The effect of the stress of forcible submergence on the diving response in muskrats (*Ondatra zibethica*)

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The effect of the stress of forced submergence in the laboratory on a number of cardiovascular adjustments to diving was examined. Free muskrats, diving in large holding tanks, always displayed bradycardia and heart rate fell from a predive rate of 320 ± 6 to 34 ± 3 beats · min^{-1} after 20 s submergence. In the laboratory this degree of bradycardia was only seen during whole-body submergence of anesthetized animals; restrained unanesthetized muskrats showed significantly less bradycardia. Forced submergence of only the head caused about half the bradycardia seen during whole-body submergence in both unanesthetized restrained and anesthetized muskrats. Decerebrated animals gave identical responses to those of anesthetized muskrats. Cardiac output was redistributed in forced diving to favour the heart and brain even when diving heart rate was three times that in free dives. However, many other tissues still received a significant proportion of cardiac output and must have placed a drain on the blood oxygen store which restricted apnoeic tolerance. Struggling during forced submergence was frequent and has a reflex or unconscious component since even decerebrated animals struggled during a dive. The present data suggest that the additional stresses imposed on muskrats during forcible submergence in the laboratory reduce rather than potentiate the cardiovascular adjustments to diving.

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L'effet du stress entraîné par la submersion forcée sur quelques ajustements cardio-vasculaires durant la plongée a été étudié en laboratoire. A chaque plongée, des rats musqués libres dans de grands réservoirs subissent toujours une bradycardie et le rythme cardiaque passe de 320 ± 6 battements/m avant la plongée à 34 ± 3 après 20 s de submersion. En laboratoire, la bradycardie n'atteint ce degré que chez les animaux anesthésiés entièrement immergés; des rats musqués non anesthésiés gardés en captivité subissent des bradycardies significativement moins fortes. La submersion forcée de la tête seulement cause une bradycardie environ deux fois moins importante que dans le cas d'une immersion totale, aussi bien chez les animaux non anesthésiés captifs que chez les rats musqués anesthésiés. Les animaux décérébrés ont les mêmes réactions que les rats musqués anesthésiés. Au cours d'une plongée forcée, le débit cardiaque est réparti de façon à favoriser le cœur et le cerveau, même si le débit cardiaque atteint trois fois la valeur enregistrée au cours d'une plongée volontaire. Cependant, plusieurs autres tissus reçoivent une proportion importante du débit cardiaque, ce qui utilise beaucoup de l'oxygène emmagasiné dans le sang et diminue par conséquent la tolérance à l'apnée. Durant la submersion forcée, en général l'animal lutte et cette lutte est de nature réflexe ou inconsciente puisque même les animaux décérébrés luttent pendant la plongée. Ces résultats semblent démontrer que le stress supplémentaire occasionné par une submersion forcée en laboratoire diminue les ajustements cardio-vasculaires de la plongée plutôt que de les augmenter.

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Introduction

During forced submergence aquatic mammals display a series of cardiovascular and respiratory adjustments that are collectively described by the term the diving response. Cessation of breathing is accompanied by intense bradycardia and an increase in peripheral resistance. The circulatory adjustments shunt blood flow away from tissues that can withstand a period of anoxia and deliver blood primarily to the brain, heart, and other tissues that require a continuous supply of oxygen (Scholander 1940). In this way the animal's oxygen demand is reduced and underwater survival time, the time until oxygen stores are functionally exhausted, is extended in comparison with survival times of non-aquatic mammals.

The time course for full development of the diving reflexes is very different in different species. In ducks, bradycardia develops rather slowly and full cardiac retardation may not occur for 60 s, whereas seals and muskrats show extremely rapid development of bradycardia, full bradycardia developing within 1 s of forced submergence (Dykes 1974; Drummond and Jones

