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THE INFLUENCE OF BODY MASS ON THE ENDURANCE TO RESTRAINED SUBMERGENCE IN THE PEKIN DUCK

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SUMMARY

Pekin ducks, ranging in mass from 0·05 to 3·5 kg, were force-dived to determine the maximum tolerance to diving asphyxia. The size of the respiratory and blood oxygen storage compartments and oxygen utilization during the dive were also measured. By the end of a maximum dive, less than 4% of the original O_2 store remained in the blood, whereas almost 25% remained in the respiratory system. In contrast, the level of arterial glucose did not change significantly during diving.

The relationship of a number of measured variables to body mass was analysed using linear regression analysis on $\log_{10}$-transformed variables to generate power equations of the form $Y = aX^b$ (Y, any variable; X, body mass; a, mass coefficient; b, mass exponent). The mass exponent was 1·19 for the total oxygen stores and 0·64 for maximum diving duration. Using measurements of brain and heart mass and literature estimates of the scaling of O_2 consumption, it was also possible to predict a mass exponent aerobic metabolism by these organs during a maximum dive. Allometric cancellation of mass exponents for O_2 availability and predicted utilization resulted in a residual mass exponent almost identical to the measured value for maximum dive duration. Thus it is possible to predict the relationship of maximum underwater endurance to body mass in Pekin ducks from a knowledge of the oxygen consumption by, and availability to, the central aerobic organs.

INTRODUCTION

The large capacity for underwater endurance exhibited by diving birds and mammals is a consequence of a series of cardiovascular adjustments to head submersion, the so-called diving response (Scholander, 1940), which conserves oxygen for hypoxia-intolerant tissues, such as the heart and brain, while peripheral organs are essentially eliminated from the central circulation. Although the forced-diving condition evokes a maximal cardiovascular response against the threat of asphyxia, i.e. a large selective vasoconstriction accompanied by a profound bradycardia, it is now apparent that most voluntary dives are aerobic in nature and do not involve anywhere near such extensive cardiovascular adjustment (Kooyman *et al.*

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1980; Kanwisher, Gabrielsen & Kanwisher, 1981; Woakes & Butler, 1983). Still, the success of diving vertebrates must partly reside in the fact that they can survive unusually long periods of apnoeic asphyxia, and that occasionally they must do so when diving under natural conditions (Butler & Jones, 1982). Certainly, when 'trapped' underwater during unrestrained diving, heart rate may fall to levels usually only seen in forced dives (Butler, 1982; Farilla & Jones, 1985). Thus, although the metabolic and physiological responses to forced submersion may not be characteristic of most natural dives, forced diving presents a useful model for understanding natural tolerance limits to asphyxia.

The maximum dive time that can be safely endured by an animal depends on the amount of oxygen stored in the body at the start of submersion, the capacity for mobilization and selective utilization of the O_2 store, and the rate of tissue O_2 consumption. Several studies have shown correlations between the maximum dive length and the time to when available O_2 stores are consumed during both forced and voluntary dives (see Butler & Jones, 1982), which implies that O_2 determines the limits to endurance in these divers, although this idea has been challenged for the Weddell seal (Hochachka, 1981).

As recently pointed out (Butler & Jones, 1982), it is the largest animals that seem to excel in diving performance, whether a comparison is made between diving species or within a single species with respect to growth and development. Hypothetically, if oxygen stores are related to the first power of body mass (M_B), and oxygen uptake is proportional to $M_B^{3/4}$, then dive endurance should scale to $M_B^{1/4}$ (Scholander, 1940; Calder, 1969), i.e. diving endurance should increase with M_B based on allometric considerations of O_2 stores and metabolic rate. Although this hypothesis may help to explain the relationship of diving time to M_B in aerobic, voluntary dives, it should fail to predict the situation during prolonged or forced diving, since the mass relationship of the organs expected to receive most of the stored O_2 (e.g. brain and heart) is not considered. If the main factor limiting endurance to submersion is the relationship of O_2 stores to brain and heart metabolism (Elsner, Shurley, Hammond & Brooks, 1970), then the mass exponent for maximum dive time should be predictable from a knowledge of the mass exponents for centrally available O_2 stores and the rate of O_2 consumption by these 'vital' organs during the dive.

MATERIALS AND METHODS

Experiments were performed on Pekin ducks (*Anas platyrhynchos*), ranging in mass from 0·05 to 3·5 kg and varying in age from 2 days to 3 years. Young ducklings were held in air-conditioned rooms at a temperature of 20–22 °C with free access to an infrared heat source. Adult ducks were maintained in an outdoor enclosure, but were acclimated to room temperature for at least 1 week before an experiment.

Surgical procedures were performed under local anaesthesia (Lidocaine HCl, 2%) and the ducks allowed 2 h recovery before an experiment. All wounds were periodically infiltrated with lidocaine during experiments. Systemic arterial blood pressure (ABP) was recorded from a heparinized (40 i.u. ml^{-1}) polyethylene cannula

that was inserted into the right brachial artery and advanced into the brachiocephalic artery. The cannula was also used to withdraw blood samples for blood gas or glucose analysis. Ducks weighing less than 0·25 kg were cannulated in the left sciatic artery and the cannula advanced into the descending aorta. In some of the ducks, mixed venous blood was sampled from a site near the right atrium *via* a second cannula in the right brachial vein. Heart rate (fH) was determined from either an electrocardiogram (ECG) or the ABP pulses using a rate meter. Brain electrical activity (EEG) was monitored from two stainless steel screws cemented about 0·7 cm apart in the right frontal region of the skull approximately over the sagittal elevation. Core body temperature (T_c) was monitored using a colonic thermistor probe and maintained at 40–41 °C with external heating or conductive cooling as necessary.

Blood gas tensions and pH were determined on iced blood samples using a Radiometer BMS3 blood gas analyser (Radiometer, Copenhagen) calibrated with gas mixtures and buffers of certified composition. All calibrations and measurements were made at T_c. The O_2 content of arterial (Ca_{O_2}) and mixed venous ($C\bar{v}_{O_2}$) blood samples was measured using a fuel cell analyser (Lexington Instruments, Waltham, Mass.). The plasma glucose concentration (C_{glu}) was determined in centrifuged arterial blood samples using an automatic biomedical analyser at a local hospital.

