 20 μl of the
supernatant was injected into the HPLC.

A Spectra Physics SP 8700 HPLC controller and pump were used to provide an isocratic flow of 2.0 ml/min through a Beckman 10 x 0.46 cm column of 10um ODS Ultrasphere reverse phase packing. The mobile phase consisted of 50mM citrate, 100 mM sodium acetate, 40 mM acetic acid, and about 1 uM sodium heptane sulfonic acid added as an ion pairing agent to increase separation. The detection system used was a Bioanalytical Systems BAS LC-4A electrochemical detector, using a BAS Plexiglas TL-3 electrode, with +0.67V to +0.7V applied across the silicone grease/graphite active surface.

The catecholamine peaks were measured and concentrations were calculated by relating the ratios of catecholamine peaks to DHBA peak in the standard to the ratios in the plasma samples. The limit of sensitivity (signal:noise >30) of the system was about 1 femtomole for NE and EP. There was a linear ratio response over the range of .5 nM to 500 nM. The coefficient of recovery was between 63 and 75% .Plasma levels of dopamine do not change significantly during the dives (Mangalam et al 1987), so only the values for NE and EP are reported.

After the post-operative recovery period plasma Na⁺ and K⁺ concentrations were analysed in ADX ducks and their sham operated controls, using a flame photometer (Instrumentation Laboratory Incorporated, Boston Mass.). Plasma Cl⁻ was measured by isometric titration with a Buckler digital chloridometer (4-2500, Fort Lee, New Jersey), and plasma glucose using an enzymatic assay kit (# 16-UV Sigma Chemical co., St Louis, MO). Arterial blood pH (pHa) was determined
using an Instrumentation Laboratory 813 PH/ Blood gas analyzer.

ECG and arterial blood pressure signals were displayed on a chart recorder (Physiograph 6, E&M Instrument Co INC Houston Texas).

5-ANALYSIS OF DATA:

Comparisons were made between intact ducks and sham-operated controls to estimate the effects of surgical and post-surgical trauma. Comparison between SH-adx and ADX was made to estimate the effects of the adrenalectomy per se, and comparison between SH-dnx and DNX to establish the effects of the adrenal denervation per se. A paired t-test was used to test the difference between pre-dive and 2 minute dive blood pressures. For comparisons between more than two values, an ANOVA, and Newman-Keuls test were used. A significant difference between two values was assumed if p<0.05. In the text and graphs values are represented by their means ± standard error of the mean.
RESULTS.

The main differences in pre-dive levels of plasma catecholamines among the 5 groups of ducks were the absence of plasma Epinephrine (EP) in ADX (Figure 6,A), and significantly lower plasma levels of Norepinephrine (NE) and Epinephrine (EP) in DNX (Figure 7,A). A significant increase in plasma catecholamine levels was observed after 3 minutes diving in all the 5 groups (Figures 6,B & 7,B) except for plasma EP in DNX ducks. Furthermore, no EP was detectable in the plasma of ADX ducks after 3 minutes submergence as was the case pre-dive. During the dive, levels of catecholamines in ADX and DNX were significantly lower than those found in their sham-operated controls. After 3 minutes diving, the levels of NE and EP in SH-adx were significantly higher than in the intact ducks, but no differences were observed between SH-dnx and intacts. No significant differences in the diving levels of NE and EP were observed between ADX and DNX. No difference in diving levels of plasma NE was observed between SH-adx and SH-dnx, but diving levels of plasma EP in SH-adx were higher than in SH-dnx.

The heart rate dropped significantly during the dive in all 5 groups (Figure 8). There was no significant difference in pre-dive or dive heart rate between intact, SH-adx, SH-dnx and DNX. However pre-dive and dive heart rate of ADX were significantly higher than those of intact, SH-adx, SH-dnx and DNX (Figure 8).
FIGURE 6: Plasma levels of norepinephrine (NE) and epinephrine (EP) in intact (open, n=5), sham-operated (filled, n=5), and adrenalectomised (cross-hatched, n=6) ducks before (A) and after 3 minutes of forced diving (B).

Significantly different from intact ducks: o, from SH-adx: ●, from ADX: *, from pre-dive value: +.
PLASMA CATECHOLAMINES
(nanomoles per liter)

A

PRE-DIVE

B

3 minutes DIVE

NE
EP

CD

57
FIGURE 7: Plasma levels of norepinephrine (NE) and epinephrine (EP) in intact (open, n=5), sham-operated (filled, n=7), and adrenal denervated (cross-hatched, n=8) ducks before (A) and after 3 minutes of forced diving (B).
Significantly different from intact ducks: o, from SH-dnx: ●, from DNX: *, from pre-dive value: +.
FIGURE 8: Heart rate during forced dives.

A- Intact (open circle, n=7), sham-adrenalectomised (open triangle, n=6), adrenalectomised (open square, n=7).
B- Intact (open circle, n=7), sham-adrenal denervated (filled triangle, n=7), adrenal denervated (filled square, n=8).
Significantly different from the other groups: *, from pre-dive value: +.
In all 5 groups the mean arterial blood pressure dropped significantly after 2 minutes of diving. No significant differences in the pre-dive or dive values were observed among the 5 groups (Figure 9).

There was no difference between ADX and SH-adx in plasma levels of Na\(^+\) (138 ± 1.4; 137 ± 0.9 meq/L respectively), K\(^+\) (3.7 ± 0.2; 3.1 ± 0.2 meq/L respectively), glucose (155 ± 18; 197 ± 10 mg/100ml respectively) or in arterial pH (7.38 ± 0.02; 7.42 ± 0.02 respectively). However, the plasma level of Cl\(^-\) in ADX (105 ± 0.9 meq/L) was significantly higher than in SH-adx (102 ± 0.9 meq/L).
FIGURE 9: Mean arterial blood pressure before and after 2 minutes forced diving.
A- Intact (open, n=5), Sham-operated (filled, n=6), and adrenalectomised (cross-hatched, n=7) ducks before and at 2 minutes forced diving.
B- Intact (open, n=5), Sham-operated (filled, n=7), and adrenal denervated (cross-hatched, n=8) ducks before and at 2 minutes forced diving.
Significantly different from pre-dive value: +.
DISCUSSION.

Plasma levels of catecholamines measured in adrenalectomised ducks (ADX) showed that during the dive 100% of EP originate from the adrenal glands. The NE released during diving in ADX on the other hand must have arisen from the discharge of autonomic sympathetic nerves innervating the vasculature, which are strongly stimulated during the dive (Jones et al. 1979). Adrenal denervation also significantly decreased plasma EP levels during dives by 98%. Since adrenal denervated ducks (DNX) did not release more NE than ADX ducks during the dive, and because there was no increase of EP in the DNX ducks during dives, then adrenal catecholamine release during dives must be mediated only by a neural mechanism. Non-neural mechanisms did not appear to play any role.

Evaluating the proportion of NE released by the adrenal glands during diving is more difficult. Comparisons between intact and sham-operated ducks allow an estimation of the effects of surgical and post surgical procedure per se on the adrenal release of catecholamines. Surgery did not appear to have damaged the adrenal glands because at rest and during diving plasma catecholamines in sham-operated ducks were not below those of intact ducks. The higher catecholamines levels in sham-operated ducks, compared with intact ducks, may have been an effect of post surgical stress which enhances the overall release of circulating catecholamines by the adrenal glands during diving. Such an effect cannot occur in ADX and
DNX because either there is no adrenal glands or there is no way to cause adrenal catecholamine release. If this is so, it may not be accurate to compare catecholamine levels of ADX with those of SH-adx in order to determine the proportion of catecholamines released by the adrenal glands during "diving" per se. However, it is surprising that an increase of plasma catecholamines caused by post surgical stress was not also observed before the dive in these sham-operated ducks. Hudson and Jones (1982) recorded huge individual variations in plasma catecholamine levels among ducks during diving. This variation could contribute importantly, if not explain, the heterogeneity of diving catecholamine levels in Intact, SH-adx and SH-dnx ducks. When compared with Intact and SH-adx the percentage drop of NE in ADX was 42% and 70% respectively. A similar comparison between Intact, SH-dnx and DNX revealed values of 70% and 80% which are quite close. Thus, the percentage of NE release by the adrenal glands is variable and seems to fluctuate between 42 and 80%.

Plasma EP and NE in DNX decreased by 98% and 70-80% which was greater than the 90% and 50% reduction in EP and NE found by Mangalam et al. (1987). This discrepancy could be due to a regrowth of adrenal nerve fibers in Mangalam’s adrenal denervated ducks. This would trigger a small release of adrenal catecholamines during forced diving. In order to prevent any regrowth in the present study, a 3 to 5 mm section of the adrenal nerves was removed. Plasma levels of NE and EP measured at rest and after 3 minutes submergence in intact ducks were similar to values found in resting and diving intact
ducks by others (Sturkie et al., 1970; Huang et al., 1974; Hudson and Jones 1982; Wilson and Butler 1983a; Mangalam et al., 1987). After adrenalectomy EP was not detected in the plasma at rest, which agrees with results of Butler and Wilson (1985) and Wilson and Butler (1983b,c).

During forced dives, all groups of ducks showed bradycardia and hypotension. Certainly it is remarkable that, during dives, such large differences in plasma catecholamines between DNX and SH-dnx were not reflected by any marked differences in their cardiovascular performance. Furthermore, diving heart rate of ADX was significantly higher than that of DNX although their diving levels of catecholamines were similar. Consequently, autonomic nerves play the main role in the establishment and maintenance of the cardiovascular responses to diving (Kobinger and Oda 1968; Butler and Jones 1971; Wilson and West 1985). Adrenal catecholamines do not seem to participate in cardiovascular adjustments, at least not during short dives. However, Pekin ducks can be forced dived for 20 minutes without any physiological damage (Jones and Furilla 1987) and a possible role of adrenal catecholamines on maximal underwater tolerance has yet to be investigated.

The difference in cardiovascular adjustments between ADX and the 4 other groups of ducks during diving could be due to the lack of endogenous corticosteroid. Heart rate in resting ADX was twice that of SH-adx. The effects of corticosteroids on the cardiovascular system are not fully understood (Fowler and Chou, 1960; Lefer et al., 1968; Sevy et al., 1974; Rovet-
Cardiovascular collapse, one consequence of adrenalectomy, can be a result of hypotension resulting from decreased Na\(^+\) reabsorption in the kidneys, caused by a lack of mineralocorticoids. However, the level of Na\(^+\) (and other plasma ions) and the pre-dive mean arterial blood pressure in ADX and SH-adx were similar and in the range of values usually recorded in ducks (Butler and Wilson 1985; Roberts and Hughes, 1984; Shimizu and Jones, 1987) so this difference in heart rate has to involve some other mechanism. The effect of adrenalectomy on the cardiovascular system of ducks has been studied by Butler and Wilson (Butler 1985; Butler and Wilson 1985). They showed that in ADX there was a significant increase in heart rate associated with a decrease in blood pressure and stroke volume. They did not observe any alteration in peripheral resistance or blood and plasma volumes. Injection of betamethasone prevented hypotension in their ADX, as did prednisone in our study. However, these authors did not give any information about heart rate and stroke volume in their ADX betamethasone injected ducks. An increase in heart rate, associated with a normal mean arterial blood pressure, may reflect an adaptation of the cardiovascular system to a decrease in stroke volume (due to a decrease of myocardial performance (Lefer et al., 1968; Sevy et al., 1974; Rovetto 1974)) in chronically adrenalectomised ducks supplemented with exogenous corticosteroid such as prednisone. In other words, even though prednisone maintains the ionic balance it still may not be an adequate replacement therapy for corticosterone and aldosterone, the endogenous corticosteroids.
CHAPTER 2:

INFLUENCE OF ADRENAL CATECHOLAMINES ON MAXIMUM DIVE TIME.
INTRODUCTION.

The previous chapter showed that circulating catecholamines do not seem to have any significant role during diving. However, Pekin ducks were forced dived for 3 minutes only. Ducks such as these (2 to 3.5 kg) could easily survive 10 to 14 minutes of submergence (Hudson and Jones 1986). Three minutes may have been too short a dive to register differences between ducks whose release of circulating catecholamines had been prevented, and their controls.

The role of circulating catecholamines in determining the maximal tolerance to underwater submergence is investigated in this chapter. It was not possible to use adrenergic blockade for this study, because this would not allow discrimination between the roles of the sympathetic nerves and adrenal catecholamines. Preventing catecholamine release from the adrenal glands by putting blood flow occluders around the adrenal veins or arteries was abandoned because the adrenal glands are closely applied to the aorta and vena cava and there is no place to fit an occluder around adrenal arteries and veins.
Moreover, because cortical and medullary tissues are intermingled in birds, adrenal demedullation cannot be done. Consequently maximum dive times (MDT) were compared between ducks whose adrenal catecholamine release had been prevented (by adrenalectomy "ADX" or by denervation of adrenal glands "DNX") and their sham-operated controls (SH-adx, SH-dnx). These MDT values were also compared with the MDT of ADX and DNX perfused with synthetic catecholamines. Heart rate, blood pressure, blood gas levels and concentrations of lactate, glucose and ions were also measured.
MATERIAL AND METHODS.

