Blood flow and volume distribution during forced submergence in Pekin ducks (*Anas platyrhynchos*)

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Received July 31, 1987

Heieis, M. R. A., and Jones, D. R. 1988. Blood flow and volume distribution during forced submergence in Pekin ducks (*Anas platyrhynchos*). Can. J. Zool. **66**: 1589–1596.

Blood is the major oxygen store in ducks forced to dive, and underwater endurance depends on how much of this store can be used by oxygen-sensitive tissues such as the heart and brain. Arterial injection of macroaggregated albumin labelled with technetium-99m, which is trapped and held by capillaries, showed that circulation in dives was restricted to the thoracic and head areas. However, tracing red blood cells labelled with technetium-99m as they were injected during dives showed not only that the time required for the activity to reach equilibrium was 4–10 times longer than when labelled cells were injected into resting ducks but also that blood flow continued in the leg and visceral regions. Tracing red blood cells, labelled with technetium-99m and mixed in the circulation before a dive, during the dive showed that labelled red blood cells were redistributed from the peripheral and visceral areas to the central cardiovascular area. Measurement of circulating red blood cell volume during and after diving showed that, on average, 75.24 ± 4.56% of the total red blood cell volume was circulated during forced submergence. Hence, in forced dives, red blood cell volume is positioned in such a manner that the heart and brain have access to the oxygen stored there, and the residual blood flow in the periphery ensures that most of the red blood cell volume is circulated.

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Le sang est la principale source d'oxygène durant la plongée forcée chez le canard. L'endurance à la submersion dépend de la proportion d'oxygène utilisée par les tissus sensibles à l'hypoxie, tels le cerveau et le coeur. Des injections artérielles de macro-agrégats d'albumine marqués au technétium-99 m et retenus dans les capillaires semblent montrer que la circulation durant la plongée est restreinte aux régions thoraciques et cérébrales. Cependant, l'observation suivie des érythrocytes marqués au technétium-99 m et injectés au cours de la plongée montre, non seulement que le temps requis pour que leur activité atteigne un équilibre est 4 à 10 fois plus long que lorsque les agrégats sont injectés chez les canards au repos, mais aussi que le sang continue de circuler dans les pattes et les régions viscérales. L'observation suivie des érythrocytes marqués au technétium-99 m et intégrés à la circulation avant la plongée montre que ces érythrocytes sont redistribués des régions périphériques et viscérales à la région cardiovasculaire durant la plongée. La mesure du volume des érythrocytes en circulation durant et après les submersions forcées indique qu'en moyenne 75,24 ± 4,56% du volume total des érythrocytes circule durant la plongée forcée. Durant la plongée forcée, le volume d'érythrocytes est donc réparti de manière à rendre accessible au coeur et au cerveau l'oxygène qui y est stocké; le débit du sang résiduel, en périphérie, assure la mise en circulation de la majeure partie du volume des érythrocytes.

Introduction

During forced submergence, diving birds and mammals display bradycardia and a massive peripheral vasoconstriction (Scholander 1940). This peripheral vasoconstriction prevents blood flow to hypoxia-insensitive tissues so that lung and blood oxygen stores are saved for the heart and brain, both of which are crucially dependent on a continued oxygen supply. In birds and mammals, maximum endurance is set by the oxygen availability to, and consumption by, the central aerobic organs (Packer et al. 1969; Hudson and Jones 1986). In Pekin ducks, approximately 50% of non-myoglobin-bound oxygen is carried in the blood, while the remaining amount is stored in the lung and air sac system (Hudson and Jones 1986). During forced submergence, Pekin ducks use approximately 75% of the oxygen contained in the respiratory system (Hudson and Jones 1986) and almost all of the blood oxygen. Hence, the contribution of the blood oxygen store to the survival of the animal will be proportionally greater than is suggested by pre-dive oxygen storage distribution (Jones and Furilla 1987), but only if the total blood oxygen store is accessible to the central aerobically metabolizing organs.

