# Cerebral energy metabolism in mallard ducks during apneic asphyxia: the role of oxygen conservation

ROBERT M. BRYAN, JR., AND DAVID R. JONES
*Zoology Department, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5*

BRYAN, ROBERT M., JR., AND DAVID R. JONES. *Cerebral energy metabolism in mallard ducks during apneic asphyxia: the role of oxygen conservation.* Am. J. Physiol. 239 (Regulatory Integrative Comp. Physiol. 8): R352–R357, 1980.—Cerebral energy metabolism during apneic asphyxia was studied in ducks. Fluctuations in the reduced form of respiratory chain nicotinamide adenine dinucleotide (NADH) were monitored from the left cerebral hemisphere and used as an indicator of mitochondrial hypoxia. Electroencephalogram (EEG) and surface $P_{O_2}$ were recorded from the right hemisphere. Forced dives of 4- to 7-min duration on restrained ducks were characterized by bradycardia and an accumulation of NADH, which increased throughout the diving period. NADH returned to the preasphyxic level when breathing was resumed. In later experiments, asphyxia was produced by stopping artificial ventilation in paralyzed ducks. Asphyxia produced by this means caused similar changes in the measured variables (heart rate, blood pressure, and NADH fluorescence) to those obtained in forced submergence of nonparalyzed ducks. The inhibition of cardiovascular adjustments by atropine caused NADH to increase faster and tissue $P_{O_2}$ to decrease faster during apneic asphyxia than in nonatropinized ducks. We conclude that the oxygen-conserving cardiovascular adjustments play a key role in the increased cerebral tolerance to apneic asphyxia in ducks.

diving; *Anas platyrhynchos;* redox state of the respiratory chain

DIVING MAMMALS AND BIRDS are known to tolerate periods of apneic asphyxia that are detrimental to terrestrial animals (1). The increased tolerance has been related to the refinement of oxygen-conserving cardiovascular adjustments (28) that are thought to protect the heart and the brain (20, 28). During a dive, the resistance of most vascular beds is greatly increased, and cardiac output is reduced as a result of intense bradycardia (6, 14, 15, 18, 28). Because vasoconstricted tissues are effectively isolated from the central circulation, they place no demands on the blood oxygen store, thus conserving oxygen for tissues such as the brain. Although basically the same response is seen in nondivers during apnea, it has been considerably refined in divers to meet the needs of prolonged apneic survival. The importance of the cardiovascular adjustments to continued brain function during diving was shown by Kerem and Elsner (21). Harbor seals, which tolerated dives lasting 18.5 min before the onset of hypoxic EEG (electroencephalogram) patterns, tolerated dives lasting only 5.5 min after the cardiovascular adjustments were inhibited with atropine.

Although oxygen conservation during diving is well documented, it has not been directly related to the biochemical events involved with adenosine triphosphate (ATP) production of the brain. Because oxygen is linked to ATP production via the respiratory chain, the effects of its conservation should be reflected in the redox state of the respiratory chain carriers. During hypoxia, the reducing equivalents enter the respiratory chain faster than they can be removed by oxygen resulting in a net reduction of the respiratory chain carriers. The oxidation-reduction state of any respiratory chain component can therefore serve as an indicator of mitochondrial hypoxia.

The purpose of the present investigation was to examine the redox state of the first component of the respiratory chain, nicotinamide adenine dinucleotide (NAD), from the cerebral cortex of ducks during apneic asphyxia. Ducks are known to tolerate extended periods of apneic asphyxia (28). In particular, we wished to examine the role played by the cardiovascular adjustments to apneic asphyxia in maintaining redox balance and tissue oxygen tension in the brain when the cardiovascular adjustments were inhibited with atropine. Furthermore, we recorded the EEG as an indication of maintenance of cerebral integrity and related this to the redox state of the brain during forced dives.

## METHODS

Experiments were done on a total of 45 mallard ducks, *Anas platyrhynchos*, of both sexes and weighing between 1.0 and 2.2 kg. Surgery was performed using general anesthesia by unidirectionally ventilating the ducks with 1% halothane in air. For unidirectional ventilation a cannula was inserted in the trachea towards the lung using local anesthesia (2% wt/vol Xylocaine, Astra Pharmaceutical, Mississauga, Ontario) and the clavicular air sac was punctured. The halothane and air mixture was humidified and passed through the tracheal tube at a rate of 1 l/min. Ducks were allowed at least 45 min to recover from the general anesthesia before experiments were started.