1-ANIMALS:

The experiments were done on 39, two to three month old male Pekin ducks (Anas platyrhynchos) ranging in mass from 2 to 3.5 Kgs. They were kept in conditions similar to those described in chapter I. Eight ducks were adrenalectomised (ADX) and 6 were sham-operated (SH-ADX) following the procedure used for adrenalectomy. In 9 ducks, adrenal glands were denervated (DNX) while in 9 others, sham-operation was performed following the procedure for adrenal denervation (SH-dnx). Seven ducks were intact.

2-OPERATIVE AND POST-OPERATIVE PROCEDURES:

The surgical techniques and post-operative procedures used for adrenalectomy and adrenal denervation are described in chapter I.

3-MINOR SURGERY AND EXPERIMENTAL PROCEDURE:

Cannulation of the brachial artery and vein was done with P.E. 90 (Intramedic Polyethylene tubing, Clay Adams) tubing, under local anaesthesia (Lidocaine hydrochloride; Xylocaine 2% Astra). The birds were given one day to recover. The position of the cannulae was checked at post mortem: the tip of the arterial cannula was positioned at the junction of the brachi-
ocephalic artery and aorta; the tip of the venous cannula was positioned in the vena cava.

The bird was placed in a sitting position and secured with tape to an operating table as described in chapter I. Only one dive was performed each day, and at least 48 hours elapsed between dives.

Maximum underwater tolerance, without any risk of permanent physiological damage or distress to the animal, is indicated by a flattening of the EEG and a sudden rise in heart rate towards pre-dive levels (Figure 10; Hudson & Jones 1986). In the present experiments, this very characteristic increase of heart rate was taken as an indication that the maximum dive time had been reached.

The venous cannula was connected to a Harvard infusion/withdrawal pump (Model 901, Millis, Mass.) for continuous infusion of catecholamines (Norepinephrine bitartrate: Levophed: Winthrop, Aurora, Ontario, Canada; Epinephrine chloride: Park-Davis, Scarborough, Ontario, Canada) or saline into ADX and DNX ducks during forced dives, beginning as soon as the duck's head was under water. During the dive, the perfusion rate was set to generate the plasma level of catecholamines measured in diving Pekin ducks by Hudson and Jones (1982). In this set of experiments, for each duck five dives were carried out, and in the first and fifth dive, the perfusion was done with the carrier (saline) only. Seven DNX ducks and two ADX ducks were perfused with catecholamines.
FIGURE 10: Sample chart recording from an experiment to determine maximum endurance to forced submersion in Pekin ducks (from Hudson and Jones 1986).

Symbols are: HR, heart rate; ABP, arterial blood pressure; EEK, electroencephalogram. The solid diamond indicates the beginning of head submersion; the hollow diamond dive termination.
4-MEASUREMENT AND ANALYSIS OF THE PHYSIOLOGICAL VARIABLES:

ECG and arterial blood pressure signals were amplified and displayed on a chart recorder (Physiograph 6, E&M Instrument Co, Houston Texas).

Arterial blood samples (on average: 1.5 ml) were taken anaerobically before the dive, at 3 and 5 minutes in the dive and at the end of the dive. 0.3 ml was used to measure PaO$_2$, PaCO$_2$ and pHa using an Instrumentation Laboratories 813 pH/Blood gas analyser (Lexington MA). 0.1 ml was used to determine CaO$_2$ following the method developed by Tucker (1967) using a radiometer gas analyser (Radiometer PHM71 MK2, Copenhagen, Denmark). The rest of the blood was centrifuged and the plasma was collected and used to determine lactate and glucose. Lactate and glucose levels were measured using enzymatic assay kits (# 826-UV; # 16-UV, Sigma, St Louis, MO). Other arterial blood samples (1 ml) were taken before the dive and at 5 minutes into the dive to determine plasma levels of Na$^+$, K$^+$ and Cl$^-$. Plasma levels of free catecholamines were measured in 1 ml of blood taken anaerobically before the dive and at 3, 5 and 10 minutes in the dive (if the dive lasted long enough) and at the end of the dive. Plasma Na$^+$ and K$^+$ were analysed using a flame photometer (Instrumentation Laboratory Incorporated Boston Mass.). Plasma Cl$^-$ was measured by isometric titration with a Buckler digital chloridometer (4-2500, Fort Lee, New Jersey). Plasma free catecholamines were measured by HPLC following the technique described in chapter I. Plasma levels of dopamine do not change significantly during dives (Mangalam et al. 1987), so only the values for norepine-
phrine (NE) and epinephrine (EP) are reported.

Fourteen ml of arterial blood were taken from 3 intact ducks at rest. Two aliquots of 7 ml were equilibrated simultaneously at 40°C with a hypoxic and hypercapnic gas mixture (PO2=54 mm Hg; PCO2=64 mmHg) in a two chamber tonometer. After 30 minutes, PaO2, PaCO2, pHa and CaO2 were measured. One microgram of both NE and EP were added to one chamber, and an equivalent volume of saline (100 ul) was added to the other. PaO2, PaCO2, pHa and CaO2 were then measured after 30 seconds, 2 and 5 minutes.

**5-ANALYSIS OF DATA:**

Comparisons were made between intact ducks and sham-operated controls to estimate the effects of surgical trauma. Comparison between SH-adx and ADX was made to estimate the effects of the adrenalectomy *per se*, and comparison between SH-dnx and DNX to establish the effects of the adrenal denervation *per se*. Statistically significant differences were based on results of Student’s t test, or analysis of variance followed by the Newman-Keuls test. The critical limit for significant difference was set at a probability of 5% (p<0.05). In the text and graphs, values are given as mean ± standard error of the mean (SE). N is the number of ducks, and n is the number of dives in a given group.

In all the dives, heart rate and mean arterial blood pressure were recorded for every minute until 1 minute before the end of the dive. Because of differences in MDT among ducks of the same group, fewer values were recorded for the longer
dives. In other words, because the length of the dive for each animal is different, the sampling time for n decreases. Consequently, the mean and SE for values recorded during the dive are only included as data for analysis when the number of dives remaining is more than, or equal to, half of the original number of dives. However, mean ± SE of heart rate and blood pressure were calculated for all ducks at the end of all dives, and one minute before the end of these dives. These values are also reported graphically. Statistically significant differences among values at the end of the dive and at one minute before the end of the dive were also based on results of Student’s t test, or analysis of variance followed by the Newman-Keuls test, and the critical limit for significant difference was set at a probability of 5%.
RESULTS.

1-EFFECTS OF ADRENALECTOMY AND ADRENAL DENERVATION ON MAXIMUM DIVE TIME (MDT):

Maximum dive time (MDT) of adrenalectomised ducks (ADX: 5 min 19 ± 20 sec) was significantly below that of their sham-operated controls (SH-adx: 9 min 58 ± 45 sec). The MDT of SH-adx was also significantly lower than in the intact ducks (Intact: 12 min 14 ± 53 sec), showing that surgery affected underwater endurance (Figure 11). In contrast, MDT of SH-dnx was not significantly different from MDT of intact ducks (Figure 12). MDT of adrenal denervated ducks (DNX: 7 min 10 ± 13 sec) was significantly reduced compared with sham-operated controls (SH-dnx ducks: 12 min 10 ± 28 sec) (Figure 12).

MDT of DNX ducks perfused with saline (7 min 21 ± 17 sec) was not significantly different from that of non-perfused DNX (Figure 12). In DNX ducks perfused with catecholamines, MDT increased significantly (9 min 46 ± 20 sec, Figure 12). However, MDT was still significantly below the values observed in SH-dnx ducks. Two ADX ducks perfused with catecholamines showed a similar increase in MDT (Figure 11). The levels of catecholamines generated during these dives are given in table 3. Perfusion of catecholamines in DNX significantly increased diving levels of plasma catecholamines almost to the values found in their sham-operated controls which had levels similar to those previously recorded during forced dives by Hudson and Jones (1982) (Table 3).
FIGURE 11: Maximal dive time in intact, sham-operated and adrenalectomised ducks with and without infusion of catecholamines.

(I: intact ducks, N=7, n=14; SH-adx: sham-operated ducks, N=6, n=12; ADX: adrenalectomised ducks, N=8, n=16; ADX+sal.: adrenalectomised ducks infused with saline, N=2, n=2; ADX+cat.: adrenalectomised ducks infused with catecholamines, N=2, n=2.)

I, S, A, significantly different from I, SH-ADX, and ADX ducks respectively.
FIGURE 12: Maximal dive time in the intact, sham-operated and adrenal denervated ducks with and without infusion of catecholamines.  

(I: intact ducks, N=7, n=14; SH-dnx: sham-operated ducks, N=9, n=18; DNX: adrenal denervated ducks, N=9, n=18; DNX+sal.: adrenal denervated ducks infused with saline, N=7, n=14; DNX+cat.: adrenal denervated ducks infused with catecholamines, N=7, n=21.)  

I, S, D, Ds, Dc, significantly different from I, SH-dnx, DNX, DNX+sal., and DNX+cat. ducks respectively.
MAXIMUM DIVE TIME (minutes)
TABLE 3: Levels of plasma catecholamines during dives in adrenal denervated ducks infused with saline (DNX+sal.), adrenal denervated ducks infused with catecholamines (DNX+cat.), and their sham-operated controls which were unperfused (SH-dnx). 

0, *, ●, significantly different from SH-dnx, DNX+sal. and DNX+cat. respectively.
### NORADRENALINE (nanomoles per liter)

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### EPINEPHRINE (nanomoles per liter)

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2-EFFECTS OF ADRENALECTOMY AND ADRENAL DENERVATION ON HEART RATE (HR) AND BLOOD PRESSURE (BP):

Mean pre-dive heart rate of ADX ducks was significantly above that of SH-adx ducks. However, pre-dive mean arterial blood pressure was not significantly different. During the dive, ADX ducks had significantly higher heart rates than SH-adx ducks, yet blood pressure in the ADX ducks fell significantly lower (Figure 13). Diving heart rates in ADX and SH-adx were significantly different from their respective pre-dive heart rates. Blood pressure during diving in ADX was significantly different from its pre-dive value, which was not the case for SH-adx.

Mean pre-dive heart rate and blood pressure of DNX and SH-dnx ducks were not significantly different. During the dive, however, heart rates in DNX ducks were significantly higher than in SH-dnx ducks. The drop in mean blood pressure occurring during dives was greater in DNX ducks, and was significantly below that of SH-dnx (Figure 14). Diving heart rates in DNX and SH-dnx were significantly different from their pre-dive values. Diving blood pressure in DNX was significantly lower than the pre-dives value. From 2 to 7 minutes into the dive, blood pressure in SH-dnx was also significantly lower than its pre-dive value.

There were no differences in pre-dive heart rate and blood pressure among SH-dnx ducks, DNX ducks which were to be perfused with saline and DNX ducks to be perfused with catecholamines (Figure 15). Diving heart rates in all those ducks
FIGURE 13: Heart rate and mean arterial blood pressure during diving in adrenalectomised ducks (ADX, N=8, n=8, hollow squares) and their sham-operated controls (SH-adx, N=6, n=6, hollow triangles).
*, significantly different from SH-adx.
FIGURE 14: Heart rate and mean arterial blood pressure during diving in adrenal denervated ducks (DNX, N=9, n=18, filled squares) and their sham-operated controls (SH-dnx, N=9, n=18, filled triangles).

*, significantly different from SH-dnx.
MEAN ARTERIAL BLOOD PRESSURE
(mm Hg)

HEART RATE
(beats per minute)

DIVE TIME (minutes)
FIGURE 15: Heart rate and mean arterial blood pressure during diving in adrenal denervated ducks infused with saline (DNX+sal., N=7, n=14 for heart rate, N=5, n=10 for blood pressure, filled circles), or catecholamines (DNX+cat., N=7, N=21 for heart rate, N=5, n=15 for blood pressure, hollow circles), and in sham-operated ducks (SH-dnx, N=9, n=18, filled triangles).

*, significantly different from the other groups.
were significantly below their respective pre-dive values. Their blood pressures during diving were also significantly lower than their respective pre-dive values, except in DNX perfused with catecholamines in which diving blood pressures at 6 and 7 minutes diving and at one minute before the end of the dive were not significantly different from their pre-dive value. Heart rate of DNX ducks during the dive remained significantly higher than in the SH-DNX ducks despite perfusion with catecholamines (Figure 15). Blood pressure of DNX ducks perfused with catecholamines was significantly higher than in DNX ducks perfused with saline after 2 minutes and did not differ from the values of SH-dnx ducks (Figure 15).