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1979). It has been observed in both birds and mammals diving voluntarily that a somewhat different pattern of development of diving responses may occur. In some short voluntary dives seals exhibit tachycardia, not bradycardia (Jones *et al.* 1973), and on short voluntary dives in birds maximum diving bradycardia is often obtained immediately upon submergence. Heart rate then increases during the rest of the dive (Butler and Woakes 1979). The discrepancy between diving responses as expressed in forced and voluntary dives has led to the view that a psychogenic reaction (fear) is an important component of the forced-diving response (Gaunt and Gans 1969; Smith *et al.* 1974; Smith and Sweet 1980; Kanwisher *et al.* 1981), although many years ago it was shown that pain, abrupt noise, or threatening gestures elicited bradycardia (Scholander 1940; Irving *et al.* 1942; Elsner *et al.* 1966). If the diving response is a specialized visceromotor "defense–alarm" reaction (Folkow *et al.* 1965) then extreme cardiovascular adjustments would be made, and be appropriate, as part of an "escape" or "flight" reaction. Consequently, it is possible that diving bradycardia, as recorded in enforced submergence, has a component which is related more to the stress of restraint than to being underwater *per se*.

The objective of the present research was to measure the effect of the additional stress of forcible submergence on a number of cardiovascular variables during diving. To do this we compared heart rates obtained during natural unrestrained dives with those obtained from forced dives in the laboratory on unanesthetized, anesthetized, and decerebrated restrained muskrats. Furthermore, we made recordings of electroencephalogram (EEG), heart rate, and blood pressure during a dive by anesthetized or decerebrated, restrained muskrats which we compared with similar data from unanesthetized animals. As there can be no perceived stress under anesthesia or after decerebration we felt that this comparison would yield unequivocal data with regard to the stress of forcible submergence. The maximum underwater survival time was taken as the period between the start of the dive and when the EEG became isoelectric (Bryan and Jones 1980*a*, 1980*b*). This is a much better measure of apnoeic tolerance than time to last heart beat (Blix and Øristsland 1970) or last struggle (Andersen 1966) because not only does it have biological validity but also it is not terminal since animals can be resuscitated by giving artificial ventilation. Finally we attempted to see if the extent of bradycardia was an adequate measure of the total cardiovascular response to diving by measuring regional distribution of blood flow in muskrats in which much of the bradycardia was prevented by using sodium pentobarbital as an anaesthetic.

Methods

The experiments were done on 43 adult muskrats (*Ondatra zibethica*); 32 muskrats were trapped in Delta, B.C., and the others were taken in the vicinity of Winnipeg, Manitoba, and shipped air freight to Vancouver, B.C. The average weight of the muskrats was 0.78 ± 0.08 kg (SE). Muskrats were kept in pairs at the Vivarium of the University of British Columbia in holding tanks which allowed the animals to move freely in and out of water. They were fed daily with carrots, apples, or parsnips supplemented with high protein cat chow. Animals were instrumented before the experiments under halothane anesthesia (2% Fluothane, Ayerst, Montreal, P.Q.). In the majority of experiments cannulae and electrodes were chronically implanted and brought to a miniature connector which was fixed to the skull with dental cement (Yates Acrylic, Yates Co., Chicago, IL, U.S.A.). The frontal and parietal bones were exposed by a midline incision in the skin after the fur was first shaved from the top of the head.

The electrocardiogram (EKG) was recorded between electrodes located in the tail and near the heart. Two thin insulated wires were threaded from the skull incision, under the skin, until the noninsulated tips were positioned at the base of the tail and near the heart respectively. The wires were connected to the female side of the miniature connector. An incision was made in the skin to expose the femoral artery and a polyethylene cannula (PE 90; Clay–Adams, Parsippany, NJ, U.S.A.) was inserted into the artery to record arterial blood pressure. The cannula was fed under the skin to the incision over the skull and sealed with a metal plug, after first being filled with heparinized saline (40 IU · mL^{-1}).

The electroencephalogram (EEG) was recorded from stainless steel screw electrodes fixed to the parietals 3 mm caudal to the coronal suture and 1 mm lateral to the sagittal suture. Two burr holes were made in the skull bones using a dental drill, and the tips of the electrodes were sealed into the skull with dental cement. Wires from the stainless steel screws were attached to the head connector. In the early experiments four EEG electrodes were implanted, two on each side, so that the EEG pattern could be obtained either unilaterally or bilaterally. In subsequent experiments, it was apparent that no rhythms could be resolved from the EEG which allowed us to evaluate change in the animal's state so only two electrodes were implanted.