Dive time determination

Before diving experiments, ducks were restrained in a supine position with the wings and legs held near the body with masking tape. Care was taken to avoid undue compression of the respiratory system or hindrance to breathing movements. For diving, the head was lowered into a large water-filled funnel to the level of the posterior border of the eye. The dive was terminated by draining the funnel while raising the head to an erect position. Each duck was subjected to a short introductory dive, before the endurance dive, and birds that exhibited atypical fH, ABP or excessive struggling ($N = 5$) were not used. The maximum diving time (t_d), was obtained in a single dive 1 h later. The dive was stopped when EEG amplitude approached background noise levels or when diving bradycardia broke and fH accelerated to resting levels or above. This stage was often accompanied by marked cardiac arhythmia and erratic fluctuations in ABP, signalling impending cardiovascular collapse.

To verify that no physiological injury was incurred during the t_d determination, a second prolonged dive was done after 1 hour's recovery. We accepted return of fH, ABP, ECG and EEG to pre-dive levels during recovery, together with a diving endurance within 90% of t_d, as evidence that no irreversible physiological impairment had occurred during the original test. In some of the birds, blood samples for gas, pH and glucose analysis were collected before and during this dive.

Blood volume

The total volume of the red cells in the circulation (V_{rc}) and the total blood volume (V_{tb}) were measured in ducks not subjected to prior blood sampling. V_{rc} was

estimated from the vascular dilution of erythrocytes labelled with ^{51}Cr (sodium dichromate, New England Nuclear, Boston, Mass.). Whole blood (3–5 ml), collected from a donor duck, were mixed 5:1 with acid-citrate-dextrose (ACD) and centrifuged at 500 ***g*** for 5 min. The plasma fraction was aspirated and discarded and the cells reconstituted to the original volume with an ACD-^{51}Cr solution containing 5–10 $\mu Ci\,ml^{-1}$ of isotope. The red cells were incubated at 40 °C for 40 min, centrifuged and washed until the total activity in the supernatant was less than 1% of the cell-bound activity. The ^{51}Cr-tagged red cells were diluted to the HCT_v of the recipient duck using saline and 0·25–1·0 ml of labelled cells injected into the dorsal metatarsal vein. The precise volume injected depended upon the size of the duck and was determined by mass difference assuming a specific gravity for blood of $1{\cdot}036\,g^{-1}$.

After 20 min, duplicate blood samples were withdrawn from the arterial cannula and injected into counting tubes containing 2 ml of a saponin-distilled water solution. The precise amount of blood sampled was determined by mass difference and the tubes counted, correcting for background and coincidence, to 1% error, in an automatic gamma scintillation counter. Triplicate 50-μl aliquots of the injected cell mixture were also counted. Haematocrits were determined by a micro-method for avian blood, assuming a value for trapped plasma of 2·12% (Cohen, 1967*a*). V_{rc} was calculated from the ratio of the injected to recovered activity, the sample volume and HCT according to Jones (1970). V_{tb} was calculated from V_{rc} and estimated whole-body HCT, assuming an f-cells ratio of 0·88 (Cohen, 1967*b*). Total O_2 stored in the blood vascular system at the beginning of a dive (V_{tbO_2}) was estimated from V_{tb}, Ca_{O_2} and $C\bar{v}_{O_2}$ assuming that one-third of the V_{tb} was arterialized.

Respiratory volume

Respiratory system volume (V_{rs}) in the 'diving' posture was measured on the day following t_d determination using an inert gas dilution method (Piiper, Pfeifer & Scheid, 1969). The duck's trachea was cannulated under local anaesthesia and a stopcock-syringe assembly was attached to a T-manifold on the distal end of the tracheal cannula. To measure V_{rs}, the open side of the manifold was closed at end-exhalation and a precise volume of argon (the exact volume depended upon the body size) was injected into the respiratory system from the syringe. Gases were mixed by ventilating the duck with the syringe. A gas sample was withdrawn from the system and the various fractional dry gas concentrations measured (F_{O_2}, F_{CO_2}, F_{N_2} and F_{Ar}) using a mass spectrometer (MGA 200, Twentieth Century Electronics, Ltd, Croydon, U.K.). V_{rs} (BTPS) was calculated from argon dilution after correction for the volume introduced, respiratory gas exchange and apparatus dead space. The V_{rs} estimate includes tracheal, bronchial and parabronchial volumes as well as the air sac volumes.

The volume of O_2 actually available during head submersion (V'_{rsO_2}) is the product of pre-dive respiratory system O_2 (V_{rsO_2}) and the amount of O_2 extracted during the dive (E_d). V_{rsO_2} at end-exhalation was calculated according to the equation

$$V_{rsO_2}\,(\text{STPD}) = V_{rs}\,(\text{BTPS}) \times k \times F_{\bar{rs}O_2},$$

where $F_{\bar{rs}O_2}$ is the average fractional dry gas concentration of O_2 in the respiratory system and k is the reduction factor from BTPS to STPD. $F_{\bar{rs}O_2}$ could not be measured directly, but a reasonable approximation was obtained according to the equation:

$$F_{\bar{rs}O_2} = \Sigma(F_{xO_2} \times V_x/V_{rs}),$$

where F_{xO_2} is the fractional O_2 concentration of each respiratory component volume, V_x, of V_{rs}. For this calculation, V_{rs} was subdivided into four major components corresponding to the major air sac volumes (i.e. interclavicular, anterior thoracics, posterior thoracics and abdominals), while minor components such as tracheal, bronchial and parabronchial volumes were ignored. V_x/V_{rs} was calculated for each air sac from values given in the literature (Scheid, Slama & Willmer, 1974) and F_{xO_2} computed from measurements of the minimum exhaled fractional O_2 concentration (FE_{minO_2}) and the empirical relationship between FE_{minO_2} and air sac P_{O_2} reported for the chicken (Piiper, Drees & Scheid, 1970). The average fractional concentration of C_{O_2} ($F_{\bar{rs}CO_2}$) was calculated in a similar manner.