Towards the end of a dive, all ducks showed an increase in heart rate (Figure 13, 14, 15). Sham-operated control ducks, also showed a rise in blood pressure toward the pre-dive values. However at the end of dives, blood pressure in ADX, DNX, and DNX perfused with saline remained significantly below their pre-dive values (Figure 13, 14, 15), and blood pressure of DNX perfused with catecholamines rose toward the pre-dive values and then fell significantly below the pre-dive value (Figure 15).

3-EFFECTS OF ADRENALECTOMY AND ADRENAL DENERVATION ON BLOOD GAS AND pH:

Pre-dive PaO₂ and CaO₂ were not significantly different in DNX and SH-dnx ducks. During the dive PaO₂ dropped faster in DNX than in SH-dnx ducks, and the mean values were signif-
significantly different after both 3 minutes and 5 minutes submergence. PaO₂ recorded in DNX ducks and in SH-dnx ducks at the end of dives were not significantly different (Figure 16). The fall in CaO₂ in DNX and SH-dnx ducks followed the same pattern as PaO₂ (Figure 16). In ADX also, PaO₂ dropped faster than in SH-adx (Table 4A). Moreover, PaO₂ recorded before and at the end of the dives were significantly higher in ADX than in their sham-operated controls.

Plotting pre-dive and dive values of arterial O₂ content against their associated PO₂ (Figure 17), shows that in DNX, the O₂ affinity of the blood during the dive was not different from blood O₂ affinity in SH-dnx in which circulating catecholamines rose spectacularly. There was no difference in PaO₂, CaO₂ and pHa between DNX after 3 minutes diving and SH-dnx after 5 minutes diving, however PaCO₂ was significantly higher in SH-dnx. At the end of dives, no difference in PaO₂, CaO₂ and pHa between DNX and SH-dnx were observed, but PaCO₂ was significantly higher in SH-dnx.

Resting PaO₂, PaCO₂ pHa, CaO₂ in the 3 ducks used in the tonometry study were 74 ± 1 mmHg, 32 ± 1 mmHg, 7.51 ± 0.01, and 13.3 ± 0.9 ml per 100 ml of blood respectively. After equilibration by tonometry, no significant differences were observed before (PaO₂ = 54 ± 2 mmHg, PaCO₂ = 64 ± 3 mmHg, pHa = 7.30 ± 0.01, CaO₂ = 62 ± 7%) or after the addition of saline (PaO₂ = 54 ± 3 mmHg, PaCO₂ = 64 ± 2 mmHg, pHa = 7.29 ± 0.02, CaO₂ after 30 sec, 2 min, 5 min = 63 ± 6 %, 59 ± 7 %, 61 ± 6 %). Because the hematocrit in one of the ducks was lower (33%) than in the other ducks (45%), CaO₂ is expressed as a
**FIGURE 16**: PaO₂ and CaO₂ in adrenal denervated ducks (DNX, N=8, n=16, filled squares) and their sham-operated controls (SH-dnx, N=6, n=12, filled triangles) during diving. +, *, significantly different from pre-dive value and from SH-dnx value respectively.
TABLE 4: Pre-dive and dive values of (A) PaO$_2$, PacO$_2$, pH$_a$, and (B) plasma levels of lactate and glucose in adrenalectomised ducks (ADX) and their sham-operated controls (SH-adx).

+, *, significantly different from pre-dive value and from SH-adx value respectively.
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</table>
FIGURE 17: Arterial content of O2 as a function of PaO2 in denervated ducks (DNX: N=8, n=16, filled squares) and in sham denervated ducks (SH-dnx: N=6, n=12, filled triangles). PaCO2 (mmHg), and pHa for each of these points are written on the graph: on the right side for DNX, on the left side for SH-dnx.
ARTERIAL O2 CONTENT (ml per 100 ml blood)

ARTERIAL PARTIAL PRESSURE OF O2 (mm Hg)

SH-dnx

DNX

32±0.6
7.49±0.01

31±0.6
7.49±0.01

45±1.1
7.31±0.01

49±1.1
7.30±0.01

45±0.8
7.34±0.01

51±1.3
7.23±0.02

66±2.2
7.15±0.02

57±1.4
7.15±0.02

0
20
40
60
80
100
120
percentage of the resting value. No differences were observed either before \((\text{PaO}_2 = 53 \pm 3 \text{ mmHg}, \text{PaCO}_2 = 63 \pm 3 \text{ mmHg}, \text{pHa} = 7.28 \pm 0.02, \text{CaO}_2 = 60 \pm 7 \%)\) or after the addition of catecholamines \((\text{PaO}_2 = 53 \pm 3 \text{ mmHg}, \text{PaCO}_2 = 65 \pm 2 \text{ mmHg}, \text{pHa} = 7.28 \pm 0.02, \text{CaO}_2 \text{ after 30 sec, 2 min, 5 min} = 58 \pm 7 \%, 59 \pm 8 \%, 59 \pm 9 \%)\).

No difference in PaCO\(_2\) between DNX and SH-dnx during the dive was observed, except at the end when PaCO\(_2\) in DNX ducks was significantly lower than in their SH-dnx (Figure 18). No difference in pH\(_a\) was observed except at five minutes into the dive when pH\(_a\) in DNX was significantly lower than in SH-dnx (Figure 18). Similar results were observed in SH-adx and ADX (Table 4A).

4-EFFECTS OF ADRENALECTOMY AND ADRENAL DENERVATION ON STRONG IONS AND BLOOD GLUCOSE:

In DNX, pre-dive values of plasma lactate and glucose were not significantly different from their respective sham-operated controls (Table 5), neither were the plasma levels of lactate and glucose in ADX and SH-adx (Table 4B). After 5 minutes diving in DNX, plasma levels of glucose were significantly higher and plasma levels of lactate significantly lower than in SH-dnx (Table 5). This is similar to the trend observed in the ADX and SH-adx (Table 4B).

However, during diving, differences in pH\(_a\) between ADX and SH-adx as well as between DNX and SH-dnx did not always match the associated differences in PaCO\(_2\) and plasma lactate.
FIGURE 18: PaCO$_2$ and pH$_a$ in adrenal denervated ducks (DNX, N=8, n=16, filled squares) and in their sham-operated controls (SH-dnx, N=6, n=12, filled triangles) during diving. +, *, significantly different from pre-dive value and from SH-dnx value respectively.
TABLE 5: Pre-dive and dive values of arterial plasma levels of glucose, lactate\(^{-}\), Na\(^{+}\), K\(^{+}\), Cl\(^{-}\) and Strong Ions Difference (SID) in adrenal denervated ducks (DNX) and their sham-operated controls (SH-dnx).

+, *, significantly different from pre-dive value and from SH-dnx value.
BLOOD GLUCOSE AND STRONG IONS

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose (millimoles per liter)</th>
<th>Plasma Lactate (millimoles per liter)</th>
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<tr>
<td></td>
<td>SH-dnx</td>
<td>DNX</td>
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<tr>
<td>PRE-DIVE MEAN</td>
<td>10.1</td>
<td>9.8</td>
</tr>
<tr>
<td>PRE-DIVE SE</td>
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<tr>
<td>DIV 5 minute MEAN</td>
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<td>11.7</td>
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<tr>
<td>DIV 5 minute SE</td>
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<th>Plasma K+ (millimoles per liter)</th>
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<td></td>
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<td>DNX</td>
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<tr>
<td>PRE-DIVE MEAN</td>
<td>135</td>
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<tr>
<td>PRE-DIVE SE</td>
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</tr>
<tr>
<td>DIV 5 minute MEAN</td>
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<td>140</td>
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<tr>
<td>DIV 5 minute SE</td>
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<table>
<thead>
<tr>
<th></th>
<th>Plasma Cl- (millimoles per liter)</th>
<th>Plasma SID (millimoles per liter)</th>
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<tbody>
<tr>
<td></td>
<td>SH-dnx</td>
<td>DNX</td>
</tr>
<tr>
<td>PRE-DIVE MEAN</td>
<td>103</td>
<td>105</td>
</tr>
<tr>
<td>PRE-DIVE SE</td>
<td>1</td>
<td>1</td>
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<tr>
<td>DIV 5 minute MEAN</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>DIV 5 minute SE</td>
<td>1</td>
<td>1</td>
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</tbody>
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levels (Table 4, Figure 18 and Table 5). For example, pH$_a$ of DNX after 5 minutes diving was significantly lower than that of SH-dnx even though its PaCO$_2$ was not different from that of SH-dnx, and plasma lactate was significantly lower. In an attempt to clarify this problem, plasma ions were measured in SH-dnx and DNX before and at 5 minutes into the dive. In DNX, pre-dive values of plasma Na$^+$, K$^+$, Cl$^-$, were not significantly different from their respective sham-operated controls (Table 5). In SH-dnx, plasma levels of Na$^+$ and lactate after 5 minutes diving were significantly higher than pre-dive values. In DNX, plasma levels of K$^+$ and of lactate after 5 minutes diving were significantly higher than pre-dive values (Table 5). An approximate strong ion difference (SID = plasma levels of Na$^+$ and K$^+$ - plasma levels of Lactate$^-$ and Cl$^-$) was calculated and its variations during diving were compared with variations of pH$_a$ and PCO$_2$ in DNX and SH-dnx. The increase of plasma Na$^+$, lactate and SID at 5 minute submergence was significantly higher in SH-dnx than in DNX (Figure 19), but the absolute change in plasma K$^+$, H$^+$ and PCO$_2$ after 5 minutes diving was significantly higher in DNX (Figure 19).
FIGURE 19: Absolute changes (Dive values - predive values) of $[H^+]a$, PaCO2 and arterial levels of ions after 5 minutes submergence in adrenal denervated ducks (DNX: N=7, n=7; except for lactate: N=8, n=8) and their sham-operated controls (SH-dnx: N=8, n=8).

+, *, significantly different from pre-dive value and from SH-dnx value respectively.
CHANGE IN ARTERIAL LEVELS OF IONS
(milliequivalents per liter)

CHANGE IN $[H^+]_a$
(nanoequivalents per liter)

CHANGE IN $\text{PaCO}_2$
(mm Hg)
DISCUSSION.

The average maximum dive time (MDT) in ADX and DNX ducks was significantly below the MDT of their respective sham-operated controls. In ADX ducks MDT decreased by 47% (relative to SH-adx) while in DNX ducks MDT fell by 41% (relative to SH-dnx). Heart rate during the dive in ADX and DNX ducks was significantly higher than in their sham-operated controls, while their blood pressures dropped significantly lower. Previous work has shown that adrenalectomy reduces plasma levels of circulating NE and EP by 70% and 100% respectively compared with their sham-operated controls during diving (Chapter I). Diving levels of NE and EP were reduced by 80% and 98% respectively in DNX compared with SH-dnx (Table 1 and Chapter I). When catecholamines were infused in ADX and DNX ducks, MDT increased. In DNX ducks there was a significant increase in MDT during infusion of catecholamines, but MDT remained significantly below that of SH-dnx ducks. Infusing catecholamines in DNX ducks during dives significantly increased blood pressure to values similar to those in SH-dnx ducks. Although heart rate decreased slightly, it was not significantly different from that of DNX infused with saline. Thus, circulating catecholamines released by the adrenals do increase MDT. However, since MDT of DNX infused with catecholamines never reached MDT of their sham-controls, catecholamines may not be the only adrenal hormones involved in promoting MDT.
In mammals, a small release of cortisol (0.5 to 3 times) has been recorded after one hour of hypoxia (Jones et al. 1988; Nishijima et al. 1989; Paulick et al. 1987). Even though Ringer (1976) stated that in birds, nerve fibers do not enter the cortical tissue, there is some evidence of an innervation of cortical cells (Unsicker 1973). The action of such nerves on the regulation of adrenocortical functions is not well known (Holzwarth et al. 1987), but Engeland et al. (1985) showed that splanchnic nerves were not involved in cortisol release during hemorrhage. The release of corticosteroids is triggered humorally, and a neural release of corticosteroids seems unlikely. In DNX ducks, the humoral regulation of corticosteroid release was not prevented, but it did not seem to play a major role in promoting underwater endurance because the drop in MDT was similar in ADX and DNX (respectively 47 and 41%). This also implies that the pre-experimental differences due to a lack of endogenous corticosteroid (e.g. pre-dive heart rate, Cl$, PaO_2$ (Chapter I)) do not affect the underwater performance of ADX ducks. Other adrenal products such as enkephalines may contribute to the prolongation of MDT and may lower heart rate during dives. In mammals, enkephalins are found in the adrenal medulla, in the same cells and vesicles as the catecholamines, and are co-secreted with them (Hanbauer et al. 1982; Viveros & Wilson 1983; Wilson et al. 1982). Furthermore, enkephalins have also been shown to cause bradycardia and apnoea (Fennessy & Rattray 1971; Sapru et al. 1981).
The higher heart rate and lower blood pressure observed in ADX and DNX during diving suggests a lower stroke volume and/or lower peripheral resistance than in their sham-operated controls. Some authors have shown that adrenal catecholamines play an important role in the cardiac response to hypoxia compared with the sympathetic nerves (Baugh et al., 1959; Chiong & Hatcher, 1972; Lee et al., 1980; Nahas et al., 1954), and others have shown the opposite (Achtel, 1972; Kahler et al., 1962; Kontos & Lower, 1969; Korner & White, 1966). Hypoxia per se has a direct depressant action on myocardial contractility (McKean, 1984; Nakhjavan et al., 1971; Tyberg et al., 1970). So, catecholamines, which have a well-known positive inotropic effect on the myocardium, may compensate for this depressant action, and maintain the myocardial contractility during the dive. Oxygen stores, reflected by partial pressure and content of arterial oxygen, dropped faster in ADX and DNX animals than in their respective shams. This suggests that a decrement in peripheral vasoconstriction during dives in ADX and DNX causes an increase in blood flow and oxygen consumption in the peripheral tissues leading to a more rapid utilization of oxygen stores. This could be the reason for a reduction of MDT in ADX and DNX ducks. It has been observed that adrenalectomy perturbs the vascular responses to hypoxia (Baugh et al., 1959; Kahler et al., 1962). Circulating catecholamines released by the adrenal medulla contribute to vasomotor responses in the hind limb, renal and mesenteric beds (Allison & Powis, 1971, 1976; Berecek & Brody, 1982; Powis, 1974; Yardley and Hilton, 1987) and would appear to be more efficient for maintaining vasoconstriction in hypoxia, since neurogenic
vasoconstriction is more susceptible to inhibition by local hypoxia in blood vessels (Gooden 1980).