Thalamic decerebration was performed under halothane anesthesia. The skull was exposed and two holes, 10 × 5 mm, were cut longitudinally on either side of the sagittal suture. The cerebral hemispheres were removed to a high thalamic level using suction and cautery. After decerebration the animals were allowed from 6 to 12 h to recover. Only heart rates were monitored from decerebrates. The electrodes were inserted just before the start of the experiment, being located just under the skin of the chest and the tail.

In free-diving experiments, five muskrats, instrumented to give EKG, were placed in large holding tanks, 4 m diameter and 2 m deep, filled with water 1 m deep. A platform (1 × 1 m square) was floated on the water surface and the animals could move freely from water to "land." The male side of the connector fixed to the head was pushed home and the connector covered with silicone adhesive. A long thin insulated wire trailed from the animal to the recording apparatus.

The signal was amplified conventionally and displayed on a two-channel pen recorder along with an event marker that was activated to indicate submergence of the animal. One of us observed the animals at all times and pressed the event marker when they submerged. It appeared that the majority of the dives occurred in response to disturbances in the animal's surroundings and it is probably more appropriate to call them "free" rather than voluntary dives. To compare the performance in free and restrained dives these animals were taken into the laboratory and heart rate was recorded during restrained diving. Each animal was placed in a sieve-like Perspex box which acted as a restraint. To completely submerge the muskrat a box full of water was raised from beneath the animal box. In these experiments the dive time was 20 s, which was slightly longer than mean dive time recorded for animals in free dives (17.5 s). Dives were repeated on these animals after anesthetization with urethane ($1.25\ g \cdot kg^{-1}$, i.p.).

Total submergence was also used when studying regional distribution of blood flow during diving. Animals were surgically anesthetized (sodium pentobarbital, $50\ mg \cdot kg^{-1}$, i.p.). The right carotid artery was exposed through an incision in the skin of the ventral surface of the neck. The artery was ligated distal to the heart and clamped proximally. A polyethylene cannula was inserted through an incision in the wall of the artery and, after removing the proximal clamp, advanced until its tip lay in the left ventricle. Placement was determined by a simultaneous pressure recording. The cannula was sewn in place and the wound closed in layers. The muskrat was placed in the sieve-like box and allowed to recover to a light plane of anesthesia. Blood flow distribution was investigated using 15 ± 5 μm diameter microspheres labelled with ^{141}Ce or ^{85}Sr (3M Company, Nuclear Products Div., St. Paul, MN, U.S.A.). One label was injected before a dive and the other after 60–90 s submergence. Approximately $2 \times 10^5 \pm 10^3$, ^{85}Sr-labelled spheres and $2 \times 10^5 \pm 10^3$, ^{141}Ce-labelled spheres were injected in a carrier volume of 1.0 mL. The techniques used and precautions taken with the microspheres have previously been described in detail (Jones *et al.* 1979). However, one difference between this and the earlier study was that no attempt was made to monitor cardiac output, so blood flow distribution to each tissue (counted in their entirety) was expressed as a percentage of cardiac output derived as follows:

$$\%\ \text{cardiac output to the tissue} = \frac{\text{radioactivity of tissue (counts} \cdot \text{min}^{-1}) \times 100}{\text{total radioactivity injected (counts} \cdot \text{min}^{-1})}$$

In all other experiments the dive was effected by submerging only the head of the animal. The animal was strapped to a board, pivoted at its center, using ties around each limb. The head was restrained in a modified stereotaxic head holder attached to the board. Tipping the board down submerged the head. A shielded lead was attached to the connector on the animal's head and EEG and EKG signals were amplified conventionally and displayed on an oscilloscope. Blood pressure was monitored using a Bio-Tec BT70 pressure transducer. All signals were stored on magnetic tape (HP 3907, Hewlett-Packard, Waltham, MA, U.S.A.) for later analysis using a PDP Lab 8/e computer (Digital Equipment Corp., Maynard, MA, U.S.A.) with conventional software.