To estimate E_d, $F_{\bar{rs}O_2}$ and $F_{\bar{rs}N_2}$ were measured during several dives using the T-manifold connected to an empty, 100-ml ground-glass syringe and the mass spectrometer. Diving was initiated and the dive variables, $F'_{\bar{rs}O_2}$ and $F'_{\bar{rs}N_2}$, were measured before surfacing by filling the syringe from the respiratory system. This manoeuvre served to pull sequential aliquots of the respiratory gases past the sampling port of the mass spectrometer, which was bypassed during most of the dive to avoid altering the respiratory volume during apnoea. E_d was calculated as:

$$[1-(F'_{\bar{rs}N_2} \times F_{\bar{rs}O_2})/(F_{\bar{rs}N_2} \times F'_{\bar{rs}O_2})]$$

and V'_{rsO_2} estimated for each duck as the product of V_{rs}, $F_{\bar{rs}O_2}$ and E_d.

Mass of brain and heart

A duck was killed at the end of an experiment by intravenous administration of sodium pentabarbital followed by saturated KCl. Total body mass (M_B) was measured by direct weighing on a triple beam balance and brain mass (M_b) measured after decapitation by weighing the skinned cranium before and after brain aspiration to the level of the foramen magnum. The M_b measurement includes an amount of trapped blood and cerebrospinal fluid, but not the weight of the dural membranes. To measure heart mass (M_h), the organ was removed from the thoracic cavity by severing the caval and pulmonary veins at their entrance to the atria and cutting the aortic and pulmonary arterial trunks at the level of the superior atrial border. The heart was weighed after carefully trimming away excess fat and pericardial membrane, flushing with avian saline to remove trapped blood, and blotting on filter paper to remove standing moisture.

Statistical analysis

Linear least-squares regression analyses were performed on $\log_{10}$-transformed variables using a microcomputer to estimate the parameters in the 'allometric' power

equation, $Y = aX^b$, with Y as any variable and X the body mass, M_B, in kg. The mass coefficient, a, is the value of Y at unit mass, estimated by the regression constant, B_o; whereas b, the mass exponent, is estimated by the regression coefficient, $B_{y \cdot x}$. The above parameters were estimated using a model that assumes X is fixed and measured without error. Although not strictly true, the assumption is essentially met if, as in the present case, Y is the measure of an organ of small size compared to body mass (Tessier, 1960). The regression equations were examined for lack of fit using an approximate repeats method to determine the pure error sums of squares (Draper & Smith, 1981). For regressions showing a significant lack of fit after $\log_{10}$ transformation, a two-segment linear model was used. For analysis, the data were divided into two groups and regression lines fitted within these groups using an iterative least-squares procedure for minimizing the pooled residual mean square (Hudson, 1966). The significance of the overall regressions and individual coefficients were decided by the appropriate F- or *t*-test using a probability level of 0·05. The statistical significance of the difference between the value of a given variable measured during a dive and the pre-dive level was assessed using a repeated measures analysis of variance and a *t*-test (Winer, 1971).

RESULTS

All ducks showed a progressive negative cardiac chronotropic response to forced submergence of the head (Fig. 1A,B,C). In a typical dive, f_H declined 90% or more from pre-dive rates, while mean arterial pressure (MAP) remained at or up to 15% below pre-dive levels. During continued submergence, f_H and MAP remained constant for about 60% of the dive; however, both variables usually increased significantly ($P < 0{\cdot}05$) during the last third of the dive. f_H often doubled during this period, but still remained less than one-third of the surface rate, while MAP often increased to the pre-dive level or above.

The maximum dive time (t_d), pre-dive and diving f_H and MAP, and time to full expression of bradycardia (t_{bc}) all depended significantly ($P < 0{\cdot}01$) upon M_B. Parameters and statistics for the linear least-squares regression of these variables on M_B, using $\log_{10}$ transformations, are presented in Table 1. Except for t_d and t_{bc}, linear regression on untransformed variables gave almost as high a correlation as with $\log_{10}$ transformations. However, inspection of residuals plotted against fitted values indicated that the variances were not normally distributed unless the data were transformed. The relationship of t_d to M_B was highly positive (Fig. 2; $F = 1186$, df 1 and 41) and best described by a single power equation: $t_d = 6{\cdot}6M_B^{0{\cdot}64}$, where t_d is in

Fig. 1. (A,B,C) Sample chart recordings from three experiments to determine maximum endurance to forced submersion in Pekin ducks of different masses (kg). Symbols are: HR, heart rate; ABP, arterial blood pressure (kPa); EEG, electroencephalogram. The solid diamond indicates the beginning of head submersion; the hollow diamond dive termination. Numerals and vertical ruled lines mark the elapsed time from head submersion (time = 0) in minutes, except for (B) where elapsed time is indicated on the time line below the other traces.