Lower levels of plasma lactate in DNX suggest an increase in the ratio of aerobic to anaerobic metabolism compared with SH-dnx. This also supports the hypothesis of a lower peripheral resistance in DNX during the dive. Increase of aerobic versus anaerobic metabolism in DNX compared with SH-dnx, also implies increased CO₂ production during diving. DNX showed a slightly larger increase in PCO₂ during diving than SH-dnx (Figure 20), but the levels of PCO₂ reached by DNX were not as high as might be expected (Figure 19). Blood in DNX, being more deoxygenated than in SH-dnx, would carry more CO₂ at any given PCO₂ (haldane effect; Scheipers et al. 1975). However this does not account for much and may not satisfactorily explain why PCO₂ in ADX and DNX doesn’t rise any faster.

The increase in heart rate in ADX and DNX towards the end of dives was not associated with an increase in blood pressure to pre-dive values. This suggests that when ADX and DNX reach their maximal underwater tolerance, peripheral vasoconstriction is not maintained, in contrast to their sham-operated controls. Consequently, the resulting release of lactate from peripheral tissues into the central circulation in ADX could explain the lack of significant difference in plasma levels of lactate at the end of the dive between ADX and SH-adx whose peripheral circulation is still shut down.
It has been shown in certain teleost fishes that circulating catecholamines increase blood affinity for O$_2$ during acidosis by maintaining the intracellular pH of the erythrocytes higher than that of the plasma (Nikinmaa 1983). The tonometry experiment showed that circulating catecholamines do not have any effect on oxygen affinity in ducks during hypoxia and acidosis, supporting Nikinmaa et al. (1984) who showed that catecholamines do not have any effect on erythrocyte pH in birds. Also, there were no significant differences in PaO$_2$, CaO$_2$ and pH$_a$ between DNX at 3 minutes diving and SH-dnx at 5 minutes diving or between DNX and SH-dnx at the end of dives. This supports the contention that circulating catecholamines do not have any action on the affinity of the blood for oxygen. It is possible that in vitro an action of adrenal catecholamines on blood affinity for O$_2$ could be masked by an inverse change in affinity due to the difference in PCO$_2$ between DNX and SH-dnx. However, such a difference in PCO$_2$ at constant pH does not result in a significant change in oxygen affinity (Isaacks et al. 1986).

During dives the action of even a large increase in circulating catecholamines on hepatic glycogenolysis must be insignificant because of the restriction of blood flow through peripheral tissues and liver (Jones et al. 1979; Zapol et al. 1979). Blood glucose levels stay constant during forced dives (Mangalam et al. 1987; Robin et al. 1981) or even decrease (Murphy et al. 1980). Therefore, during forced diving, the major glucose sources available to the tissues are local glycogen stores for the peripheral tissues and local glycogen
stores and blood glucose for the heart, brain and lungs. The higher plasma glucose in diving ADX and DNX compared with the drop in their sham-operated controls may be due to a release of glucose by the liver caused by the weaker peripheral vasoconstriction observed in these ducks.

An approximate plasma SID (strong ion difference = sum of cations - sum of anions) was calculated from lactate\(^-\), Na\(^+\), K\(^+\) and Cl\(^-\). In SH-dnx, SID showed a significant increase during the dive (+2.6 mEq/L) which was also observed by Shimizu and Jones (1987). No change was observed in DNX which explains the bigger drop in arterial pH in DNX compared with SH-dnx, during the dive despite the smaller increases in plasma lactate and PaCO\(_2\). Plasma levels of lactate\(^-\) and Na\(^+\) in SH-dnx increased during the dive. This was also observed by Shimizu and Jones (1987). However in DNX, it was the plasma levels of lactate\(^-\) and K\(^+\) which increased during the dive. If the increase of plasma lactate observed during forced dives in DNX and their sham-operated controls was a consequence of anaerobic metabolism, the increases of Na\(^+\) in SH-dnx and K\(^+\) in DNX are harder to explain. However, an hypothesis can be proposed based on two sets of experiments, one which analyzes the effects of ischemia on skeletal muscle and myocardium and the other which analyzes the effects of circulating catecholamines on these muscles.

When skeletal muscles are subjected to ischemia (Irwing and Noakes 1985), and similarly when the myocardium is subjected to anoxia or ischemia (Rau et al. 1977; Wiegand et al. 1977; Whalley et al. 1985),...
1979; Hill and Gettes 1980; Shine 1981) they rapidly lose some of their intracellular potassium into the extracellular space and to the circulation. Most of these studies have been done on the heart. Weiss and Shine (1981) showed that this release is biphasic: it reaches its maximum after 5 minutes of exposure, then levels off for about 30 minutes, and then rises again. This later phase, which is irreversible, has been linked to cell necrosis (Conrad et al 1979). The early phase which is reversible on reoxygenation (Rau et al. 1977; Weiss and Shine 1981) is not due to a decrease of $K^+$ influx into the cells, but to an increase of efflux (Kleber 1983; Shine 1981; Rau et al. 1977). This increase of net $K^+$ efflux out of the cells is not due to an inhibition of the $Na^+/K^+$ pump (Kleber 1983; Rau et al. 1977), and is dissociated from the energetic state of the myocytes (Rau and Langer 1978). Efflux of anions such as lactate occur at the same time as efflux of $K^+$ in ischemic heart and skeletal muscles (Matur and Case 1973; Irwing and Noakes 1985). Crake et al (1987) showed that potassium loss from cells is secondary to the extrusion of lactate (end product of anaerobic metabolism) from the myocyte, in order to maintain electrical neutrality across the sarcolemma.

Intravenous injection of adrenaline leads to a short lived initial release of $K^+$ by the liver (Vick et al. 1972), mediated by the alpha-adrenergic receptors (Todd and Vick 1971) followed by a prolonged decrease in the plasma $K^+$ (Vick et al. 1972; Struthers and Reid 1984) due to a net uptake of potassium in skeletal muscles and myocardium (Vick et al. 1972; Clausen and Flatman 1977; Sejersted et al. 1985) mediated mostly by beta$_2$-adrenergic receptors (Clausen and Flatman
Moreover intracellular sodium activity in cardiac cells decreases during perfusion with norepinephrine (Clausen and Flatman 1977; Wasserstrom et al. 1982; Lee and Vassalle 1983). This influx of $K^+$ into the cells and this decrease of intracellular $Na^+$ are due to an increase of the $Na^+ / K^+$ ATPase activity by catecholamines (Clausen and Flatman 1977; Sejersted et al. 1985; Lee and Vassalle 1983).

Based on these results, it can be proposed that potassium, together with lactate, leaves the intracellular compartment during diving. Because of the increase of catecholamines which enhances the activity of the $Na^+ / K^+$ pump, $K^+$ is returned to the intracellular compartment, and the matching efflux of $Na^+$ causes the increase of plasma $Na^+$ observed during diving. If this increase in circulating catecholamines is prevented (as in DNX ducks) plasma levels of $K^+$ rather than $Na^+$ should increase, which is precisely what has been observed.

In forced dived ducks, the increase in peripheral vasoconstriction is due to the activity of the sympathetic branch of the autonomic nervous system. Kobinger and Oda (1969) observed changes in cardiovascular adjustments in ducks forced dived for 90 seconds, after injection of anti-adrenergic agents such as reserpine, bretylium, guanethidine, methyldopa and catapres. They concluded that the changes observed were due to an alteration of the peripheral vasoconstriction during diving due to an impairment of the autonomic sympathetic nervous system. However they did not check the extent of the
depletion of the catecholamines stores in sympathetic nerves, adrenal glands, and central nervous system after injection of the adrenergic neuron blocking agents. So, it was impossible for them to differentiate the role of peripheral sympathetic nerves, from the role of adrenal glands on the cardiovascular response to forced dives. Andersen and Blix (1974) injected adrenergic alpha-receptor blockers (phentolamines), as well as reserpine before dives in ducks which were normally able to endure forced submersion for more than 10 minutes. MDT were reduced considerably, respectively to 150 seconds and 45 seconds (Andersen & Blix 1974). The effectiveness of phentolamine and reserpine was not controlled, and so it is impossible to conclude if the reduction of MDT observed in ducks dived with reserpine is due to a depletion of catecholamines stored in sympathetic nerves only or in both sympathetic nerves and adrenal glands.

The work done in this chapter differentiates between the role of sympathetic nerves and the role of adrenal catecholamines on the cardiovascular adjustments to forced diving. I suggest that sympathetic nerves initiate the peripheral vasoconstriction at the beginning of the dive, and also partially maintain it during the dive. However, peripheral vasoconstriction controlled by sympathetic nerves is not strong enough to eliminate blood flow from peripheral tissues. Adrenal catecholamines seem to enhance and maintain peripheral vasoconstriction during the dive and thus would prevent O_2 stores (indispensable for the brain and heart) from being depleted by peripheral tissues. Therefore tolerance to underwater submersion is increased.

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CHAPTER 3:

INFLUENCE OF PROPRANOLOL ON MAXIMUM DIVE TIME.
INTRODUCTION.

The cardiovascular peculiarities observed during diving in adrenalectomised (ADX) and adrenal denervated ducks (DNX) (higher heart rate associated with a lower arterial blood pressure) suggested a reduction in peripheral vasoconstriction and consequently a faster drop in oxygen stores leading to a decrease of maximum dive time (MDT). However, as I mentioned in chapter 2, a decrease in stroke volume could also have occurred in ADX and adrenal DNX, due to a depressant effect of hypoxia (McKean 1984; Nakhjavan et al. 1971; Tyberg et al. 1970) or / and acidosis (Rau et al. 1977; Gonzalez and Clancy 1975; Cingolani et al. 1970) per se on myocardial contractility. A decrease in cardiac output might cause a drop in MDT because of a lack of an adequate cerebral and cardiac blood flow and pressure. The hypothesis that circulating catecholamines could counteract the depressant action of hypoxia, and acidosis on the heart and thus enhance MDT has to be investigated.
By injecting propranolol (beta 1 and 2 adrenergic blockade) in Pekin ducks at the beginning of the dive, most of the actions of catecholamines on the myocardium can be prevented. In this chapter, the MDT from such dives are compared with control dives in which ducks were injected with saline. This preliminary experiment established whether or not catecholamines, by their action on the myocardium, enhanced MDT. This experiment did not differentiate between the action of catecholamines from sympathetic nerves, and the action of catecholamines from the adrenal glands. On the other hand, in the absence of any significant effect on MDT, it would be likely that the enhancement of MDT by adrenal catecholamines is not achieved through myocardial actions via beta-adrenergic receptors.
MATERIAL AND METHODS.

1-ANIMALS:

These experiments were performed on 10 two to three month old male Pekin ducks ranging in mass from 2.5 to 3 Kgs. They were kept in conditions similar to those described in Chapter I.

2-MINOR SURGERY AND EXPERIMENTAL PROCEDURE:

Cannulation of the brachial artery and vein was done under local anesthesia (Lidocaine hydrochloride, Xylocaine 2% Astra) as described in chapter II. The birds were given one day to recover from surgery. The position of the cannula was checked at post mortem: the tip of the arterial cannula was positioned at the junction of the brachiocephalic artery and aorta; the tip of the venous cannula was positioned in the vena cava.

Birds were placed in a sitting position and secured with tape to an operating table. The electrocardiogram leads were inserted subcutaneously, one above the left thigh, the other below the right shoulder, and the ground lead to the web of the right foot. The arterial cannula was connected to a blood pressure transducer.