A polyethylene cannula (PE-90) was inserted into the left sciatic artery and connected to a Statham P23Gb pressure transducer for monitoring blood pressure. A second polyethylene cannula (PE-90) was inserted in the right brachial vein for drug injections. Heart rate was determined from the ECG by amplifying the potential difference between two copper wire electrodes; one inserted subcutaneously in the left side of the chest and

the other inserted in the right thigh, and passing the signal to a rate meter. Holes, 1.0 cm in diameter, were cut in the calvaria over the anterior portion of the left and right hemispheres and the dura was retracted. Tissue oxygen tension ($Pt_{O_2}$) was recorded polarographically from the surface of the right hemisphere in an area corresponding to that from which the fluorometric recordings (see below) were made on the opposite side. The electrode consisted of a platinum wire, 25 $\mu$m in diameter, fused in lead glass tubing and a silver/silver chloride reference. A detailed description of the electrode has been presented elsewhere (5).

Changes in mitochondrial NADH were monitored from the surface of the left hemisphere by a fluorometric technique (5, 7, 17), which takes advantage of the natural fluorescence of NADH. When excited with ultraviolet light, NADH fluoresces in the blue region of the spectrum, whereas its oxidized form, NAD, does not. Because hemoglobin absorbs at both the excitation and fluorescence wavelengths, changes of blood in the recording field produce artifacts in the apparent NADH fluorescence (8, 12, 17, 22, 23, 27). To correct for the hemoglobin artifact, we used a compensated differential fluorometer that monitored not only the fluorescence light but also the reflected excitation light (5, 17). The reflectance signal (R), which is a measure of hemoglobin change, was electronically subtracted from the fluorescence signal (F) giving a trace termed corrected fluorescence (CF) due solely to NADH. In addition to hemoglobin changes, other artifacts caused by movements of the cortex were eliminated by the difference method. Although in cells NAD exists in cytoplasmic and mitochondria pools, the fluorescence from the mitochondrial pool is enhanced over the fluorescence from cytoplasmic NADH to the point that fluctuations in cytoplasmic NADH contribute little to the overall fluorescence signal (9, 16, 25).

For the purpose of expressing the optical changes in a quantitative manner, the fluorescence intensity during normoxia (base line) was defined as zero and the fluorescence intesity following death by anoxia was defined as 100 arbitrary units (AU). The ducks were killed by ventilating them with 1% halothane in nitrogen; death was defined as the point when blood pressure fell to 0 kPa. Optical changes that correspond to intensities greater than the base line (NAD reduction) are designated with a positive sign; conversely, optical changes that have intensities less than the base line (NADH oxidation) are designated with a negative sign. Numerical values in Figs. 1-3 are expressed as means ± SE, and the $t$ test was used in the statistical analysis of the data with 5% ($P < 0.05$) considered the acceptable level of significance.

*Fluorescence recordings from restrained ducks.* NADH fluorescence was monitored from the left cerebral cortex of nine ducks using glass windows 1 cm in diameter to cover the exposed cortex. The windows were held in place by cementing them to the skull with dental cement (Hygienic, Akron, OH). In restrained ducks, the glass window reduced movement of the cortex during struggles and therefore eliminated artifacts that would have been too large to be compensated by the reflectance channel. After surgery, the punctured air sacs were sewn closed and artificial ventilation was stopped allowing the birds to respire on their own. The birds were secured ventral side down on a metal operating table, and the head was held motionless with the bill at 45° below horizontal by three metal screws (3 cm long) secured to the skull with dental cement and bolted to rods fixed to the metal table. In five of the nine ducks, brain electrical activity was monitored from the right frontal region of the skull. Ducks were subjected to periods of apneic asphyxia (4–7 min) produced by submerging the bill, nares, and eyes in water. Each duck was exposed to two periods of submergence with a 45- to 60-min recovery period between them.