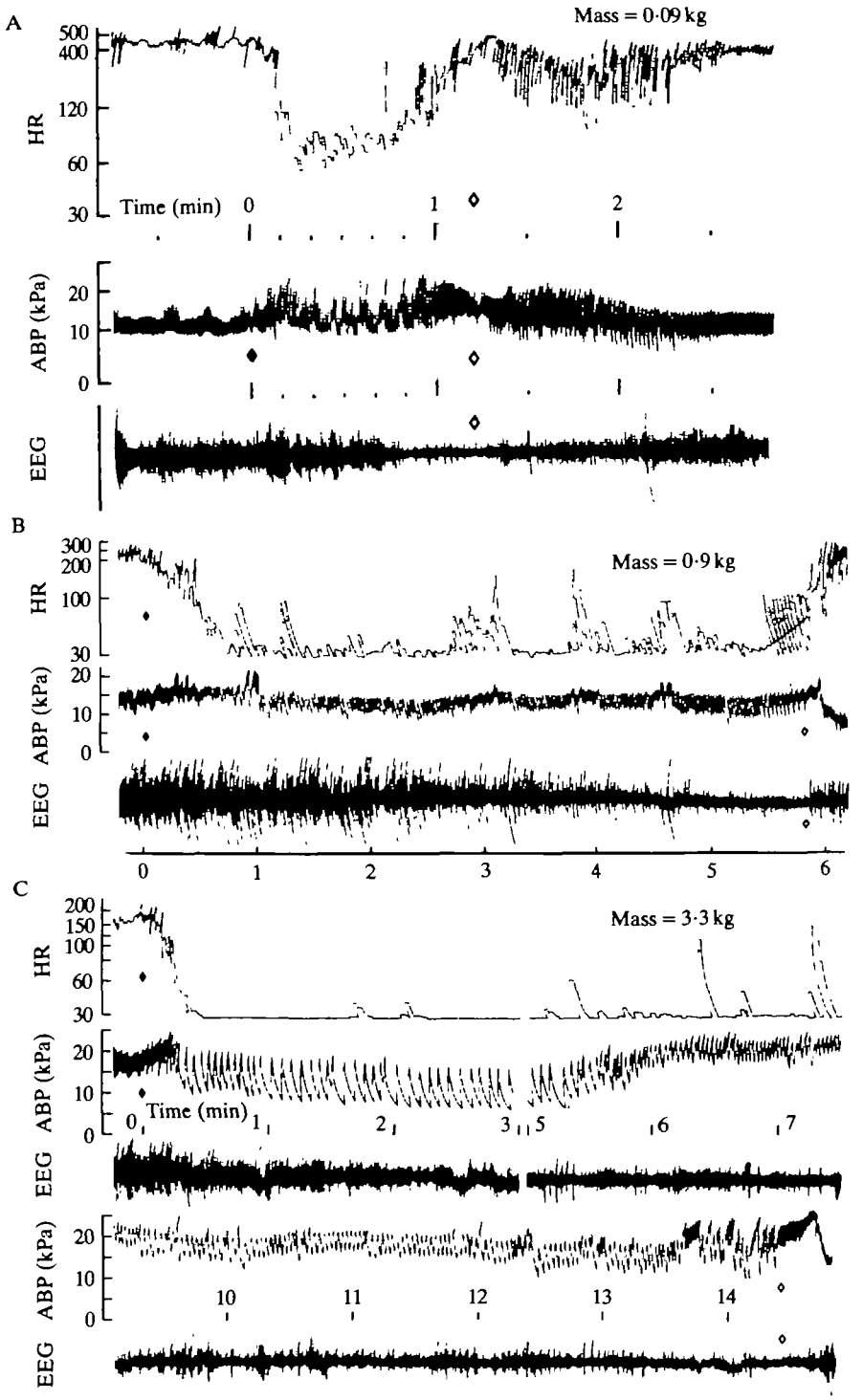
A
Mass = 0·09 kg
HR
500
400
120
60
30
Time (min) 0
1
2
ABP (kPa)
20
10
0
EEG
B
Mass = 0·9 kg
HR
300
200
100
30
ABP (kPa)
20
10
0
EEG
0
1
2
3
4
5
6
C
Mass = 3·3 kg
HR
200
150
100
60
30
ABP (kPa)
20
10
0
Time (min)
0
1
2
3
5
6
7
EEG
ABP (kPa)
20
10
0
10
11
12
13
14
EEG
Dive time (min)

Fig. 1

min and M_B in kg. Both the pre-dive and diving fH depended significantly ($P < 0{\cdot}01$) upon M_B, with negative mass exponents of $-0{\cdot}30$ and $-0{\cdot}28$ respectively ($F = 161$, df 1 and 42; $F = 89$, df 1 and 41). These exponents were both significantly different

Table 1. *Parameter estimates and statistics for power equations relating heart rate, mean arterial pressure (kPa), and maximum dive time (min) to body mass (kg) in Pekin ducks*

Variable	N	a	b	Sb	r	S.E. yx	S.E. %
f_H (min^{-1})	44	220·5	−0·30	0·023	−0·891	0·091	23·0
MAP (kPa)	44	17·7	0·13	0·014	0·815	0·058	14·0
t_d (min)	43	6·6	0·64	0·018	0·983	0·069	17·0
f_H^* (min^{-1})	43	29·3	−0·28	0·026	−0·858	0·101	26·2
MAP* (kPa)	43	15·7	0·10	0·017	0·669	0·064	15·8
t_{bc} (s)	43	31·4	0·23	0·042	0·647	0·161	45·0

The model is $Y = aX^b$, where X is body mass (kg) and Y is the co-variate in units as indicated. Statistical parameters were obtained by linear-least squares regression analysis on $\log_{10}$-transformed variates.

N = the number of ducks; a = the mass coefficient; b = mass exponent; r = the correlation coefficient; Sb = the standard deviation of the slope; S.E. yx = the standard deviation of the residuals (log); S.E. % = the percentage standard error of estimation. The Y variates are: fH (heart rate), MAP (mean arterial blood pressure), t_d (maximum dive endurance), t_{bc} (time to full expression of bradycardia). The symbol (*) indicates a variable measured during restrained submergence.