The responses of decerebrated, anesthetized (urethane, $1\ g \cdot kg^{-1}$, i.p.), and unanesthetized muskrats to 90 s submergence were investigated, each animal being allowed 60 min between dives. In an attempt to assess the maximum underwater survival time, two anesthetized and four unanesthetized muskrats were subjected to prolonged dives until the EEG became isoelectric.

Statistical analysis of data

In the text and figures numerical values, when referring to determinations of variables in a group of animals, are given as means ±SE of n observations on N animals. Data from the various groups, in each series of experiments, were compared at each sampling time using a one-way analysis of variance (ONEWAY, SPSS; Nie *et al.* 1975). Comparisons of data within a group were conducted using a two-factor analysis with repeated measures over time (ANOVAR, SPSS). In the case of significant F values ($P < 0.05$), pair-wise comparisons of means were done with either Scheffe's method (Scheffe 1959) or the LSD test (Snedecor and Cochran 1967). The significance of changes in regional distribution of cardiac output was assessed using a paired t-test and $P < 0.05$ was taken as the fiducial limit of significance.

Results

(a) A comparison between heart rate during free and restrained whole-body submergence

Heart rate of five animals held in large outdoor tanks averaged 320 ± 6 beats $\cdot$ min^{-1} ($n = 15$) and fell to 57 ± 7 beats $\cdot$ min^{-1} ($n = 11$) 1–2 s after submergence (Fig. 1).

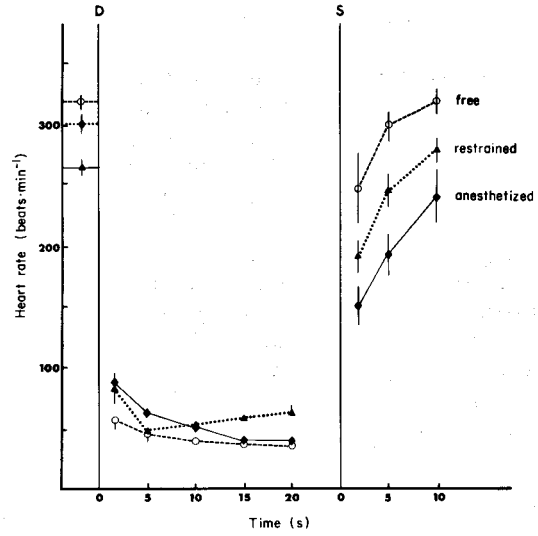

FIG. 1. Heart rates recorded during dives from free unanesthetized, restrained unanesthetized, and anesthetized muskrats. Vertical bars indicate standard error. The absence of a bar indicates that the limits of the standard error fall within the symbol. D indicates the start of the dive; S indicates surfacing.

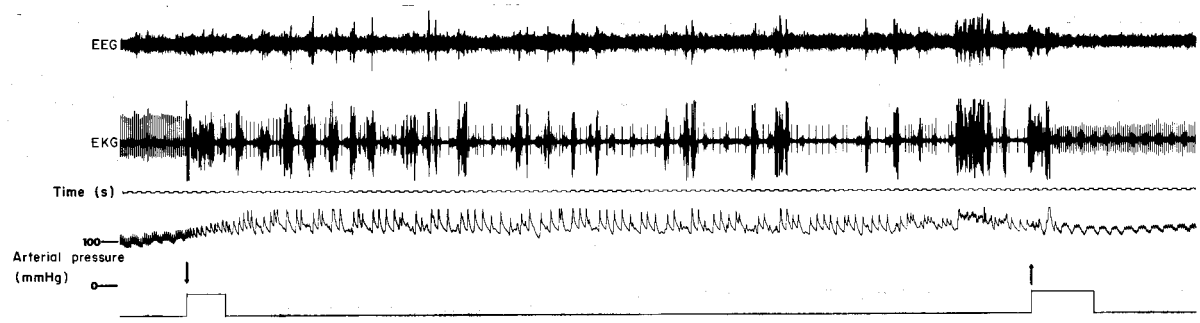

FIG. 2. A 90-s dive in a restrained unanesthetized muskrat showing increase in blood pressure. Top trace, electroencephalogram; middle trace, electrocardiogram; lower trace, arterial blood pressure. Downward pointing arrow indicates submergence, upward pointing arrow, emergence. Signal artefacts on the EEG and EKG traces indicate struggling. Time marker is in seconds.