The ducks were left undisturbed behind a screen for 15 minutes to settle down before an experiment started. The head of the duck was then gently lowered into the water. During the
first 10 seconds of the dive with beta-blockade, 4 mg/Kg of propranolol (Sigma) dissolved in 1 ml of saline was injected into the duck via the venous cannula. The cannula was flushed with 2 ml of saline. This dose of propranolol was enough to prevent, for 20 minutes, the tachycardia following an injection of 50 ug of isoproterenol hydrochloride (Winthrop lab. Ontario). In control dives, 3 ml of saline was injected following the same procedure as that for dives with beta-blockade. Three dives were performed on each duck with 48 hours of recovery between dives. In the first and third dives, only saline was injected (control dives). In the second dive, propranolol was injected. Maximum dive time was measured following the criteria established in chapter II.

3-ANALYSIS OF DATA:

Heart rate and mean arterial blood pressure were sampled every minute, until 30 seconds before the end of the dive. In the text and graphs, values are given as mean ± standard error of the mean (SE). Because the length of the dive for each animal is different, the sampling time for n (number of dives) decreases. The mean and SE for values recorded during the dive are only included as data for analysis when the number of dives remaining is more than, or equal to, half of the original number of dives. Mean and SE of values at the end of dives are also included on graphs. Statistically significant differences were based on results of analysis of variance followed by the Newman-Keuls test. A significant difference was assumed if p<0.05.

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RESULTS.

1-EFFECTS OF PROPRANOLOL ON MAXIMUM DIVE TIME (MDT):

In the first and third dives, in which the ducks were injected with saline, mean MDT was 13 min. 53 sec. + 1 min. 11 sec., and 12 min. 55 sec. + 1 min. 16 sec respectively. MDT in the second dive, in which ducks were injected with propranolol was 12 min. 5 sec. + 1 min. 1 sec. There were no significant differences among the mean MDT in the three dives (Figure 20).

2-EFFECTS OF PROPRANOLOL ON HEART RATE (HR) AND BLOOD PRESSURE (BP):

There were no significant differences in pre-dive heart rate among dives. Diving heart rates, in the three dives were significantly lower than their respective pre-dive values with exception of those at end dive (Figure 21).

In the first and third dives, a strong bradycardia developed. Heart rate dropped from 154 ± 7 beats/minute to 17 ± 3 beats/minute at 2 minutes into the dive during the first dive, and from 159 ± 11 to 19 ± 4 beats/minute in the third dive. Diving heart rates in the first and third dive were not significantly different and remained close to the levels reached at 2 minutes throughout the dive (Figure 21).

The heart rate profile during the second dive, in which ducks were injected with propranolol was quite different. Heart rate dropped from 150 ± 10 beats/minute to 24 ± 6 after
FIGURE 20: Maximum dive time in ducks injected with propranolol and saline.

(1-sal.: first dive saline injected; 2-prop.: second dive propranolol injected; 3-sal.: third dive saline injected)
MAXIMUM DIVE TIME (minutes)
FIGURE 21: Heart rate and mean arterial blood pressure during diving in ducks injected with saline (1st dive: hollow triangles, n=10 for heart rate and blood pressure; 3rd dive: hollow circles, n=8 for heart rate and n=6 for blood pressure) or with propranolol (2nd dive: filled square, n=10 for heart rate and n=9 for blood pressure).

*: significantly different from both control dives.
+: significantly different from the first control dive.
2 minutes diving, but after 3 minutes it had increased and was significantly higher than during the saline injected dives (Figure 21). After 6 minutes submergence, heart rates in the first and third dive were 18 ± 2 and 21 ± 4 beats/minute but in the beta-blocked dive, heart rate was 52 ± 8 beats/minute.

No significant differences in pre-dive blood pressure were observed among the three dives (Figure 21). Blood pressures during diving in the first and third dives were not significantly different. In propranolol injected dives, blood pressures dropped significantly below pre-dive levels after 1 minute diving and stayed lower than the pre-dive value throughout the dive. This was not the case in the saline dives: diving blood pressure was never significantly lower than the pre-dive values. Blood pressures significantly dropped from 157 ± 4 to 83 ± 7 mmHg after 6 minutes diving in propranolol dives. In the first dive, blood pressure was 170 ± 8 mmHg before the dive and 126 ± 12 mmHg at 6 minute into dives and in the third dive, it was 155 ± 10 and 120 ± 20 mmHg respectively (Figure 21).
DISCUSSION:

Preventing the cardiac actions of catecholamines did not decrease MDT even though the cardiovascular responses to diving were strongly altered. The increase in heart rate observed in the beta-blockade dives was not associated with an increase of blood pressure. This suggests that a decrease of peripheral vasoconstriction or a decrease in stroke volume occurred during the beta-blocked dives. The fact that MDT was not shorter during the beta-blocked dives implies that the oxygen stores were not depleted faster than during the control dives. That, plus the fact that propranolol if anything reinforced vasoconstriction by blocking the vasodilator beta-2 receptors, suggests that a decrease of stroke volume (not peripheral vasoconstriction) occurred in beta-blocked dives and was accompanied by an increase of heart rate. Since the beta actions of catecholamines on the heart were suppressed, and alpha effects are not expected to be great (see next paragraph) these changes must be a consequence of the direct effects of vagal activity, hypoxia, hypercapnia, and acidosis imposed on the heart during diving.

It has long been known that in mammals, the positive chronotropic, dromotropic, and inotropic effects of catecholamines on the heart are mediated through beta-adrenoceptors (Berne and Levy 1981), and this is also true for birds (Bolton 1967, Bolton and Bowman 1969; Tummons and Sturkie 1970).
However, recent studies on mammals have demonstrated the existence of alpha-adrenoceptors in the heart. It was not possible to block these alpha-adrenoceptors during the dive without suppressing as well the peripheral vasoconstriction and, consequently, the dive response. However, beta-adrenoceptors seem to be the main receptors through which the cardiac action of catecholamines is expressed in adult mammals and birds. Bristow et al. (1988) established that the relative proportion of alpha and beta adrenoceptors in a human heart is 15% and 85%. Also, Skomedal et al. (1988) showed that in the isolated papillary muscles of rat heart, the cardiac inotropic response, elicited after NE stimulation decreased by 75% after propranolol blockade. The physiological significance of alpha-adrenoceptors in cardiac regulation is not well established and appears minor compared to beta-adrenoceptors. Alpha adrenoceptors have a direct positive inotropic effect on the heart (Aass et al. 1983; Bruckner et al. 1984; Skomedal et al. 1985), they decrease the neural release of NE (Starke 1972; Yamaguchi et al. 1977) and increase NE uptake by both adrenergic nerve terminals and extraneuronal tissues (Starke et al. 1971; Iversen 1973). They also decrease neural release of acetylcholine (Wetzel et al. 1985; McGrattan et al 1986; McDonough et al. 1986), although this has not been observed in birds (Loffelholz et al. 1984). Furthermore, Bolton (1967) and Bolton and Bowman (1969) in fact failed to demonstrate the existence of any alpha-adrenoceptors in birds. After adding NE to the heart, they looked at the cardiac inotropic changes when alpha-adrenoceptor blockers were added. The fact that they did not find any alpha-adrenoceptors could be because
their activity was masked by that of cardiac beta-receptors: they should have added NE first, then a beta-1 and 2 receptor blocking agent and then the alpha blocking agent (Skomedal et al. 1988). In chickens, the effect of sympathetic nerve stimulation or NE on heart rate was nearly abolished after injection of propranolol (Bolton 1967; Tummons and Sturkie 1970). Thus it is likely that, by injecting propranolol in Pekin ducks at the beginning of the dive, I prevented most of the action of catecholamines on the heart.

In birds as in mammals, stimulation of parasympathetic nerves causes bradycardia (Furilla and Jones 1987; Lang and Levy 1989) as well as a negative inotropic effect (Folkow and Yonce 1967; Furnival et al. 1973; Lang and Levy 1989).

The main consequence of the direct action of hypoxia and acidosis on the heart is a decrease in contractility. Ng et al. (1966) showed that the effect of hypoxia on cardiac contractility depends on the severity of the hypoxia. Moderate hypoxia increases ventricular contractility, while severe hypoxia decreases ventricular contractility in mammals (Shine 1981; McKean 1984; McKean and Landon 1982; Rau et al. 1977; England and Krause 1987) as well as in ducks (Abati 1975). Acidosis also has a direct negative inotropic effect on the heart (Rau et al. 1977; Gonzalez and Clancy 1975; Cingolani et al. 1970), respiratory being more potent than metabolic acidosis (Steenbergen et al. 1977). Acidosis potentiates the depressant effect of hypoxia on myocardial functions (Downing et al. 1966). Negative inotropic effects have also been observed
during ischaemia (Katz and Hetcht 1969; Steenbergen et al. 1977).

As a result of their positive inotropic effects, catecholamines can compensate for the debilitating effects of hypoxia, hypercapnia, acidosis, ischaemia (England and Krause 1987; Nakanishi et al. 1987) and vagal activity on cardiac contractility. The prevention of the action of catecholamines during diving would cause a decrease in cardiac contractility and stroke volume. The resulting decrease in cardiac output could be compensated for, in this particular case, by an increase of heart rate invoked by the baroreceptor reflex. However, chemoreceptors during the dive tend to inhibit the baroreflex (Smith 1987). Nevertheless, the cardiac action of catecholamines is prevented by injection of beta-blockade, so the increase of heart rate can only be due to a decrease of vagal activity on the heart.

Previous work has been done on the effects of propranolol during diving (Butler and Jones 1968, 1971; Folkow et al. 1967; Gooden et al 1974), but no increase of heart rate was observed because the dive times used in these experiments did not exceed 2 minutes and, as I have shown, propranolol affects cardiovascular responses later in the dive. However, Ferrante and Opdyke (1969) working on Nutria submerged for 1 minute demonstrated that vagotomy or atropinization caused a marked increase in ventricular contractility to more than 30% above control levels and that propranolol administrated to these vagotomised or atropinised animals greatly reduced or abol-
ished this increase. This shows that very early in the dive, there is only a small effect of catecholamines on cardiac contractility which is masked by the vagal activity. This explains why injection of propranolol did not alter the cardio-vascular adjustments. Sympathetic activity on the heart increases throughout the dive (i.e. increase in circulating catecholamines). Moreover, hypoxia, hypercapnia and acidosis develop and reach the levels which would depress cardiac contractility (after 3 minutes into diving) if catecholamines were not there.

During diving the heart is controlled by powerful negative inotropic and chronotropic influences (the vagus, hypoxia, hypercapnia and acidosis). This work demonstrates a significant effect of the sympathetic system whose strong positive inotropic action mediated through beta-adrenoceptors, counterbalance these depressant influences exerted on cardiac contractility. At 2 minutes into the dive in Pekin ducks, stroke volume is not significantly different from its pre-dive value (Jones and Holeton 1972). This and the fact that catecholamines prevent stroke volume dropping later in the dive, suggest that catecholamines maintain stroke volume at its pre-dive values throughout the dive. However, the cardiac effect of the sympathetic system had no bearing on MDT.
CHAPTER 4:

RESPECTIVE ROLES OF SYMPATHETIC NERVES AND SYMPATHETIC HUMORAL AGENTS ON THE VASCULAR RESISTANCE OF THE HIND LIMB OF PEKIN DUCKS DURING DIVING.
INTRODUCTION.

It has been suggested in Chapter II that the drop in maximum dive time (MDT) observed in adrenalectomised (ADX) and adrenal denervated (DNX) ducks was caused in part by a decrease in peripheral vasoconstriction due to the absence of adrenal catecholamines during diving. Peripheral vasoconstriction could be enhanced by adrenal catecholamines exerting their effects directly on the sympathetic nerves and/or vascular smooth muscle. Indeed, Gooden (1980) suggests that a role for circulating catecholamines could be to sustain vasoconstriction during prolonged dives when neurally mediated vasoconstriction is depressed by hypoxia.

In this chapter I will examine the following questions:
1) Are the effects of circulating catecholamines and sympathetic nerve activation on peripheral vasomotor tone additive?
2) Do hypoxia, hypercapnia and acidosis reduce the degree of vasoconstriction produced by sympathetic nerve activation during diving?
In order to directly assess whether or not circulating catecholamines can enhance peripheral vasoconstriction during a dive, a system was developed to monitor the changes in vascular resistance simultaneously in both hind limbs. One leg was perfused with the duck's own blood (autoperfused leg) and the other with different blood mixtures necessitated by the experimental protocol (test leg). Because of the enormous decrease of hind limb blood flow (from 40 - 50 ml/min to 2 - 0 ml/min) and variation in blood pressure during a dive (180 mmHg to 120 mmHg), I chose not to employ classical methods of perfusion which use constant flow or pressure. Before and during the dive, the autoperfused leg was perfused at variable pressure and flows matching those normally observed in a duck under these conditions. The test leg was perfused with the same flow as the autoperfused leg. Differences in perfusion pressure between autoperfused and test legs indicated differences in their vascular resistances during the dive.