*Fluorescence recordings from paralyzed ducks.* NADH fluorescence was monitored from the left cerebral cortex of 28 ducks paralyzed with gallamine triethiodide (Flaxedil, 1 mg/kg, Poulence, Montreal, Quebec). Plastic film covered the exposed cortex to prevent drying. Ducks were subjected to two or three periods of apneic asphyxia lasting from 2 to 9 min by stopping artificial ventilation. A 30-min recovery period separated the asphyxic periods. Arterial blood samples were withdrawn from the sciatic artery before each asphyxic period and analyzed with a Radiometer PHM-71 gas monitor with oxygen and carbon dioxide electrodes and a pH microelectrode unit type E5021 (Radiometer, Copenhagen, Denmark). The air flow was adjusted to give arterial blood values for $Pa_{O_2}$ of 11.3–14.0 kPa (85–105 Torr), $Pa_{CO_2}$ of 3.3–4.4 kPa (25–33 Torr), and pHa of 7.45–7.50. Cloacal temperature was monitored by a thermistor inserted into the cloaca and maintained at 41 ± 0.5°C by a temperature-controlled feedback unit that regulated a heating pad placed over the duck.

*Inhibition of cardiovascular adjustments during apneic asphyxia in ducks.* Eight mallard ducks of either sex, weighing 1.2–2.0 kg, were used in this series of experiments and were prepared as described above except in two ducks the window method was used for optical recordings and cortical oxygen tension was measured in four ducks.

$Pt_{O_2}$, where appropriate, and CF were measured during 2-min periods of apneic asphyxia before and after the cardiovascular adjustments were inhibited with atropine sulfate (2.5 mg/kg, BDH Chemicals, Poole, England). The effects of atropine were tested before and after apneic asphyxia by injections of acetylcholine chloride (5–10 mg/kg, BDH). The hypotension and bradycardia normally produced by acetylcholine is abolished by atropine, and the absence of hypotension was considered to indicate the inhibition of cardiovascular adjustments. Each duck was exposed to four to five periods of apneic asphyxia, two to three preatropine, and two postatropine, with a 30-min recovery between each period.

## RESULTS

*NADH change in ducks during forced submergence.* Forced submergence of ducks caused an increase in fluorescence from the cerebral cortex as the dive was prolonged (Fig. 1). At the onset of submergence heart rate decreased rapidly and stabilized, after 30 s, at a rate that was maintained for the duration of the dive (Fig. 1). In the example shown in Fig. 1, mean arterial blood pressure