Heart rate continued to decline as submergence was prolonged and fell to 34 ± 3 beats·min^{-1} (n = 14) at 20 s. All animals that remained submerged for periods in excess of 5 s showed this trend of increasing bradycardia throughout the dive. Breathing started immediately on emergence and heart rate increased rapidly, returning to predive levels after 10 s (Fig. 1). Mean heart rate of five restrained muskrats in the laboratory (266 ± 3 beats·min^{-1}) was significantly lower than in unrestrained and restrained anesthetized animals. Nostril closure and apnea occurred on submergence accompanied by prominent bradycardia, heart rate falling to 83 ± 11 beats·min^{-1} after 1 s underwater. The lowest rate of 47 ± 3 beats·min^{-1} (n = 15) was reached after 5 s submergence and then rose, being significantly above that for both free and anesthetized muskrats after 15 s submergence (Fig. 1). On emergence heart rate increased markedly with the first breath and the predive rate was reached after 10 s; there was no postdive tachycardia. After anesthesia these animals were submerged again. Heart rate fell from 302 ± 8 to 87.5 beats·min^{-1} (n = 15) after 1 s and continued to decline to a rate of 39 ± 3 beats·min^{-1} (n = 15) after 20 s of submergence (Fig. 1). This rate was significantly below that in unanesthetized muskrats but not different from that seen in free dives (Fig. 1). As a rule, respiratory movements continued throughout submergence of anesthetized animals although water was not drawn into the lungs.

(*b*) *Heart rate, blood pressure, EEG changes, and behavioural responses to submergence of the head*

These experiments were performed on 12 animals although not all animals were instrumented to give records of each variable. Heart rates were recorded from unanesthetized, anesthetized, and decerebrated restrained muskrats. The predive rate in all animals was in the range of 300 beats·min^{-1} and fell rapidly on submergence. After 20 s submergence, heart rates in both anesthetized and unanesthetized restrained muskrats were significantly above those obtained during whole-body submergence (section *a*). During 90 s submergence heart rate fell to 85 ± 17 beats·min^{-1} (n = 6) in unanesthetized animals and was not significantly different from rates observed in anesthetized (71.5 ± 22 beats·min^{-1}, n = 6) or decerebrated (67 ± 9 beats·min^{-1}, n = 6) animals. Heart rate returned to the predive level immediately after surfacing in unanesthetized muskrats, whereas complete recovery took 10 to 20 s in anesthetized and decerebrated animals. In both unanesthetized and anesthetized animals mean arterial blood pressure rose significantly during diving and after 90 s submergence was 18 ± 3 mmHg (1 mmHg = 133.322 Pa) above the predive level (Fig. 2). In both groups of animals mean blood pressure remained elevated throughout the initial part of the recovery period (Fig. 2).

All animals struggled during submergence and these struggles were recorded as signal artefacts on either or both the EEG and EKG traces. When the number of struggles occurring in each 10 s of the dive were counted and averaged no pattern emerged. Bouts of struggling appeared to have little affect on arterial blood pressure (Fig. 2). Anesthetized animals made two or three struggles per 10-s interval, which was somewhat higher than the struggling frequency exhibited by unanesthetized muskrats. On some occasions the former did not become apneic during a dive, pumping water in and out of their nostrils, and some signal artefacts noted as struggles may, in fact, have been these "false" breathing movements (Drummond and Jones 1979). Decerebrated muskrats struggled 1.5 times·10 s^{-1} on average, about half the frequency of unanesthetized or anesthetized animals.