To estimate the respective roles of sympathetic nerves and humoral factors on the vascular resistance during a dive, the test leg was perfused with blood obtained from the resting animal. The gas O$_2$ and CO$_2$ tensions of this blood were adjusted before perfusion to match those found during the dive.

The "resting" blood, equilibrated with different gas mixtures, was used in a further set of perfusions in order to determine whether or not peripheral vasoconstriction, mediated by sympathetic nerves, is affected by hypoxia and/or hypercapnia.
Norepinephrine (NE) and epinephrine (E) were added to hypoxic-hypercapnic blood perfusing the test leg to match the catecholamine levels measured during diving (Hudson & Jones 1982). This demonstrated the role of circulating catecholamines on the peripheral vasoconstriction during the dive.
MATERIAL AND METHODS.

1-ANIMALS:

Male Pekin ducks 3 to 5 months old and 3 to 3.5 Kgs in mass were kept under conditions similar to those described in chapter I. Twenty six ducks were used for perfusion. Twenty six ducks served as blood donors.

2-SURGERY AND EXPERIMENTAL PROCEDURE:

In donor ducks, the brachial vein was cannulated with P.E. 190 tubing (Intramedic Polyethylene tubing, Clay Adam), under local anesthesia, following the procedure described in chapter II.

In the experimental ducks, all surgery was performed at least one day before a perfusion experiment. The brachial vein and artery were cannulated with P.E. 190 tubing (Intramedic Polyethylene tubing, Clay Adam), following the procedure described in chapter II. In order to prevent any leg impairments, and degradation of the vascular system, non-occlusive cannulations were performed on the ischiatic arteries under local anesthesia (Lidocaine hydrochloride; Xylocaine 2% Astra). The skin was cut along the caudal side of the femur, exposing the biceps femoris. An incision was made in this muscle adjacent to the femur, 1.5 cm from the hip joint to expose the ischiatic artery which was clamped in two places 1 cm apart. An incision was made in its wall between the clamps
and the ends of a single catheter (15 cm length, 3 and 3.7 mm inside and outside diameters) were inserted 1.5 cm in the vessels proximally and distally, to form a loop in which the blood flowed freely when the clamp was removed. The catheter was tied into the vessel, and muscle and skin were sutured, leaving part of the loop exposed outside the animal.

The catheter was made of Polyvinyl chloride (PVC, size V-11, BOLAB, Lake Havasu City, Arizona). It was treated with a heparin-containing solution (TD-MAC; 25%; Polysciences Inc.; Warrington Pennsylvania) to bind heparin onto the tubing wall in order to prevent blood clotting. The length of the tubing was filled with a 1:1 mixture of TD-MAC solution and toluene for 4 minutes. Then, the mixture was removed and the tubing was rinsed with 20 ml. of saline containing 500 IU of heparin per ml. The tubing was air dried for at least 4 hours. The effectiveness of this procedure was such, that blood could flow freely through the catheter for up to 4 weeks without the need to flush it or inject heparin into the animal.

The donor duck was placed on its back on an operating table and the perfused duck was placed in a sitting position on another operating table. Ten ml of saline was injected into the venous cannula of the donor, and immediately after, 10 ml of blood was withdrawn. This procedure was repeated 6 times, removing 60 ml of venous blood from a donor duck without creating a hypotension which might have lead to release of catecholamines. In order to minimize cross reactivity, 10 ml of donor blood was injected into the venous cannula of the duck to be used in the perfusion experiment, and 10 ml venous
blood was extracted. This was repeated 6 times and 60 ml of venous blood was extracted.

This blood was then poured into a blood gas chamber maintained at 41 °C (T: Figure 22). The blood was stirred very gently to equilibrate it with the inflow gas mixtures necessitated by the experimental protocol. Stirring did not cause any hemolysis.

The EEG was recorded as described in chapter I. The ischiatic loops were cut, the brachial arterial cannula, as well as the distal and proximal parts of the loops were then connected up as described in Figure 22. The duck (D: Figure 22) was left undisturbed for 5 to 10 minutes while both legs were perfused with their own blood. Ducks were dived by lowering their head gently into a beaker of cold water (16-20°C). Then, the test leg was connected to a given blood gas mixture and perfused with it throughout the dive. The dive was terminated when the blood chamber was empty or maximum dive time was reached. MDT was determined following the characteristics established in Chapter II.

Seven ducks had their test leg perfused with hypoxic-hypercapnic blood, 5 with hyperoxic-hypocapnic blood, 8 with hyperoxic-hypercapnic blood and 6 with hypoxic-hypocapnic blood. The effects of acidosis on the neurogenic component of peripheral vasoconstriction during diving are secondary to the effects of hypercapnia because the drop of pH observed in the blood during diving is due to an increase of PaCO₂ and not to a drop of SID (Shimizu and Jones 1987; Chapter II). Conse-
FIGURE 22: Diagram of the experimental set up used for the hind limb perfusion experiments (for explanations see text).
quenty, the effects of the diving acidosis on the neural vasoconstriction should be the same as the effects of hyper-capnia. Resting blood was used in these experiments, so circulating catecholamines did not exceed resting levels.

In five other ducks, test legs were perfused with hypoxic-hypercapnic blood into which NE (Norepinephrine bitartrate: Levophed Winthrop, Aurora, Ontario, Canada) and EP (Epinephrine chloride: Park-Davis, Scarborough, Ontario, Canada) were infused to yield levels matching those recorded by Hudson and Jones (1982). The procedure used for this group differed from that described above, because only the test leg was cannulated. The distal and proximal parts of the loop were connected as described in Figure 22 for the test leg. The downstream catheter was connected through a side arm, to a Harvard infusion pump (model 901 Millis Mass.; I: Figure 22) infusing at a rate of 0.136 ml/min. Saline was infused into the blood flow of the test leg for two minutes before the dive. Three minutes after the dive had commenced, the saline infusion was replaced by catecholamines (0.136 ml/min infusing 0.34 ug of NE and 0.34ug of EP per minute) and maintained until the end of dive.

3-MEASUREMENTS AND ANALYSIS OF THE PHYSIOLOGICAL VARIABLES:

The set-up used for the perfusion experiments is shown in Figure 22. Perfusion of the test and autoperfused leg was maintained with a peristaltic pump (Watson-Marlow; H.R. Flow inducer; Cornwall, England; P: Figure 22). Although the autop-
erfused leg was perfused with the duck's own blood before and throughout the dive, the test leg was only perfused with the duck's own blood before the dive. At the beginning of the dive, the stopcocks (S: Figure 22) were turned to perfuse the test leg with the blood from the thermostated chamber (T: Figure 22). In order to keep blood volume constant, an equivalent volume of blood was pumped out of the animal into a waste container (W: Figure 22). The autoperfused leg was perfused before and during the dive with variable pressures and flows matching the levels normally observed in ducks in these conditions. This was done using a pressure flow : controller (C: Figure 22). This controller compared the perfusion pressure of the autoperfused leg with the mean arterial blood pressure of the duck and increased or decreased the flow of the pump in order to maintain perfusion pressure identical to the mean arterial blood pressure. For example, if the vascular resistance increased in the autoperfused leg flow decreased in order to keep perfusion pressure similar to mean arterial blood pressure. The test leg was perfused at the same flow as the autoperfused leg although its perfusion pressure was not controlled (Figure 22). The blood used for perfusion of the test leg was stored and kept at 41°C in the chamber through which desired gas mixtures were circulated (T: Figure 22). The length of the tubing was the same in the circuits perfusing the two legs and was kept to a minimum in order to prevent cooling of blood. Because the tubing inside the pump was expansible, pressures upstream of the pump had to be the same in both circuits in order to keep the flow in each leg identical. Consequently, the blood chamber (T: figure 22) was pres-
surized at mean arterial blood pressure (±5 mm Hg). The pressure inside the chamber was checked throughout the dive with a mercury manometer (M: figure 22). The pressurization system consisted of a regulator (A: Figure 22; Figure 23) connected to the gas outflow of the blood chamber. A flowmeter (F: Figure 22) was used to set the pressure in the blood chamber to mean arterial blood pressure and the pressurization was checked over a range of 50 to 250 mm Hg before beginning the experiment.

The experimental set up for the catecholamine experiment differed from the previous description as only one leg was cannulated. In this case, the pressure flow controller simply compared the perfusion pressure of the test leg with the mean arterial blood pressure.

ECG, mean arterial blood pressure, perfusion pressure of autoperfused and test legs, as well as blood flow in these legs were recorded on a chart recorder (Watanabe III, Type WTR 281, Japan; R: Figure 22). The difference between vascular resistance of the autoperfused and test legs was reflected by any difference in perfusion pressure. Vascular resistance was calculated by the formulae \( \int r = \int p / \int f \), where \( \int p \), \( \int f \) and \( \int r \) are the average values of perfusion pressure, blood flow and vascular resistance over a 1 minute (t+1-t) interval. Venous pressure was ignored in this calculation because it did not significantly change the overall results (Jones 1973).
FIGURE 23: Pressure regulator for the blood chamber.
If the pressure in the chamber was less than the mean arterial blood pressure (MAP), the elastic membrane (m) bowed to obstruct the gas outflow tubing. Pressure inside the chamber then rose to exceed MAP and the membrane was reflected allowing the gas to escape.
GAS INFLOW
(from the blood chamber)
At the beginning and at the end of a dive, 1 ml of blood was taken in air tight syringes from the duck and from the blood chamber in order to measure blood gas values and pH using an Instrumentation Laboratories 813 pH/Blood gas analyzer (Lexington MA). In 14 random trials, blood was taken from the gas chamber and catecholamine levels were measured following the technique described in chapter I.

4-ANALYSIS OF DATA:

Heart rate, mean arterial blood pressure as well as perfusion pressure and blood flow in both legs were averaged over one minute intervals before and throughout dives for up to 8 minutes.

Significant differences in heart rate and mean arterial blood pressure among the 5 groups of ducks were established using an analysis of variance, followed in the case of significant differences by the Newman-Keuls test. Significant differences in perfusion pressure and blood flow between test and autoperfused legs, were established by using a paired Student’s t-test. Comparisons of perfusion pressure and blood flow among the 4 groups of ducks whose test legs were perfused with blood mixtures devoid of catecholamines, were done using an analysis of variance, followed in the case of significant differences by the Newman-Keuls test. In order to compare the effects that these different blood mixtures have on the peripheral vasoconstriction, linear regression between the changes of vascular resistance in the autoperfused leg and in the associated test leg during diving
were calculated in the 4 groups of ducks. Significant differences among their slopes were obtained using an analysis of covariance followed by the Newman-Keuls test. Statistically significant differences in vascular conductance between test legs perfused with hypoxic-hyperoxic blood with or without infusion of catecholamines were established by using unpaired Student's t-test.

The fiducial limit for a significant difference was set at a probability of 5% (p < 0.05). In the text and graphs, values are given as mean ± standard error of the mean. When perfusion pressure in autoperfused and test legs at rest differed by 15% and more, the ducks were not included in the experiment. Two ducks were dismissed on this criterion.
RESULTS.

1- HEART RATES (HR) AND MEAN ARTERIAL BLOOD PRESSURES (MABP):

All ducks in the 5 groups presented the classic diving bradycardia (Figure 24). There was no significant difference in pre-dive and dive heart rates or mean arterial blood pressures among the 5 groups of ducks.

2- EFFECTS OF HYPOXIC AND / OR HYPERCAPNIC BLOOD DEVOID OF CATECHOLAMINES ON THE VASCULAR RESISTANCE OF HIND LIMBS DURING DIVING:

PO₂, PCO₂ and pH of the blood perfusing test and autoperfused legs of ducks were measured at the beginning and at the end of all dives (figure 25). To ascertain that the method used to remove 60 ml of blood from ducks did not trigger a release of catecholamines, levels of NE and EP were measured in the blood perfusing the test leg. These values (2.8 ± 0.5 nM for NE and 0.3 ± 0.1 nM for EP) were below the resting plasma levels measured in ducks in chapters I and II.

After 2 minutes diving, perfusion pressure in the leg perfused with hypoxic-hypercapnic blood dropped significantly below that of the autoperfused leg, and remained significantly lower throughout the dive (Figure 26). During diving, there was no significant difference in perfusion pressure between autoperfused and test legs of ducks perfused with hyperoxic-hypocapnic blood (Figure 27) or with hypoxic-hypocapnic blood.
FIGURE 24: Heart rate and central mean arterial blood pressure during diving in ducks whose legs were perfused with hypoxic and hypercapnic blood (open triangles), with hyperoxic and hypocapnic blood (open diamonds), with hypoxic and hypocapnic (open squares), with hyperoxic and hypercapnic blood (open circles) and with hypoxic and hypercapnic blood with catecholamines (inverse open triangles).
FIGURE 25: Blood gas tensions and pH of blood perfusing autoperfused and test legs at the beginning (hollow bars) and at the end (filled bars) of dives.