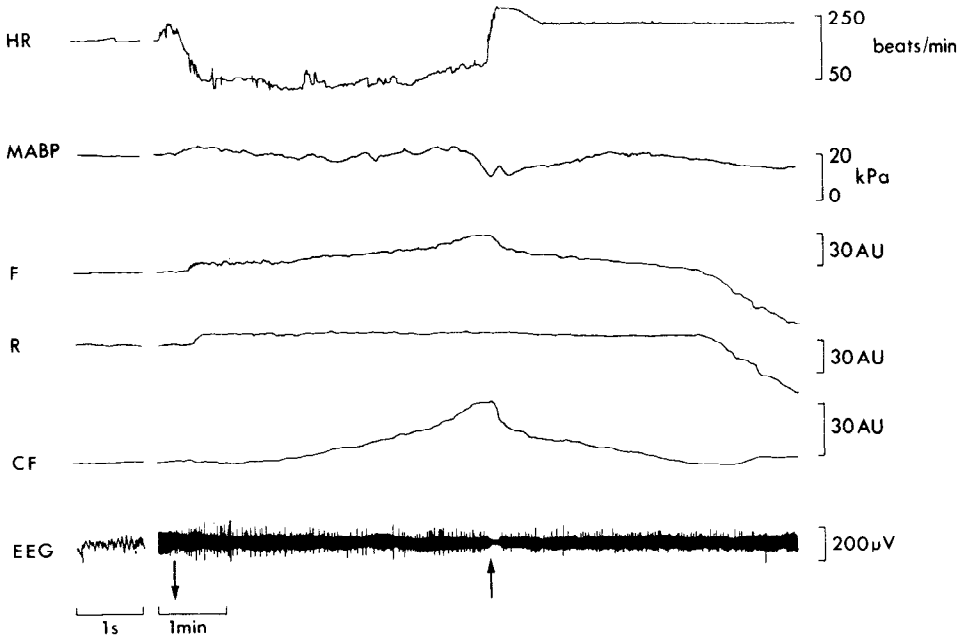


FIG. 1. A 4.7-min period of submergence asphyxia and recovery in a nonparalyzed restrained duck. *Downward pointing arrow* indicates submersion and *upward pointing arrow* indicates emersion. An upward deflection of optical traces represents an increase in light intensity and optical traces are expressed in arbitrary units (AU) where the CF change from normoxia to anoxia was defined as 100 AU. Time bars 1 s and 1 min, respectively, denote changes in chart speed. Abbreviations: HR, heart rate; MABP, mean arterial blood pressure; kPa, kilopascals; F, fluorescence; R, reflectance; CF, corrected fluorescence; EEG, electroencephalogram; μV, microvolts.

(MABP) was maintained near the predive level until 4.5 min of the 4.7-min dive. At the end of the dive, MABP fell precipitously. The third, fourth, and fifth traces from the top describe the optical signals. In all cases an increase in light detected by the photomultiplier is indicated by an upward deflection of the pen. Abrupt changes in F and R soon after submergence and during the recovery are artifacts most probably caused by slight movements of the cortex. The electronic subtraction of F−R gave the corrected fluorescence (CF) trace that was free of these artifacts and due solely to NADH. About 80 s after submersion, CF began to increase and gradually increased to 5 AU after 2 min. NADH fluorescence continued to increase relatively slowly until about 4.5 min after submersion; however, when the blood pressure dropped markedly, a slightly sharper increase in CF occurred. EEG activity diminished (Fig. 1) when CF was approximately 32 AU. In ducks that did not show a precipitous drop in blood pressure, the EEG diminished more gradually, but isoelectricity still occurred when florescence had increased from between 28 and 40 AU (34 ± 2.13 AU) in all restrained ducks.

On emersion, heart rate rose above the predive rate after several seconds and was followed by a gradual return to the predive level. Blood pressure also increased. A few seconds after emersion, corrected fluorescence decreased sharply and was followed by a more gradual decrease over 3 min, briefly dipping below the base line, before returning to the predive level. Some other ducks showed an immediate base-line overshoot, omitting the slower oxidation shown in Fig. 1.

In experiments on restrained ducks, movement artifacts added greatly to our difficulties when attempting to record NADH fluorescence. In many cases the movement was too great to be compensated adequately by the reflectance trace (R). Furthermore, it was technically difficult to submerge the ducks bill, nares, and eyes in water while recording NADH fluorescence from the cerebral cortex. In an attempt to minimize these movement artifacts and allow for easier fluorescence recordings, we investigated NADH fluorescence and cardiovascular adjustments in ducks before and after paralysis with Flaxedil. The nonparalyzed ducks were submerged in water; apneic asphyxia was produced in paralyzed animals by stopping artificial ventilation.

Heart rate, MABP, and corrected fluorescence in paralyzed and nonparalyzed ducks during the first 2 min of asphyxia and 40 s of recovery are compared in Fig. 2. Because the length of the asphyxic periods in both paralyzed and nonparalyzed ducks varied, the recovery phase in Fig. 2 starts from termination of asphyxia. Mean preasphyxic heart rate for paralyzed and nonparalyzed ducks was 255 ± 32.04 beats/min (55 periods of asphyxia in 20 ducks) and 247 ± 13.05 beats/min (11 periods of asphyxia in 6 ducks), respectively. During the 1 min of asphyxia, mean heart rate fell to 44 and 23% of the control rate in paralyzed and nonparalyzed ducks, respectively, and remained relatively stable until termination of asphyxia. Although mean heart rate in nonparalyzed ducks was lower during asphyxia, only at 60 s was the difference between paralyzed and nonparalyzed ducks significant. Nonparalyzed ducks characteristically showed a tachycardia almost immediately after emersion; heart rate increased from less than 50 beats/min to 376 ± 24.00 beats/min after 10 s and gradually returned to preasphyxic levels during the next 40 s. When ventilation was resumed in paralyzed ducks, heart rate returned to the preasphyxic level after 90–120 s without a tachycardia. Before asphyxia MABP was 20.1 ± 1.040 kPa (151 ± 7.8 mmHg) and 17.8 ± 0.660 kPa (134 ± 4.5 mmHg) in nonparalyzed and paralyzed ducks, respectively, and showed only minor changes during and after asphyxia.