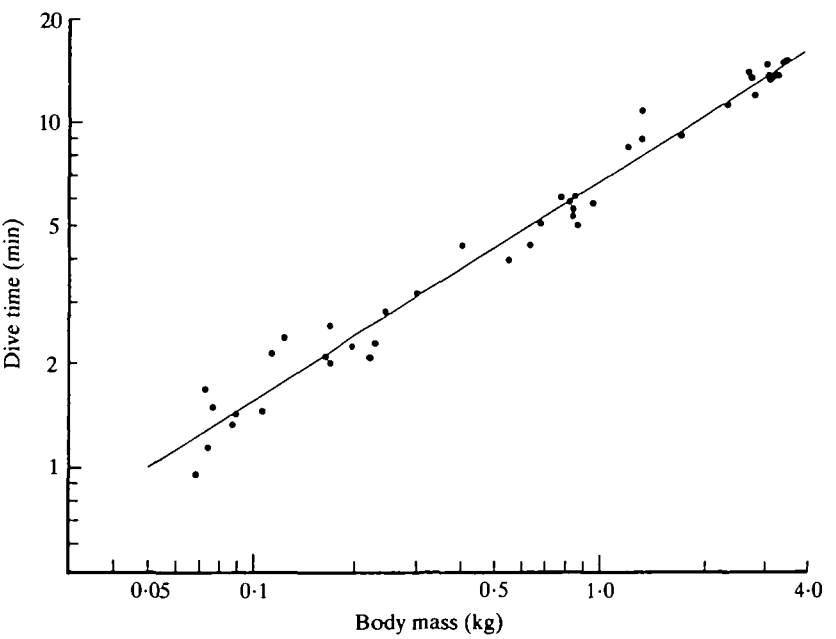

Fig. 2. The relationship between maximum endurance to forced submersion (min) and body mass (kg) in Pekin ducks. Data are presented on $\log_{10}$ co-ordinates; each point represents a single determination. The solid line represents the least-squares linear regression equation calculated using $\log_{10}$-transformed data. Equation and statistics are presented in Table 1.

from zero ($P < 0{\cdot}05$), but did not differ significantly from each other. If these exponents are truly the same, then f_H during submersion, calculated from the ratio of mass coefficients (Table 1), was a constant 13% of the pre-dive rate. MAP, before and during head submersion, showed a small, but significant ($P < 0{\cdot}01$), tendency to increase with M_B ($F = 85$, df 1 and 42; $F = 28$, df 1 and 41). The respective mass exponents, 0·13 and 0·10, were not statistically distinguishable from one another; MAP thus dropped about 10% during the dives. t_{bc} also depended significantly ($P < 0{\cdot}01$) upon M_B ($F = 24$, df 1 and 41), f_H falling more rapidly in small than in large ducks. The mass exponent, 0·23, was significantly greater than zero ($P < 0{\cdot}05$), although the standard error of estimation (Table 1) was considerable.

The wet mass of the heart (M_h) and brain (M_b), both showed a strong relationship ($P < 0{\cdot}001$) to M_B ($F = 8532$, df 1 and 55, and $F = 2529$, df 1 and 55, respectively). Single power equations best fitted the data over the entire M_B range (0·05–3·5 kg; Table 2). The mass exponent for M_b, 0·31, was significantly ($P < 0{\cdot}001$) below 1·0 and above zero, whereas M_h increased according to $M_B^{0{\cdot}97}$, which was only slightly, but significantly, below unity ($P < 0{\cdot}05$). Thus, M_b was almost 4% of the mass of a 0·05-kg duckling, but only about 0·2% of the M_B of a large 3·5-kg adult. In contrast, the heart made up 0·8–1·0% of the body mass over the entire range.

Both arterial and mixed venous P_{O_2} and O_2 content fell in a non-linear manner during prolonged head submersion (Table 3). This occurred concomitantly with a decrease in pH and rise in P_{CO_2}. The non-linear fall in Ca_{O_2} and $C\bar{v}_{O_2}$ tended to preserve the arterial-to-mixed venous O_2 concentration difference ($Ca_{O_2} - C\bar{v}_{O_2}$) until at least midway through a dive. Thus, although Ca_{O_2} decreased 60% at mid-dive, $Ca_{O_2} - C\bar{v}_{O_2}$ actually increased slightly. At t_d, however, $Ca_{O_2} - C\bar{v}_{O_2}$ was reduced to only 0·3 vol%; less than 4% of pre-dive O_2 remained in the blood, indicating an ability to use, almost completely, the blood O_2 store. The average fraction of O_2 in the respiratory system, $F_{\bar{rs}O_2}$, also decreased non-linearly during submergence, but since E_d was 0·76, almost 25% of the original O_2 store remained in the respiratory system at t_d. In contrast, Ca_{glu} decreased slightly midway through a dive (Table 3)

Table 2. *Parameter estimates and statistics for power equations relating various organ volume (ml) and mass (gm) to body mass (kg) of Pekin ducks*

Variable	*N*	a	b	Sb	*r*	S.E. yx	S.E. %
V_{rc} (ml)	37	30·9	1·00	0·019	0·994	0·063	15·5
V_{tb} (ml)	37	99·0	0·93	0·019	0·993	0·062	15·2
V_{tbO_2} (ml)	37	10·3	1·02	0·020	0·993	0·066	16·5
V_{rs} (ml, BTPS)	22	108·1	1·26	0·065	0·976	0·087	22·2
V'_{rsO_2} (ml, STPD)	22	10·1	1·26	0·065	0·976	0·087	22·2
V_{taO_2} (ml, STPD)	22	19·2	1·19	0·039	0·989	0·049	12·2
M_b (g)	57	4·91	0·308	0·006	0·989	0·028	6·8
M_h (g)	57	8·40	0·97	0·010	0·997	0·049	12·1

The model and statistical parameters are the same as in Table 1.

X = the body mass (kg); Y = variables: V_{rc} (red cell volume); V_{tb} (total blood volume); V_{tbO_2} (total blood oxygen); V_{rs} (respiratory system volume); V'_{rsO_2} (available respiratory O_2); V_{taO_2} (total available non-myoglobin O_2 stores); M_b (brain mass); M_h (heart mass).

and actually tended to increase towards dive termination. These changes were not statistically significant ($P > 0{\cdot}05$).

The regression of V_{rc} and V_{tb} on M_B were all highly significant ($P < 0{\cdot}001$; $F = 2701$, $F = 2401$, df 1 and 35) (Table 2). The mass exponent for V_{rc} was equal to unity; V_{rc} was thus a constant 3·1% of M_B over the entire mass range. However, the mass exponent for V_{tb}, 0·93, was significantly below 1·0 ($P < 0{\cdot}05$). The difference between the exponents may have been related to a significantly lower HCT_v (34·4+0·4 S.E., $N = 22$) in smaller ducks (0·91–1·4 kg) than in adults (38·6+0·6 S.E., $N = 15$). V_{tbO_2} was calculated from V_{tb} and the arterial and venous oxygen content assuming (1) that all of the blood O_2 content was extractable during a dive (see above), and (2) that the proportion of V_{tb} in the arterial and venous systems was independent of M_B. The regression was highly significant ($P < 0{\cdot}001$, $F = 2499$, df 1 and 35). However, the mass exponent, 1·02, was not significantly different from 1·0 ($P > 0{\cdot}05$).