Blood volume measurements in forcibly dived harbour seals (*Phoca vitulina*) demonstrated that virtually the entire blood volume was circulating during the dive, in spite of a presumed intense peripheral vasoconstriction (Packer et al. 1969). In nonmarine mammals, approximately 20% of the total blood volume is located in the arteries, small arteries, arterioles, capillaries, and postcapillary venules, while the remaining 80% is divided between the venules, small veins, and large veins (Wiedeman 1963). Therefore, if the pre- and post-capillary areas of all tissues except the heart and brain were instantaneously isolated from the circulation, most of the total blood volume would be available to yield its crucial oxygen supply in a dive, as long as the blood contained within the venous system was being circulated. However, from studies of blood flow distribution in forced dives (Jones et al. 1979; Zapol et al. 1979) it is difficult to see how blood from peripheral regions of the body can be incorporated into the central circulation if the circulation is largely restricted to the heart, lungs, and brain. Consequently, the purpose of the present investigation was to measure circulating red cell volume in ducks forced to dive and to determine how blood flow and blood volume distribution contribute to maximal utilization of the blood oxygen store.

Methods

General methods, monitoring of cardiovascular variables, and analysis of data

Experiments were done at room temperature (20–24°C) on unanaesthetized white Pekin ducks (*Anas platyrhynchos*) ranging in body mass from 2 to 3 kg. All surgical procedures were of a minor nature and were carried out under local anaesthesia (2% lidocaine hydrochloride; Astra Pharmaceuticals, Toronto, Ont.). The left

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brachial artery was cannulated with PE 90 tubing and one or both brachial veins, with PE 190 tubing. The cannulae were filled with saline containing 50 USP units heparin·mL^{-1} (Glaxo Canada, Toronto, Ont.). The arterial cannula was advanced until the tip was either in the left ventricle or at the base of the aortic arch, just outside the aortic valves. The venous cannulae were advanced some 10 to 15 cm until their tips lay in the great veins. Cannulae were filled with X-ray opaque material and the location of their tips in the cardiovascular system was confirmed by radiography.

The ducks were restrained on an operating table for blood volume studies, and on a Plexiglas template shaped in the form of a duck for gamma scanning studies. In the former, the animal's head was restrained by a Plexiglas assembly that could be lowered into a container of water to start a dive. Forced dives in the gamma scanning studies were done by immersing the duck's head manually into a beaker of water.

Arterial blood pressure was monitored from the brachial artery cannula using a Biotec BT-70 (Narco Biosystems, Houston, TX) pressure transducer. Blood pressure traces were recorded on a two-channel chart recorder, using rectilinear coordinates. Heart rate was obtained from the blood pressure traces. Values are given as means ± standard error of the mean (SEM). Paired *t*-tests were used to determine significant differences ($P < 0.01$) between paired sets of data.

Blood volume determinations

Total red blood cell (RBC) volume was determined in five ducks by labelling the cells with chromium-51 (^{51}Cr). Approximately 6 mL of blood was withdrawn from the brachial artery. Arterial haematocrit was determined by the microcapillary technique. In eight ducks, arterial haematocrit was monitored before and during dives of up to 4 min in duration. In two ducks, arterial haematocrit was measured before and during dives in which RBC volume was also measured. One millilitre of anticoagulant, acid–citrate–dextrose (ACD) solution (dextrose 132 mg, sodium citrate 250 mg, citric acid 80 mg, water 10 mL), and approximately 1.5 MBq of sodium [^{51}Cr]dichromate were added to the blood sample. The blood–ACD–^{51}Cr solution was then gently mixed and incubated for 25 min at room temperature. During this period the chromium, which is in a hexavalent state, freely passes into the red cells where it binds to haemoglobin and is reduced to a trivalent state which cannot pass through the cell membrane. After incubation, approximately 25 mg of L-ascorbic acid was added to the solution to stop the tagging process. This procedure reduces any free chromium present in the incubating solution, or within the red cells, thereby preventing any further movement of ^{51}Cr into or out of the RBC. The solution was incubated and gently agitated for a further 10 to 15 min. The labelled cells were then washed 2 or 3 times with saline. Tagged red cells were resuspended in saline and haematocrit was determined for the resuspended cells. Two millilitres of the suspension was then withdrawn into a syringe for injection into the animal. Standards were prepared from the resuspended solution and, after centrifugation, from the suspension saline. Five 100-μL aliquots of both the resuspended RBCs (injection standard) and suspension saline (saline standard) were counted in a Picker Place II gamma counter (Picker, Northford, CT). The average count for the five aliquots of the resuspended RBCs and suspension saline was obtained.