An increase of CF occurred in both paralyzed and nonparalyzed ducks during asphyxia. Moreover, the rates of increase were similar and nearly constant between sampling intervals. After 120 s of asphyxia, CF increased by 15 ± 1.94 AU and 18.5 ± 6.35 AU in paralyzed and nonparalyzed ducks, respectively. When asphyxia was

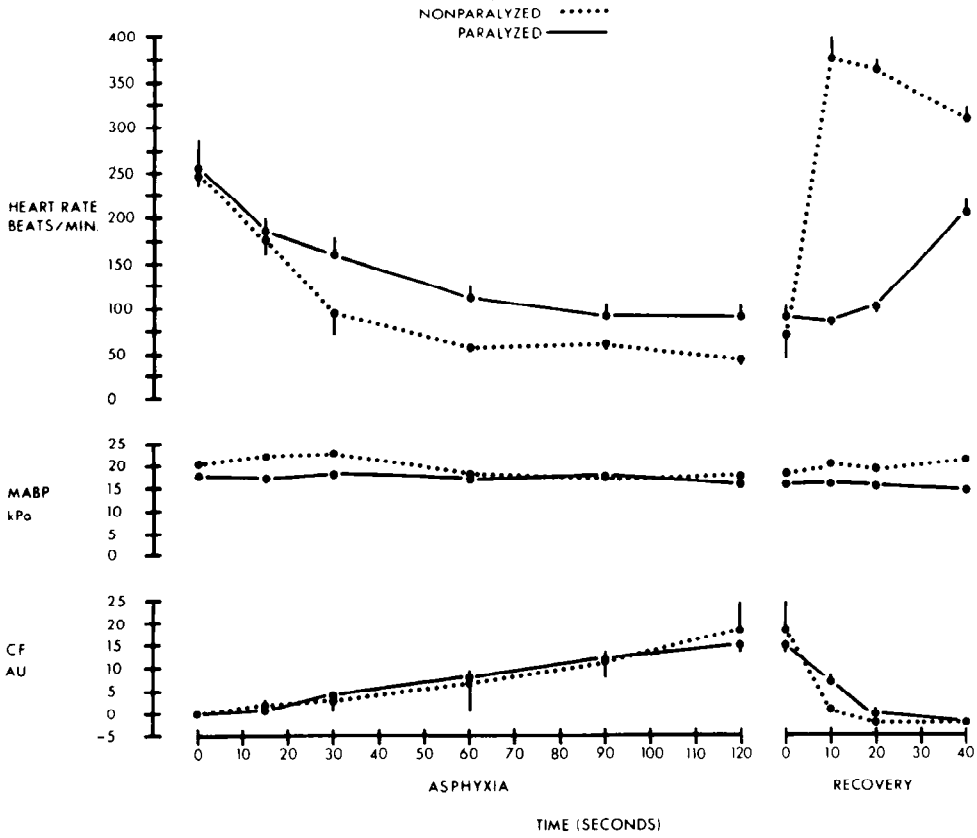


FIG. 2. Comparison between heart rate (HR), mean arterial blood pressure (MABP), and corrected fluorescence (CF) during 11 periods of apneic asphyxia in 6 nonparalyzed ducks (*dotted line*) and 55 periods of apneic asphyxia in 20 paralyzed ducks (*solid line*). Asphyxia was produced by submerging face in water and by stopping artificial ventilation in nonparalyzed and paralyzed ducks, respectively. Elapsed time (*abscissa*) during asphyxia and recovery is given in seconds. CF is expressed in arbitrary units (AU) where CF change from normoxia to anoxia was defined as 100 AU. Each *point* represents mean ± SE and the SE is contained within the point when not shown.

terminated, mean CF decreased and fell below the base line 40 s after asphyxia in both groups. In both paralyzed and nonparalyzed ducks, recovery of CF after asphyxia was always associated with a transitory overshoot of the base line, which generally returned to the preasphyxic level after 5–10 min.