In contrast to the blood variables, V_{rs} (BTPS) and the volume of extractable O_2 in the respiratory system, V'_{rsO_2}, both scaled as $M_B^{1{\cdot}26}$, which was significantly greater than unity ($P < 0{\cdot}05$; Table 2). Comparison of mass coefficients and predicted values for V_{tbO_2} and V'_{rsO_2} indicated that the ratio of available blood to respiratory O_2 was about 1:1 for a duck of 1 kg. However, in small ducks (0·1 kg) more than two-thirds of the total available non-myoglobin O_2 is in the blood vascular system, whereas in a 3·5-kg duck almost 60% of the available O_2 is stored in V_{rs}. The

Table 3. *Various respiratory and cardiovascular variables before and during restrained submersion in adult Pekin ducks (mass range 2·0–3·5 kg)*

Variable	N	Pre-dive	Mid-dive	End-dive
f_H (min^{-1})	10	144 (10)	18 (1)	40 (4)
MAP (kPa)	10	19·9 (0·5)	17·1 (0·9)	22·3 (1·2)
Pa_{O_2} (kPa)	8	12·9 (0·4)	7·6 (0·4)	4·0 (0·2)
$P\bar{v}_{O_2}$ (kPa)	6	8·4 (0·2)	4·7 (0·2)	3·0 (0·07)
Ca_{O_2} (mmol)	9	6·8 (0·18)	2·8 (0·13)	0·3 (0·13)
$C\bar{v}_{O_2}$ (mmol)	6	4·95 (0·18)	0·85 (0·09)	0·18 (0·04)
Pa_{CO_2} (kPa)	8	4·0 (0·13)	5·5 (0·2)	8·4 (0·5)
$P\bar{v}_{CO_2}$ (kPa)	6	5·2 (0·4)	6·1 (0·2)	8·8 (0·1)
pHa	9	7·45 (0·016)	7·27 (0·010)	7·11 (0·022)
pH$\bar{v}$	6	7·38 (0·018)	7·23 (0·018)	7·04 (0·027)
C_{glu} (mmol)	8	12·9 (0·8)	11·8 (0·6)	13·5 (1·3)
FE_{minO_2} (%)	9	13·3 (0·2)		
FE_{maxCO_2} (%)	9	5·6 (0·4)		
$F_{\bar{r}sO_2}$ (%)	9	15·5 (0·5)	7·3 (0·3)	4·1 (0·2)
$F_{\bar{r}sO_2}$ (%)	9	4·7 (0·8)	6·6 (0·2)	9·5 (0·3)

Values are means with standard errors in parentheses; N = number of ducks.

P is the partial pressure and C the concentration of gases in arterial or mixed venous blood; C_{glu} is the plasma glucose concentration; $F_{\bar{r}sCO_2}$ and $F_{\bar{r}sO_2}$ are the average fractional concentrations of O_2 and CO_2 in the respiratory system.

Pre-dive values for $F_{\bar{r}s}$ were calculated from the minimum (FE_{minO_2}) and maximum (FE_{maxCO_2}) exhaled values (see Materials & Methods for details).

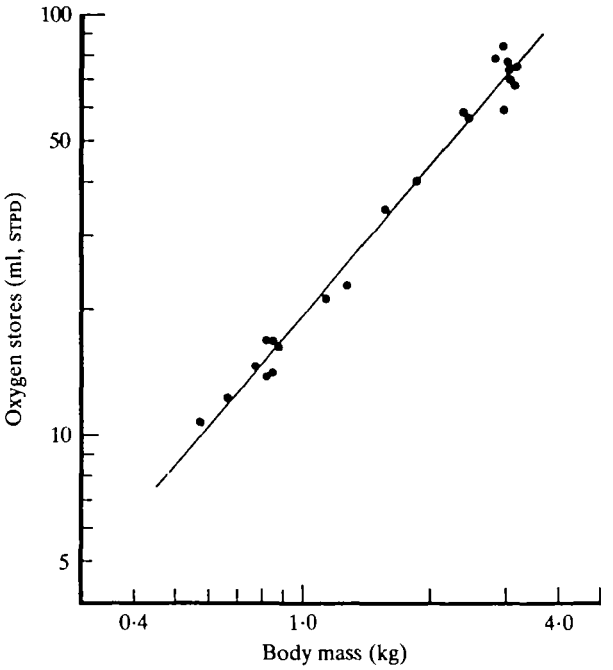

Fig. 3. The relationship between the total volume (ml) of oxygen stores available at the beginning of a dive and body mass (kg) in Pekin ducks. Each point represents the sum of available respiratory and blood oxygen calculated for each duck. The solid line represents the least-squares linear regression equation calculated using $\log_{10}$-transformed data. The equation and statistics are presented in Table 2.

relationship of total available O_2 to M_B was obtained by adding estimates of V_{tbO_2} and V'_{rsO_2} for each duck and plotting these sums, V_{taO_2}, against M_B on $\log_{10}$ coordinates (Fig. 3). The mass exponent, 1·19, was significantly greater than 1·0 ($P < 0{\cdot}05$), primarily because of the influence of V'_{rsO_2}. A single equation (Table 2) best fitted the data over the range of M_B measured. However, a complete data set was unavailable since we were unable to make reliable V_{rs} measurements in small ducks.

DISCUSSION

Our data have confirmed the quite remarkable tolerance of dabbling ducks to enforced submergence. A 1-kg duck can survive 6·6 min under water which, if the same scaling factors apply, means that a duck the size of a Weddell seal could dive for 9 h. A somewhat less bizarre comparison is provided by the observation that a large duck (3–4 kg) can dive as long as a harbour seal of some 20 times greater body mass (Andersen, 1966).