Dives were prolonged in two anesthetized and four unanesthetized muskrats, being terminated when the EEG became isoelectric; the time to isoelectric EEG was between 3.5 and 4 min. As submergence was prolonged, struggling frequency tended to diminish, and by the time EEG amplitude had declined significantly, struggling

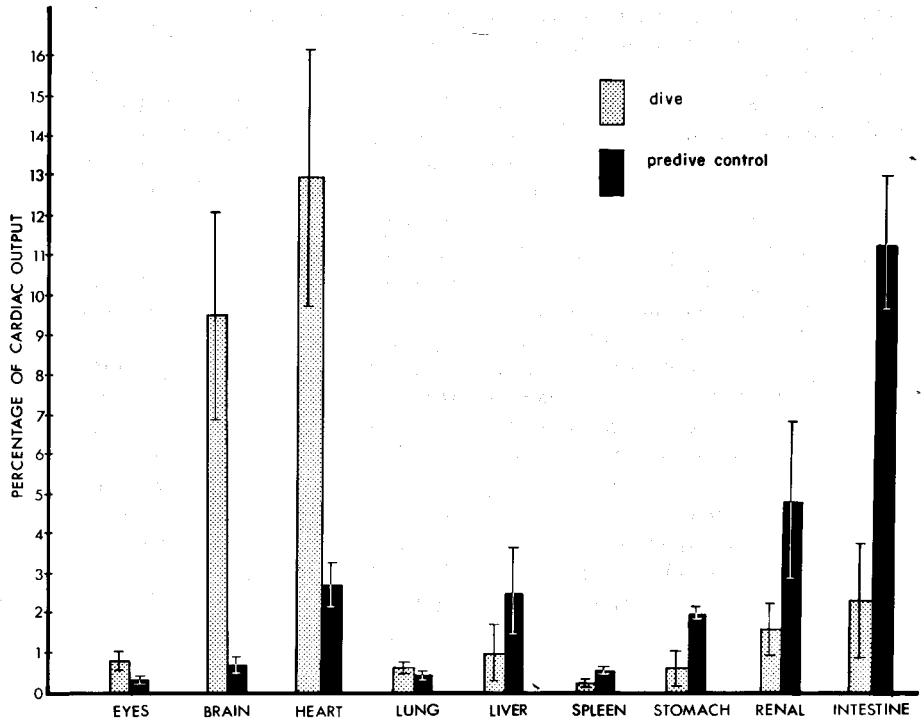

FIG. 3. Percentage of cardiac output going to various organs and tissues before and during diving in anesthetized muskrats. Bars indicate standard errors of the mean for observations on seven animals ($n = 7$ except for the eyes and spleen where $n = 3$, and lungs and liver, where $n = 4$).

had virtually ceased. Diving bradycardia was maintained throughout the period underwater. The heart continued to beat after the EEG was isoelectric and animals were resuscitated by giving artificial ventilation.

(c) *Regional distribution of blood flow during diving in muskrats anesthetized with sodium pentobarbital*

The underwater survival time of anesthetized or unanesthetized muskrats in the above experiments was about one-third of what is often quoted for the muskrat (Irving 1939; Drummond 1980). Bradycardia was much reduced compared with that obtained in whole-body submergence yet the increase in mean arterial blood pressure suggested some peripheral vasoconstriction was occurring. Consequently, we wanted to know the extent of the cardiovascular adjustments other than bradycardia in forced dives. Regional distribution of blood flow was studied in whole-body submergence of muskrats anesthetized with sodium pentobarbital. The choice of diving and anesthetic techniques was dictated by the need to obtain far more consistent heart rate responses during dives than was obtained by submerging only the head of muskrats anesthetized with urethane. Predive heart rates were in the range of 295 ± 10 beats·min^{-1} (n, $N = 7$) and fell rapidly to 112 ± 12 beats·min^{-1} during submergence. On average, a slight increase in heart rate (about 20 beats·min^{-1}) occurred soon after injection of the microspheres (at 90–120 s) and this faster rate was maintained for the rest of the dive, which was usually terminated between 3 and 3.5 min. The percentage of cardiac output going to a range of tissues (Fig. 3) changed dramatically during the dive. The proportion of cardiac output going to the brain and heart significantly increased. The brain's share increased by 15 times and the heart's by nearly 5 times, as compared with predive values (Fig. 3). Except for the lung, the proportion of cardiac output going to all other tissues fell markedly, but fell significantly from predive conditions only in the case of the intestine and stomach (Fig. 3). In six animals the proportion of cardiac output going to the left kidney was plotted against that going to the right as an indication of whether the spheres were evenly mixed in the circulation. A good one to one correspondence was obtained both before and during diving.