*Inhibition of the cardiovascular adjustments during apneic asphyxia in ducks.* Eight ducks were exposed to 2-min periods of apneic asphyxia before and after the cardiovascular adjustments to apneic asphyxia were inhibited with atropine. Over 75% of the hypertension and bradycardia produced by injection of acetylcholine in untreated ducks was abolished by atropine. Table 1 shows the increase in eight ducks after 60 and 120 s of apneic asphyxia before and after atropine treatment. In every duck, CF was greater after 120 s of asphyxia following treatment with atropine than in asphyxia before atropine treatment and the difference between means was significant. Note that CF was similar in the normal (8 ± 2.29 AU, $n = 8$) and atropinized (12 ± 2.90 AU, $n = 8$) ducks after 1 min of asphyxia and only in the 2nd min were the effects of cardiovascular adjustments in maintaining a more oxidized respiratory chain apparent. Figure 3 shows $Pt_{O_2}$ and CF traces during apneic asphyxia before (C) and after (A) an atropine injection. After 2 min of asphyxia $Pt_{O_2}$ decreased by 61% and CF increased 25 AU before atropine injection while, after atropine, $Pt_{O_2}$ decreased by 72% and CF increased 44 AU in the same period.

TABLE 1. *Increase in CF in paralyzed ducks after 60 and 120 s of apneic asphyxia before and after atropine treatment*

| Duck No. | Corrected Fluorescence | | | |
|---|---|---|---|---|
| | Apneic asphyxia before atropine treatment | | Apneic asphyxia after atropine treatment | |
| | 60 s | 120 s | 60 s | 120 s |
| 1 | 8 (2) | 39 (2) | 8 (2) | 47 (2) |
| 2 | 3 (1) | 6 (1) | 1 (2) | 16 (2) |
| 3 | 5 (3) | 23 (3) | 11 (2) | 44 (2) |
| 4 | 4 (2) | 19 (2) | 10 (2) | 36 (2) |
| 5 | 8 (3) | 21 (3) | 8 (2) | 43 (2) |
| 6 | 18 (3) | 23 (3) | 23 (2) | 50 (2) |
| 7 | 19 (2) | 31 (2) | 25 (2) | 45 (2) |
| 8 | 3 (2) | 29 (2) | 7 (2) | 32 (2) |
| Mean ± SE | 8 ± 2.29 | 23 ± 3.39 | 12 ± 2.90 | 39 ± 3.89 |

CF, corrected fluorescence. Nos. in parentheses are the nos. of asphyxic periods to which each duck was subjected. Mean ± SE AU are given at the bottom of the table.

## DISCUSSION

Apneic asphyxia in restrained ducks was characterized by a reduction of NAD after 1–2 min. The critical fluorescence increase above which EEG ceased in restrained ducks (34 AU) was similar to that previously reported in paralyzed ducks (38 AU) and chickens (34 AU) during apneic asphyxia (5). When asphyxia was terminated by removing the ducks head from water, NADH was reoxidized and eventually stabilized at the preasphyxic level.

Apneic asphyxia produced in paralyzed ducks by stopping unidirectional ventilation simulates the onset of cardiovascular adjustments and changes in respiratory chain NADH in nonparalyzed restrained ducks during forced submersion. Although bradycardia during as-

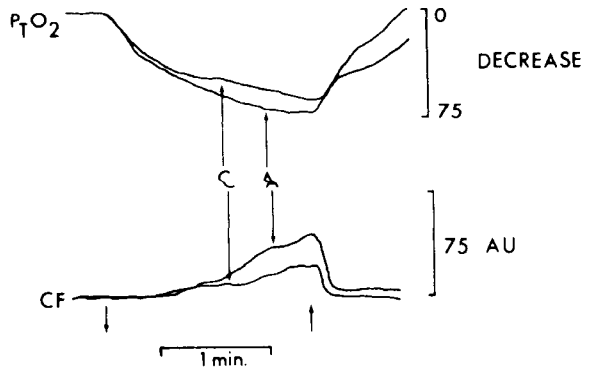


FIG. 3. $Po_2$ of right cortical surface ($Pt_{O_2}$) and corrected fluorescence (CF) from left cortical surface during 2 min of apneic asphyxia (indicated by *arrows*) in a paralyzed duck before (C) and after an atropine injection (A). $Pt_{O_2}$ is expressed as a percent decrease of electrode current when decrease from normoxia to anoxia (death) was defined as a 100% decrease. CF was expressed in arbitrary units (AU) when CF change from normoxia to anoxia was defined as 100 AU.