Despite the high tolerance to asphyxia, blood volume and other blood O_2 storage parameters were rather unremarkable. Red cell volume was virtually a constant 3·1 % of M_B, which is within the range previously reported for adult Pekin ducks having a similar venous HCT (Rodnan, Ebaugh & Spivey-Fox, 1957). Our mass exponent for total blood volume, 0·93, was significantly less than the exponent of 0·98 reported for

the mallard duck (West, 1981). In the latter study, the small mass range, differences in technique, and lack of statistics inhibit comparison. Nevertheless, our equation predicts, to within 11%, the total blood volume of 1-kg adult mallards reported by Keijer & Butler (1982). Since V_{rc} is an important determinant of total blood O_2 capacity, it is not surprising that the estimated total blood O_2 scales to almost the same power as does V_{rc}. Our calculation, since it is based on measured values of arterial and venous O_2 content, incorporates any changes in O_2 capacity due to changes in either HCT or Hb concentration with age. However, we assumed that a one-third to two-thirds volume distribution existed between arterial and venous systems. Although this assumption may, or may not, be well grounded, any systematic deviation from the assumed values should principally affect the regression constant, rather than the mass coefficient, unless this distribution changes with size or age.

The Pekin duck's ability to tolerate forced submersion results largely from the capacity to store, conserve and selectively utilize O_2 from the extensive air-sac system during the apnoea. Indeed, air-sac O_2 stores make up over half of the non-myoglobin O_2 available to a 3-kg duck, which is much greater than would be the case for a mammal of similar mass. The air-sac O_2 store is reduced to one-third in a 0·1-kg duck, however, since V_{rs}, and consequently the available respiratory O_2, scaled to the 1·26 power. However, respiratory system O_2 utilization was not complete; about 25% of the original O_2 store still remained in the air-sacs when the birds were no longer able to tolerate the asphyxia. The inability to use the remaining respiratory O_2 was most probably a consequence of a decrease in blood O_2 affinity due to acidosis. The pronounced Bohr effect, which has been described as advantageous for maintaining adequate blood-tissue O_2 diffusion gradients at low saturation during a dive (Andersen & Lovo, 1967), impairs O_2 loading from a fixed pool when air-sac P_{O_2} approaches that of the mixed venous blood, since the blood can have a P_{O_2} of 3–4 kPa with virtually no O_2 content (Table 3).

It has been reported that younger ducks reach a sustainable bradycardia faster than older ducks (Rey, 1971; West, 1981). However, speculation on maturational changes in central and peripheral mechanisms controlling f_H are premature, since age-related changes in periodic or frequency variables cannot be characterized as developmental as long as body mass is a confounding variable. Any process that includes external time as a dimension is inevitably size-dependent, with a mass exponent between 1/4 and 1/3 (Lindstedt & Calder, 1981). Since our mass exponent for T_{bc} was within this range, the cardiac chronotropic response to forced submersion in ducks may not change with age differently from that which would be expected from consideration of physiological time scaling.

If a constant rate of aerobic metabolism is maintained in the 'vital' organs during the late stages of diving asphyxia, the decrease in $Ca_{O_2}-C\bar{v}_{O_2}$ should force augmentation of cardiac output, when the duck has reached its maximum capability for redistributing blood flow. An increase in cardiac output, and hence myocardial metabolism, must prove an additional burden to already strained O_2 stores. This presents a paradox, since increasing O_2 convection requirements in the face of

decreasing $Ca_{O_2}-C\bar{v}_{O_2}$ will further deplete, rather than conserve, the limited O_2 pool. The increased cost of O_2 delivery is thus a contributing factor limiting endurance to forced submersion. Circumstantial evidence to support this idea can be obtained from our Table 3 and Fig. 1, which show an increase in f_H accompanying the drastic decrease in $Ca_{O_2}-C\bar{v}_{O_2}$ in the last one-third of a dive.

In the present study, the mass exponent for maximum endurance to forced diving was found, empirically, to be 0·64, which is considerably greater than predicted (see Introduction). Since total available O_2 scaled according to $M_B^{1·19}$, endurance ought to be proportional to $M_B^{1·19}/M_B^{3/4}$, or $M_B^{0·44}$. Part of the remaining discrepancy between the theoretical and observed t_d mass exponent can be accounted for if the organs using the major share of the O_2 store in a dive do not scale to the first power of M_B, or if the proposed mass exponent for metabolism, 3/4, does not accurately reflect intraspecific variation in energy metabolism or changes in metabolism during growth.

Empirical evidence that the first condition is true can be obtained by re-examining our organ scaling data (Table 2). M_h increased almost to the first power of M_B, although M_b clearly did not. Our mass exponent for M_b, 0·31, was significantly below unity and almost identical with previous reports in mallards (West, 1981). Thus, the brain makes up a larger proportion of M_B in a small duckling than in an adult. To get an approximation of the combined influence of these organs on the relationship of O_2 storage to utilization, we added values of M_b and M_h for each duck and plotted these sums (M_{bh}) as a function of M_B on $\log_{10}$ coordinates (Fig. 4). A single regression line fitted to $\log_{10}$-transformed variables showed significant ($P<0·05$) lack of fit as well as irregularities in the pattern of the residuals; a two-segment linear model was therefore employed. The iterative regression procedure divided the data into two groups above and below about 0·6 kg on the x axis (Fig. 4) and fitted separate line segments below (A; $F = 1063$, df 1 and 24) and above (B; $F = 1312$, df 1 and 29) the break. The mass exponent, 0·56, for line segment A was significantly below ($P<0·001$) the mass exponent, 0·85, for segment B and residual plots gave a normal distribution.