Group with hypoxic and hypercapnic hind limb perfusion (A), hyperoxic and hypercapnic perfusion (B), hypoxic and hypocapnic perfusion (C), hyperoxic and hypocapnic perfusion (D), hypoxic and hypercapnic perfusion with catecholamines (E).
FIGURE 26: Perfusion pressure and blood flow in hind limbs of ducks whose legs during diving were perfused with their own blood (autoperfused leg, hollow triangles), and with hypoxic and hypercapnic blood (test leg, filled triangles). +: significantly different from control legs.
In the lower graph, the filled triangles overlap the open triangles.
TEST LEG: HYPOXIA AND HYPERCAPNIA
WITHOUT CATECHOLAMINES

PERFUSION PRESSURE (mm Hg)

PERFUSION FLOW (ml/min.)

DIVE TIME (minute)
FIGURE 27: Perfusion pressure and blood flow in hind limbs of ducks whose legs during diving were perfused with their own blood (autoperfused leg, hollow diamonds), and with hyperoxic and hypocapnic blood (test leg, filled diamonds).
In the lower graph, the filled diamonds overlap the open diamonds.
TEST LEG: HYPEROXIA AND HYPOCAPNIA
WITHOUT CATECHOLAMINES

PERFUSION PRESSURE (mm Hg)

PERFUSION FLOW (ml/min.)

DIVE TIME (minute)
(Figure 28). However, after 2 minutes of diving, perfusion with hypoxic-hypercapnic blood caused perfusion pressure in test leg to fall significantly below that of the autoperfused leg (Figure 29).

Before diving, the perfusion pressure in autoperfused legs was not significantly different from that in the test legs (Figures 26, 27, 28, 29). No differences in blood flow were observed before and during diving between the autoperfused leg and test legs in any of the 4 groups of ducks (Figures 26, 27, 28, 29), except before diving in ducks perfused with hypoxic-hypocapnic blood (Figure 28).

Hind limb blood flow during diving dropped drastically in all ducks (Figures 26, 27, 28, 29). No differences in blood flow were observed before and during dives among the 4 groups of ducks, except for the ducks perfused with hypoxic-hypercapnic blood which at 3 minutes into the dive had a significantly higher blood flow than ducks perfused with hypoxic-hypercapnic blood (Figures 26, 27). Before diving, perfusion pressure in test legs was the same in all 4 groups of ducks (Figures 26, 27, 28, 29). Perfusion pressure in autoperfused legs before and during diving was not significantly different among the 4 groups of ducks (Figures 26, 27, 28, 29).

An example of traces recorded during a dive when the duck was perfused with hypoxic-hypercapnic blood is given in figure 30A. Perfusion pressure in the test leg was lower than in the autoperfused leg. Another dive was completed 30 minutes later.
FIGURE 28: Perfusion pressure and blood flow in hind limbs of ducks whose legs during diving were perfused with their own blood (autoperfused leg, hollow squares), and with hypoxic and hypocapnic blood (test leg, filled squares).

+ : significantly different from control legs.

In the lower graph, the filled squares overlap the open square.
TEST LEG: HYPOXIA HYPOCAPNIA
WITHOUT CATECHOLAMINES

PERFUSION PRESSURE (mm Hg)

PERFUSION FLOW (ml/min.)

DIVE TIME (minute)
FIGURE 29: Perfusion pressure and blood flow in hind limbs of ducks whose legs during diving were perfused with their own blood (autoperfused leg, hollow circles), and with hyperoxic and hypercapnic blood (test leg, filled circles).

+: significantly different from control legs.

In the lower graph, the filled circles overlap the open circles.
TEST LEG: HYPEROXIA AND HYPERCAPNIA
WITHOUT CATECHOLAMINES

![Graphs showing perfusion pressure and flow over time.](image-url)
FIGURE 30: Examples of traces recorded during 2 dives: mean arterial blood pressure (MAB), hind limb perfusion pressure and blood flow (\( P_{\text{perf}} \)). In the first dive (A), only one leg (autoperfused leg) was perfused with the duck's own blood and the other (test leg) was perfused by hypoxic hypercapnic blood without catecholamines. In the second dive (B), both legs were autoperfused.
but in this case both legs were autoperfused (Figure 30B) and no differences occurred between the perfusion pressures during this dive.

Resting vascular resistance for autoperfused and test legs were 4 ± 0.2 and 4 ± 0.2 PRU (Peripheral Resistance Units in mmHg x min x ml⁻¹) respectively for hypoxic-hypercapnic perfusion, 4 ± 0.4 and 4 ± 0.5 PRU for hyperoxic-hypocapnic perfusion, 3.8 ± 0.2 and 4 ± 0.1 PRU for hypoxic-hypocapnic perfusion and 4.8 ± 0.3 and 4.9 ± 0.2 for hyperoxic-hypercapnic perfusion. The relationship between the absolute increase in vascular resistance during diving (resistance during the dive - resistance during pre-dive) in the test leg and the absolute increase of vascular resistance during diving in the autoperfused leg is shown in Figure 31. The absolute increase in vascular resistance in test legs was positively correlated with that in autoperfused legs.

The regression line for ducks perfused with hypoxic-hypercapnic blood (A: Figure 31) was not significantly different from that of ducks perfused with hyperoxic-hypercapnic blood (C: Figure 31). Furthermore, there was no significant difference between the regression line for ducks perfused with hyperoxic-hypocapnic blood (B: Figure 31) and the regression line for ducks perfused with hypoxic-hypocapnic blood (D: Figure 31). However, the regression lines of the two groups of ducks perfused with hypercapnic blood (A and C: Figure 31) are significantly different from regression lines of ducks perfused with hypocapnic blood (B and D: Figure 31).
FIGURE 31: Relationship between the absolute increase in vascular resistance during diving in the test legs and in autoperfused legs.

Ducks perfused with hypoxic and hypercapnic blood (A: filled triangles), hyperoxic and hypocapnic blood (B: hollow diamonds), hyperoxic hypercapnic blood (C: filled circles), and hypoxic and hypocapnic blood (D: hollow squares). The linear regression equation, the standard error of the slope (in parentheses) and the coefficient of determination are shown on each graph.

The slopes of the regression in A and C are significantly different from the slopes in B and D.
2-EFFECTS OF CIRCULATING CATECHOLAMINES ON HIND LIMB VASCULAR CONDUCTANCE (1 / RESISTANCE) DURING DIVING:

Vascular conductance was measured in legs perfused with hypoxic and hypercapnic blood during diving. Saline (0.136 ml.min\(^{-1}\)) infused into the blood perfusate before and during diving did not have any significant effect on vascular conductance (Figure 32). However, infusing saline containing catecholamines after 3 minutes submergence caused a significant drop in conductance (Figure 32) within 5 minutes. Because blood flow during diving was very low (0-2 ml/min.), at least 1 minute was required for the infused catecholamines to reach the vascular bed of the hind limb.
FIGURE 32: Vascular conductance in hind limbs perfused with hypoxic and hypercapnic blood without (filled triangles), and with catecholamines (inverse filled triangles) during diving. ▼: switching from saline infusion to catecholamine infusion. *: significantly different from hind limbs perfused without catecholamines.
VASCULAR CONDUCTANCE (ml/min.mmHg)

DIVE TIME (Minute)
DISCUSSION.

Comparison of the results of perfusion of hind limbs with hypoxic and hypercapnic blood with and without catecholamines demonstrated that circulating catecholamines played an important role in increasing peripheral vasoconstriction during diving. This supports the findings in chapter II: circulating catecholamines increase MDT by enhancing peripheral vasoconstriction. Perfusion with hyperoxic and hypocapnic blood demonstrated that sympathetic nerves were effective enough to achieve the normal level of peripheral vasoconstriction during diving. Vascular resistance of hind limbs perfused with hypercapnic blood during dives was always lower than those of control legs. This was not the case when perfusion was done with hypoxic mixtures; perfusion with hypoxic and hypocapnic blood did not affect vascular resistance during dives. Furthermore, the slope of the linear regression for hypoxic and hypercapnic perfusion was not significantly below that of hyperoxic and hypercapnic perfusion. This suggests that circulating catecholamines are needed to increase peripheral vascular resistance during diving because of the depressant effect of hypercapnia on the neural component of peripheral vasoconstriction. Consequently they would enhance MDT by preventing the peripheral tissue from having access to the O₂ stores.
Blood flow and vascular resistance measured in autoperfused legs before diving and at 2 minutes after submergence were similar to the values recorded with electromagnetic or ultrasonic flow probes (Butler and Jones 1971; Jones 1973; Smith 1987). This confirms that the present preparation did not affect the physiological values observed at rest or interfere with cardiovascular adjustment during the dive. This preparation presents advantages compared with traditional methods of perfusion which use constant flow or pressure. By allowing blood flow and perfusion pressure to be kept at physiological values, this preparation is adequate to perform any perfusions involving strong variations of vascular resistances, without creating any artifact in the measurement of vascular resistance, or causing any discomfort for the unanesthetised animal.

The results obtained in this chapter contrast with those of Gooden (1980) who suggested that hypoxia inhibited neurogenic vasoconstriction during diving. The experiments done by Gooden were very different from those described in this chapter: duckling and chicken mesenteric arteries were isolated, perfused and exposed to intravascular and extravascular hypoxia for 45 minutes in order to mimic diving. He observed that hypoxia depressed vasoconstriction mediated by intravascular injection of NE or mediated by nerve stimulation. However, nervously mediated vasoconstriction was depressed to a significantly greater extent, and surprisingly did not appear to be more resistant to hypoxia in duckling than in chicken. Gooden speculated that circulating catecholamines during diving
sustain vasoconstriction and thus compensate for the strong depression by hypoxia of the neurally mediated vasoconstriction. However, since 45 minutes of hypoxic exposure is far longer than any MDT observed in Pekin ducks, the results obtained in these experiments may not be representative of physiological responses that occur in forced dived ducks.

Peripheral vasoconstriction is mediated by the sympathetic branch of the autonomic nervous system (Butler and Jones 1971; Andersen and Blix 1974). Peripheral vasoconstriction during diving is extremely powerful because it can shut down circulation in the peripheral organs in order to spare O\textsubscript{2} stores for the heart and brain (Jones \textit{et al.} 1979; Heieis and Jones 1988). Folkow \textit{et al.} (1966; 1971) demonstrated that peripheral vasoconstriction in diving birds (ducks) and mammals (nutria) is far more efficient than that of non-divers such as turkeys and cats. In ducks, the larger arteries situated outside the muscles are narrower and their sympathetic innervation is far more abundant than in non-divers which explains their strong vasoconstrictor capabilities (Folkow \textit{et al.} 1966). Moreover, in spite of production of vasodilator metabolites by peripheral tissues during diving, vasoconstriction is maintained in ducks. Folkow \textit{et al.} (1966) induced a peripheral vasoconstriction in anaesthetised vagotomised ducks, comparable to that observed in intact ducks during forced dives, by giving them a hypercapnic-hyperoxic mixture to breath combined with controlled bleeding. They observed not only that blood flow to the leg almost stopped during vasocon-
striction, but also that exercising the leg during intense vasoconstriction did not increase blood flow. These results contrast with those obtained with cats, where vasodilatation produced by exercise overcame the intense neurogenic constriction (Kjellmer 1965; Folkow et al. 1966). Folkow et al. (1966) suggested that ascending vasodilatation is not observed in ducks during diving because an intense vasoconstriction occurs in the larger and richly innervated arteries which are situated outside the skeletal muscles, and hence out of reach of vasodilator metabolites produced by these muscles. Folkow et al. concluded that the vasoconstriction observed in ducks was neurogenic only, and they did not attribute any participation of adrenal catecholamines to the peripheral vasoconstriction even though hypercapnia and hemorrhage could have triggered a release of catecholamines by the adrenal glands (Engeland et al. 1981; Mangalam et al. 1987). My experiments differentiated between neurogenic and humoral vasoconstriction, and showed that the neurogenic vasoconstriction is not maintained against the vasodilating action of CO$_2$. The ability of the peripheral vasoconstriction to withstand the metabolic dilator influence in ducks appears to reside primarily in the action of circulating catecholamines directly at the site of the vasoconstriction.

It has been shown that hypercapnia can depress vasoconstriction induced by the sympathetic branch of the autonomic nervous system. Hypercapnia acts on isolated blood vessels inducing a slight relaxation whereas hypocapnia causes a slight contraction (Roger et al. 1965; Kontos 1971; Edvinsson
and Sercombe 1976). These effects are markedly exaggerated when the alpha-adrenergic receptors in the vascular walls are activated by catecholamines: hypercapnia depresses and hypocapnia potentiates the vasoconstrictor action of circulating catecholamines or of sympathetic nerves (Bygdeman 1963; Roger et al. 1965; Edvinsson and Sercombe 1976; Hjemdahl and Fredholm 1976). In fact, it is the changes of blood pH resulting from hypercapnia and hypocapnia which reduce or potentiate the vasomotor action of catecholamines (Roger et al. 1965; Edvinsson and Sercombe 1976). This means that more catecholamines are needed for a given intensity of vasoconstriction when vessels are subjected to hypercapnia. The sympathetic nerves appear to be unable to supply the extra catecholamines required for the maintenance of the peripheral vasoconstriction during diving. This extra dose of catecholamines is supplied by the adrenal glands.