phyxia in paralyzed ducks was not severe as in nonparalyzed ducks, there were no significant differences between the two groups except at the 60-s sampling interval. The major difference was found in the recovery period where the paralyzed group lacked the typical postasphyxic tachycardia; instead heart rate gradually returned to the preasphyxic rate over a period of 90–120 s. Bamford and Jones (2) reported similar results for paralyzed and nonparalyzed ducks and later claimed it is caused by the absence of rhythmic neural input from the lungs in unidirectionally ventilated animals (3). NADH fluorescence in both groups gradually increased during the first 2 min of asphyxia and returned to the base line after a transitory overshoot when ventilation was resumed. The NADH fluorescence was almost identical in both groups during the entire cycle. Only 10 s after the termination of asphyxia did NADH fluorescence differ significantly due to a faster recovery rate in the nonparalyzed animals.

The importance of the cardiovascular adjustments for protecting the brain during apneic asphyxia was demonstrated by monitoring NADH fluorescence and brain $Pt_{O_2}$ during apneic asphyxia in ducks before and after the cardiovascular adjustments were inhibited with atropine. Apneic asphyxia following inhibition of the cardiovascular adjustments produced a greater reduction of NAD and a greater decrease in brain $Pt_{O_2}$ than before inhibition. However, the effects of the cardiovascular adjustments on CF and $Pt_{O_2}$ were not apparent during the 1st min of asphyxia. For example, after 1 min of asphyxia CF was 12 AU and 8 AU in atropinized and nonatropinized ducks, respectively (Table 1 and Fig. 3). This result was expected even in nonatropinized ducks, since the cardiovascular adjustments were not complete until 40–60 s after artificial ventilation was stopped. Only in the 2nd min, when bradycardia was maximal, did large differences in $Pt_{O_2}$ between the periods of asphyxia before and after atropine injections become apparent. Furthermore, the relationship between NADH fluorescence and $Pt_{O_2}$ is such that very little change in NADH fluorescence occurs until $Pt_{O_2}$ has decreased by 50% (5). After $Pt_{O_2}$ has decreased by 50%, small changes in tissue oxygenation caused large increases in CF. Since $Pt_{O_2}$ did not fall below 50% in either the atropinized or nonatropinized animals during the 1st min of asphyxia, a large difference in CF would not be expected.

$Po_2$ of the brain, and thus the redox state of respiratory chain, NAD, is dependent not only on arterial $Po_2$, but also the rate at which blood perfuses the brain. Published reports indicate that there is not a general consensus as to cerebral blood flow (CBF) changes during diving (4, 10, 11, 13, 18, 19, 29), however it does appear quite certain that in ducks CBF increases markedly (18, 19). In fact, Jones et al. (19) found that CBF increases in ducks as the dive is prolonged and reaches a level of about eight times the predive flow rate after 2–3 min submergence. The exact mechanisms that alter CBF are controversial and are not well understood. Although it has been known for some time that local mechanisms predominate in regulation of CBF, recent studies indicate that neural control through cholinergic fibers may also contribute to vasodilation (24, 26). The administration of atropine in the present experiments would undoubtedly affect the functioning of the cholinergic fibers. However, the local mechanisms that respond to hypoxia, hypercapnia, and a fall in pH (either from hypercapnia or lactate) are still functional and should produce the necessary vasodilation to increase CBF and thus oxygen delivery.

The nervous system of birds, whether in divers or nondivers, appears to be equally dependent on oxidative metabolism for its survival (5). Therefore, birds such as the duck that can withstand extended periods of apneic asphyxia must ensure that oxygen supply to the brain is maintained throughout the duration of a dive. The present study shows that oxygen conservation through cardiovascular adjustments during diving plays a key role in prolonging the apneic survival time.

Present address of R. M. Bryan, Jr.: Dept. of Physiology, Duke University Medical Center, Durham, NC 27710.

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