In addition to 'vital' organ masses not scaling in direct proportion to M_B, there is also evidence that the mass exponent for metabolism changes during growth and development. The relationship of O_2 consumption to body mass in fowl, measured from hatching to maturity, generally fits a multiphase log model (Kibler & Brody, 1944; Freeman, 1963). The overall pattern consisted of an initial period of rapidly increasing metabolic rate immediately after hatching, followed by a second phase, lasting about 3–4 weeks, during which the O_2 consumption was directly proportional to M_B, and which was succeeded by a third phase of proportionally reduced O_2 consumption (Freeman, 1963). The second and third phases in the fowl correspond to the developmental stages of the ducks represented by line segments A and B, respectively (Fig. 4), describing the increase in M_{bh} with M_B. The estimated mass exponent during the second phase, calculated as the average of the reported regression coefficients for fowl of both sexes on 'standard diets' is 0·96 (range 0·92–1·0) (Kibler & Brody, 1944; Freeman, 1963). This value, which is probably

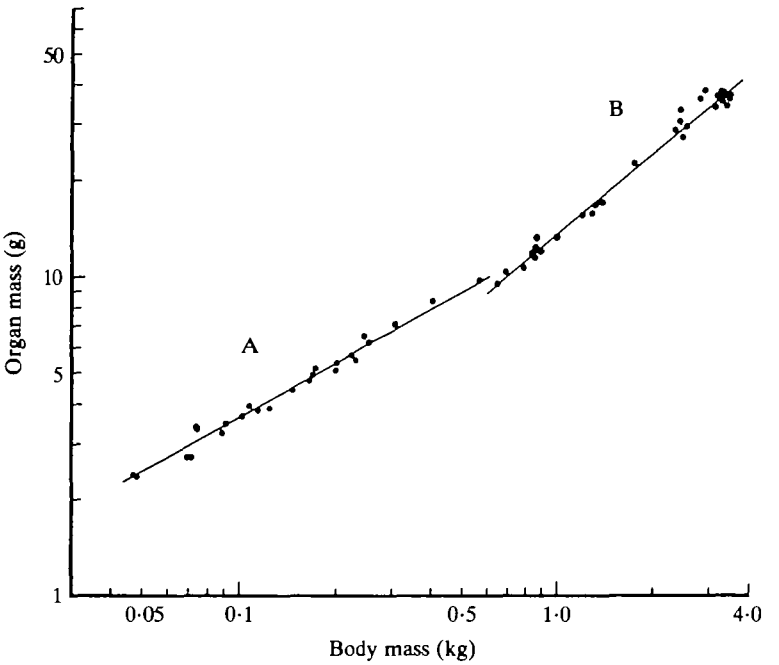

Fig. 4. The relationship between the mass of the brain and heart (g) and body mass (kg) in Pekin ducks. Data are presented on $\log_{10}$ coordinates; each point represents the sum of brain and heart mass of an individual duck. The solid lines (A and B) represent least-squares linear regression equations for $\log_{10}$-transformed data. Groups A and B were statistically delineated assuming a two-segment linear model, iterating all possible regressions, and choosing the two equations with the lowest pooled residual mean square between groups. Equation A: $Y = 12{\cdot}3M_B^{0{\cdot}56}$, $S_b = 0{\cdot}017$, $S_{y{\cdot}x} = 0{\cdot}0245$, $N = 26$; equation B: $Y = 12{\cdot}7M_B^{0{\cdot}85}$, $S_B = 0{\cdot}023$, $S_{y\,x} = 0{\cdot}0335$, $N = 31$.

not different from unity, may be expected in animals undergoing changes in body composition, form or function (Heusner, 1984). The average mass exponent for the third phase is 0·69 (range 0·5–0·86), or about 2/3. The range in the reported regression coefficients was large, but there is additional justification, both experimental and theoretical, for choosing a 2/3 mass exponent to describe intraspecific changes in metabolic energy expenditure with size, where intensive properties such as body structure are relatively constant, but mass may not be (Heusner, 1982; Feldman & McMahon, 1983).

Using the above assumptions, can we predict the empirical relationship between t_d and M_B using a knowledge of the relationship between M_B, the available O_2 stores, and their rate of utilization during submersion? Considering ducks in which intensive properties of form and density are probably constant (i.e. group B; Fig. 4), the actual rate of metabolism, assuming it increases to the same power as overall metabolism, would be $(M_B^{0{\cdot}85})$ raised to the 2/3 power, or $M_B^{0{\cdot}57}$, and forced-diving endurance would be expected to scale as $M_B^{1{\cdot}19}/M_B^{0{\cdot}57}$, or $M_B^{0{\cdot}62}$. This is very close to the mass exponent of 0·64 actually measured. In group A (Fig. 4), a similar analysis is complicated by the fact that we do not have a complete range of O_2 storage estimates. However, if we extrapolate V_{taO_2} over this range, endurance ought to be

proportional to $M_B^{1.19}/(M_B^{0.56})^{1.0}$, or $M_B^{0.63}$, which again is almost identical with the empirical mass exponent for t_d. The analysis suggests that the changing relationship of available O_2 to the aerobic metabolic rate of the brain and heart can account for the increasing asphyxic tolerance with mass or age observed within a species. In the analysis we have neglected metabolic contributions of other organs, such as the spinal cord, retina, adrenal glands and lung. These organs receive normal or increased blood flow during a dive (Jones *et al.* 1979) and might be expected to metabolize aerobically. However, the contribution of these organs to overall O_2 consumption during a dive may be low, compared to the brain and heart, due to either a small relative mass or a lower absolute rate of tissue metabolism (Jones, 1984).

Finally, the constancy of blood glucose in diving ducks contrasts with the significant depletion of blood glucose reported for the Weddell seal during both forced (Murphy, Zapol & Hochachka, 1980) and voluntary diving (Kooyman *et al.* 1980). Interestingly, the pre-dive glucose level in our ducks was almost triple that reported for the Weddell seal. The minimum centrally available glucose at the start of a dive was calculated from blood glucose and blood volume measurements. In the duck, estimated blood glucose, $1{\cdot}2\,\text{mmol}\,\text{kg}^{-1}$, is twice the calculated pool size of $0{\cdot}6\,\text{mmol}\,\text{kg}^{-1}$ in the Weddell seal (Hochachka, 1981). Plasma glucose levels are usually much higher in birds than in mammals; however, the basis of the differences in the pattern of blood glucose regulation during diving is not immediately apparent. The harbour seal, with a high pre-dive blood glucose level ($1{\cdot}6\,\text{mmol}\,\text{kg}^{-1}$), shows little change in blood glucose in dives which are the same proportion of maximum endurance as those experienced by Weddell seals (Robin *et al.* 1981; Davis, 1983).

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