Discussion

The prominent cardiovascular features of the diving response in muskrats (bradycardia and hypertension) were qualitatively similar in all animals studied. Quantitatively, it seemed that simulating dives by submerging only the head caused about half the bradycardia seen with whole-body immersion in both unanesthetized and

anesthetized muskrats. Nevertheless, what is clear is that the cardiovascular responses to diving shown by the muskrat in the laboratory are reduced, not potentiated. In other words, any "fear" component is adverse with respect to underwater survival. This conclusion seems confirmed by the fact that the heart rate responses to diving in anesthetized and decerebrated animals were similar to each other and, in the case of wholebody submergence, significantly below rates in forced submergence of unanesthetized animals. This argues against the idea of the forced diving response as a "defence" mechanism (Folkow *et al.* 1965) but supports suggestions that the "psychological condition" of the animal before and during diving may have a marked effect on the response. Irving *et al.* (1941) found disposition and nervous state of seals to be more important for full development of responses to forced diving than physical factors, better responses were obtained from relaxed animals. Also, "calm" ducks show more intense diving responses than "alarmed" animals (Folkow *et al.* 1967). Even in man, mental stress or mental activity, in the form of mental arithmetic, prevents most, if not all, of the cardioinhibitory effects of face immersion (Paulev 1969; Wolf *et al.* 1975; Wolf 1978; Ross and Steptoe 1980). However, we are not proposing that the lack of bradycardia shown by seals and ducks in short voluntary dives (Jones *et al.* 1973; Butler and Woakes 1979) bears any relation to effects of mental stress but rather suggest that it represents extinction of the forced-dive type of response by habituation. However, it may not be entirely appropriate to argue our case based on experiments done on an animal, the muskrat, which has never been observed to dive in voluntary dives without bradycardia.

The short underwater endurance encountered in laboratory dives when bradycardia was reduced suggests that blood flow redistribution and consequent oxygen conservation may not have been as extreme as occurs when maximum bradycardia develops. However, the fact that mean arterial blood pressure rose during diving suggests that the proportionate increase in total peripheral resistance was greater than the reduction in cardiac output. Certainly, high cardiac output during diving will contribute to foreshortened underwater endurance since the heart will consume far more oxygen than will be the case when bradycardia is more intense. The proportion of cardiac output going to the heart increased spectacularly during dives but assuming that the decline in heart rate reflects the fall in cardiac output then tissue blood flow per unit mass would only increase by just over two times. It seems more probable that the failure to conserve oxygen was due to the fact that blood supply to such tissues as intestine, kidneys, and skeletal muscle was not completely suspended. In seals and ducks, forced to dive, blood flow to most of these regions is negligible (or, more strictly, too low to be measured accurately by the microsphere technique, Blix *et al.* 1976; Jones *et al.* 1979). Of interest is the extremely large increase in the proportionate share of cardiac output going to the brain, being two to four times greater than in large diving mammals such as seals (Blix *et al.* 1976; Zapol *et al.* 1979). This difference is probably related to the fact that in muskrats we assessed regional redistribution of cardiac output close to half way through maximum dives, whereas in seals, redistribution has been assessed at no more than one-quarter of the way through a maximum dive.

An analysis of the behavioural response to forced submergence, as assessed by the number of struggles made by the animal during a dive, is of considerable interest when looking at the emotional or "psychic" impact of forced diving in the laboratory. Decerebrated animals struggled as violently and at about half the frequency of unanesthetized rats during dives, yet decerebrates can have no conscious conceptualization of their situation. In other words, struggling during forced submergence has a reflex or unconscious component which is provoked by the diving situation and is not a direct conscious response to it. In fact, anesthetized animals appeared to struggle more often than even unanesthetized muskrats in a dive. Furthermore, there was no apparent increase in struggling as the isoelectric EEG was approached in prolonged dives. If nothing else, this limited behavioural observation at least highlights the dangers of applying anthropomorphic interpretations to animal behaviour.

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