Several mechanisms could be proposed to explain the depression of sympathetic vasoconstriction by hydrogen ions. 1) Acidosis acts by reducing the release of NE by the sympathetic nerve endings (Shepherd and Vanhoutte 1985). 2) Acidosis could increase the activity of Catechol-O-Methyltransferase, and therefore the removal of NE from extraneuronal sites would be increased. However, the optimal pH for this enzyme is between 7.5 and 8.2 (Axelrod and Tomchick 1958) which does not lend credence to this hypothesis. 3) Gende et al. (1985) showed that H⁺ decreases the affinity of cardiac beta-adrenoreceptors for agonists. This may also occur in the alpha receptors in blood vessels when submitted
to acidosis. 4) Increased concentrations of hydrogen ion can interfere with excitation-contraction coupling (Nakamaru and Swartz 1972; Inesi and Hill 1983). Therefore, contractility of the vascular smooth muscle would decrease due to muscle fatigue.
GENERAL DISCUSSION.
This work demonstrates the importance of the adrenal glands in aquatic air breathing animals during forced submersion, and the following mechanism of action of adrenal catecholamines is proposed. As soon as a Pekin duck's head is submerged, $\text{PaO}_2$ and $\text{pHa}$ drop, and $\text{PaCO}_2$ increases. This stimulates the chemoreceptors and triggers a drop in heart rate, mediated by the vagus, and an increase of peripheral resistance mediated by the sympathetic nerves. These cardiovascular adjustments allow a redistribution of $\text{O}_2$ blood stores toward the heart and brain. As the dive continues, the increase of $\text{PaCO}_2$ (which is responsible for the large drop of $\text{pHa}$ observed during diving: Shimizu and Jones 1987) depresses the neurogenic component of the vasoconstriction. Without the release of catecholamines from the adrenal glands (as in adrenalectomised (ADX) and adrenal denervated (DNX) ducks), the peripheral tissues would have access to the $\text{O}_2$ blood stores, and the maximum dive time (MDT) would be reduced. However, in intact ducks, catecholamines released by the adrenal glands compensate for the depressant action of hypercapnia on the neural
component of the peripheral vasoconstriction. Thus, peripheral resistance is maintained during diving in spite of the increase of hypercapnia. Oxygen stores are not depleted by the peripheral tissues which explains how MDT is enhanced. The function of the sympathetic nerves could be to initiate a rapid increase in peripheral resistance at the beginning of dives, in order to prevent O\(_2\) stores from being prematurely depleted by peripheral tissues. Several intriguing questions, some of which have been discussed in the previous chapters, have been raised by the results obtained in this work.

Adrenal catecholamines, which comprise 100% of the epinephrine and 40 to 80% of the norepinephrine found in the blood during diving, promote MDT but they do not seem to act alone. Other adrenal products whose release is also triggered by the adrenal nerves may be implicated. These substances have not yet been identified but it was suggested in Chapter 2 that adrenal opiates are the most likely contenders.

Adrenal catecholamines do not promote MDT by protecting the heart against the depressant effects of hypoxia, hypercapnia or acidosis, or even by maximizing pulmonary uptake of O\(_2\) by the blood by increasing blood affinity for O\(_2\) during acidosis. Instead adrenal catecholamines enhance MDT primarily by increasing peripheral resistance during diving. They act directly on the site of vasoconstriction itself and compensate for the depressant effect of CO\(_2\) on the neurogenic component of the vasoconstriction elicited during diving. However, two other mechanisms by which adrenal catecholamines could enhance
peripheral vasoconstriction during diving, must also be considered:

1) In studies on rats, circulating catecholamines appear to protect the brain against ischemic damage by a direct action on the brain itself (Koide et al. 1986). The mechanism of such an action is not known. If the rise of catecholamine levels is prevented during diving, normal functions of the cardiovascular centers may be adversely affected in hypoxic conditions and consequently peripheral vasoconstriction would be impaired.

2) It is known that circulating catecholamines increase the activity of the carotid chemoreceptor nerve fibers (Folgering et al. 1982; Milsom and Sadig 1983), and that the diving response of Pekin ducks is mainly triggered by the hypoxic stimulus on the peripheral chemoreceptors (Jones et al. 1982). This suggests that during diving, circulating catecholamines may potentiate the carotid body response to hypoxia and consequently may enhance the peripheral vasoconstriction. If the release of circulating catecholamines is suppressed, a weaker diving response would occur, resulting in a shortened MDT. The action of circulating catecholamines on carotid chemoreceptors, however, is mediated by beta-adrenoceptors (Folgering et al. 1982; Milsom and Sadig 1983). Since no significant decrease of MDT was observed in beta-receptor blocked dives compared with control dives, the effect of circulating catecholamines on MDT would not appear to be mediated through an additional excitation of the chemorecep-
tors during dives. The possibility that the action of circulating catecholamines on chemoreceptors is mediated by alpha-adrenergic receptors has not been studied. However, this does not seem very likely because Milsom and Sadig (1983) demonstrated that the effects of norepinephrine on chemoreceptors were completely abolished by beta-blockade.

The main role of the sympathetic nerves and of the adrenal glands during diving appears to be to provide a strong increase of peripheral vascular resistance in order to preserve O₂ stores for the heart and brain. Some of the experiments in this thesis also suggest that the sympathetic nerves and / or the adrenal glands counterbalance the depressant influences exerted on cardiac contractility by vagal activity, hypoxia, hypercapnia and acidosis. Consequently one of the action of catecholamines appear to maintain stroke volume near the pre-dive values throughout the dive.

Is the maintenance of stroke volume an adaptative advantage for coping with apnoeic asphyxia; or is it simply an indirect effect of the high levels of circulating catecholamines whose prime function is to enhance peripheral resistance? From the series of experiments described in Chapter 4, it would seem that the second suggestion is more likely because preventing the action of catecholamines on the heart did not diminish the ducks' ability to tolerate long periods of submersion.
The effects of the sympathetic and parasympathetic systems on heart rate have long been studied in mammals (Rosenblueth and Simeone 1934; Samaan 1935; Levy and Zieske 1969; Warner and Russell 1969). It has been established that their antagonistic effects are not additive, but that interactions take place between the two systems (Levy 1971, 1984; Berne and Levy 1981; Chassaing et al. 1983). Furilla and Jones (1987) examined the relationship of heart rate to bilateral stimulation of the cardiac parasympathetic and sympathetic nerves in Pekin ducks (Figure 33). By comparing heart rate during diving in control and beta blocked ducks from the present study with values obtained by these authors, an estimation of the relative contributions of the two components of the autonomic nervous system can be evaluated. During the propranolol dives there was little or no sympathetic activity: the estimated level of vagal activity decreased from 100% at 2 minutes into the dive (A: figure 33; when bradycardia was fully developed) to approximately 63% after 10 minutes (B: figure 33). However during the control dives, heart rates at 10 minutes into the dive were not significantly different from those at 2 minutes, and sympathetic activity was at its maximum (100%) because of the huge release of circulating catecholamines. But, according to figure 33, such low heart rates cannot be produced with this degree of sympathetic stimulation. Three hypotheses are offered to explain the divergence between my results and those of Furilla and Jones.

1) The maximum level of stimulation of parasympathetic nerves may not have been reached during Furilla and Jones'
FIGURE 33: The relationship of heart rate to bilateral stimulation of the distal cut ends of the vagus and cardiac sympathetic nerves. 100% represents the frequency of stimulation above which no further changes in heart rate occurred with increases in stimulation frequency (from Furilla and Jones 1987a).

Heart rate and cardiac sympathetic and parasympathetic nerves activities in propranolol injected ducks at 2 minutes (A) and 10 minutes (B) into diving.
2) There is no cardiac sympathetic chronotropic activity during diving (the action of the cardiac sympathetic nerves is minimal), and the action of circulating catecholamines is only selectively inotropic. However, it is difficult to see by what mechanism circulating catecholamines could be completely prevented from acting on the sinoatrial node.

3) Hypoxia and ischaemia also depress the sinoatrial nodal rate and conduction (Billette et al. 1973; Bellardinelli et al. 1980, 1981; Weiss and Shine 1981; McKean and Landon 1982; Stowe et al. 1985). Thus, during diving, the action of the vagus on the heart rate could be potentiated by hypoxia and acidosis (Courtice et al. 1983; Potter et al. 1986). This could explain the discrepancy between values in control dives, and values in figure 33 because in their experiments, Furilla and Jones did not subject the ducks' hearts to conditions of hypoxia, hypercapnia and acidosis similar to those encountered during diving.

In order to fully understand the regulation of heart during diving, the relationship of heart rate, stroke volume and cardiac contractility to bilateral stimulation of cardiac sympathetic and parasympathetic nerves should be studied when the heart is perfused with blood of different gas tensions, with and without circulating catecholamines.

There is a steady increase of circulating catecholamines during diving, to over 1000 times pre-dive levels. One minute after the end of the dive the levels of circulating catechola-
mines are still high: approximately 100 times pre-dive levels (Hudson and Jones 1986). This raises some questions about the ability of the blood flow to resume in the peripheral tissues after the dive. Some of the ducks in Chapter 4 were dived until they reached their maximum limit of underwater tolerance characterised by an increase of their heart rates toward pre-dive values (Figure 34). At the end of dives and during the first minute of post dive recovery, the increase in heart rate was not associated with an increase of hind limb blood flow (Figure 34). Thus, circulating catecholamines appeared to maintain peripheral vasoconstriction throughout the dive and continued to do so, even after emersion, in the early stages of the recovery period. It is quite clear that the increase of heart rate at the end of diving is not caused by a collapse of the peripheral vasoconstriction. An hypothesis can be advanced to explain why heart rate increases when the maximum underwater tolerance is reached.

The flattening of the EEG seen when maximum underwater tolerance is reached (Hudson and Jones 1986), indicates that brain function has deteriorated. Central cardiovascular nuclei should also be affected and consequently the activity of the sympathetic and parasympathetic nerves may also be impaired. This could explain why the bradycardia is not maintained anymore. Peripheral vasoconstriction at MDT would be maintained, however by the circulating catecholamines. Associated with this rise of heart rate, a decrease of stroke volume must occur because, at MDT mean arterial blood pressure is not significantly higher than it was pre-dive, even though peripheral vasoconstriction is at its maximum. Thus, deterioration
FIGURE 34: Heart rate (HR), mean arterial blood pressure (MAB), hind limb perfusion pressure and blood flow ($P_{\text{perf}}$, $F_{\text{perf}}$) in a duck during forced dive and recovery.
of brain function as well as cardiac failure caused by the strong hypoxemia and acidosis may determine the maximum underwater tolerance. Under these circumstances, the heart and brain could be protected by the circulating catecholamines which continue to maintain peripheral vasoconstriction, even after emersion, in the early stages of the recovery period. This would allow the heart and brain to recover before being hit by the discharge of lactate flushed from the peripheral tissues. After 3 minutes of recovery, hind limb blood flow and vascular resistance are back to their pre-dive values. The high level of heart rate associated with a lower blood pressure indicate however that stroke volume is still low and that the heart has not fully recovered yet.

To prevent oxygen stores from being prematurely depleted, vasoconstriction of the peripheral tissues is quickly established at the start of the forced dive by the sympathetic nerves. As the dive progresses, vasoconstriction may slacken owing to the depressant action of hypercapnia; however, this effect is counteracted by the release of adrenal catecholamines which serve to maintain vasoconstriction. This action differs from that which happens in most voluntary dives. The stimuli which lead to a release of circulating catecholamines (such as the variation in blood gas tension and the stimulation of nasoreceptors) are triggered during voluntary diving (see General Introduction). Most of the dives observed for free swimming birds and mammals are aerobic (see General Introduction) and consequently, are without much need of
peripheral vasoconstriction, nor of a large increase in circulating catecholamines. This raises some questions: is there, or is there not, a release of adrenal catecholamines during voluntary aerobic dives? If there is, how could it be prevented from causing peripheral vasoconstriction? If there isn’t, what are the mechanisms which prevent this release? In free diving mammals, the forced dive pattern of responses has been observed during long anaerobic exploratory dives (see General Introduction). It is not unreasonable to suggest that during these long exploratory dives, adrenal catecholamines could have a similar action to that observed in forced dives: the prevention of $O_2$ depletion by peripheral tissues.
BIBLIOGRAPHY.

ABATI A. (1975). A comparison of the effect of anoxia on left ventricular function in the isolated perfused heart of the chicken (a non-flying bird), the pigeon (a flying bird), and the duck (a diving bird). Ph.D. thesis, Rutgers university, New Brunswick, New Jersey.


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