Labelled RBCs were injected into the ducks through the venous cannula 1–2 min after the start of a dive. Arterial blood samples (2 mL) were taken 2–5 min after injection and then 20 min postdive. Five aliquots of whole blood (whole blood sample) and five aliquots of plasma (plasma sample), each of 100 μL, for both dive and postdive samples were counted. The average count of both sets of five aliquots was taken and used, along with the injection and saline standards, in the following formula to calculate total red cell volume.

$$\text{RBC volume} = \frac{\text{volume injected} \times [\text{injection std} - (\text{saline std} \times \text{Plct(injection std)})]}{\text{whole blood sample} - (\text{plasma sample} \times \text{Plct(arterial)})} \times \text{Arterial Hct}$$

where

std = standard
Hct = red cell volume/total blood volume
Plct = decimal plasmacrit = 1 − Hct

Blood flow distribution methods and procedure

Blood flow distribution was visualized using two techniques. In one method, blood flow distribution was determined by organ trapping of gamma-labelled macroaggregated albumin (MAA) (Frosst Pharmaceuticals, Kirkland, Que.). The MAA technique is similar to the glass microsphere technique in that the MAA is trapped and held in the capillary circulation, and the gamma scan reveals the blood flow distribution. In the other technique, the movement of gamma-labelled red cells is observed over time. In both studies, the gamma-emitting radionuclide used was technetium-99 m (^{99m}Tc) which was generated from molybdenum-99 (^{99}Mo) (Minitec, Frosst Pharmaceuticals, Kirkland, Que.). The physical half-life of the radionuclide is 6.02 h.

Labelled MAA

Nine ducks were injected with ^{99m}Tc-labelled MAA. First, a low dose of approximately 4 MBq of MAA in a volume of 1–2 mL was injected slowly via the arterial cannula into ducks 2 min after the start of a forced dive. Following the dive scan, a second and much higher (40 MBq) dose of MAA, in a volume of 1–2 mL, was injected either within the same scan session or 24 h later to obtain blood flow distribution during rest. The injected MAA is trapped at the first capillary bed and remains trapped for approximately 2 h before being broken down. Consequently, the animal can be injected and scanned again 24 h later or, alternatively, repeated injections of MAA can be made under different experimental conditions with little time between scans, provided the subsequent activity dose is approximately 10 times higher than the previous dose. The average particle size of the MAA was 40 μm.

Labelled red blood cells

Injections were given to 14 ducks. Red blood cells, withdrawn from the duck about to be scanned, were labelled in vitro with ^{99m}Tc as follows: 0.3 mL of gluceptate (Frosst Pharmaceuticals, Kirkland, Que.) was injected into the duck via the arterial cannula and 15 min later, 6 mL of blood was withdrawn. Haematocrit was determined by the microcapillary technique. One millilitre of ACD and 400 MBq of ^{99m}Tc were added to the blood sample which was then incubated for 10 min at room temperature. After centrifugation and separation, both plasma and RBC fractions were counted by a dose calibrator (Picker, Northford, CT) to determine the amount of ^{99m}Tc labelling. The amount of free ^{99m}Tc in the plasma was usually less than 5%. The RBC fraction was then resuspended to the haematocrit of the blood sample with physiological saline. Approximately 2 mL (~80 MBq) of ^{99m}Tc-tagged RBCs were used for bolus injection.

To obtain time–activity curves of injected tracers, two ducks were injected with labelled RBCs 2 min after the start of a dive. The dive duration for these animals was 7 min. Three ducks were injected with a 2-mL bolus of labelled RBCs during rest. These animals were scanned from the start of the injection for 10 min. In one duck, a veno-venous shunt was made in an effort to determine the rate at which injected radioactivity mixed in the circulation. Both right and left brachial veins were cannulated. Blood was pumped from left to right through the extracorporeal circuit by a Wiz pump–diluter–dispenser (Isco, Lincoln, NE) at a rate of 2 mL·min^{-1}. Labelled RBCs were injected over a 60-s period through the pump.

To observe changes in vascular capacitance, ^{99m}Tc-labelled red blood cells were injected into seven ducks 10 to 20 min before a dive. This was done to ensure that the labelled RBCs had completely mixed within the animal's cardiovascular system. The ducks were scanned while being held in the dive position before, during, and after 4-min dives.

Gamma camera equipment and scanning procedure

The gamma scanning instruments used for both techniques were a Picker Dyna Camera 4/15 with attached Micro Z processor, Image

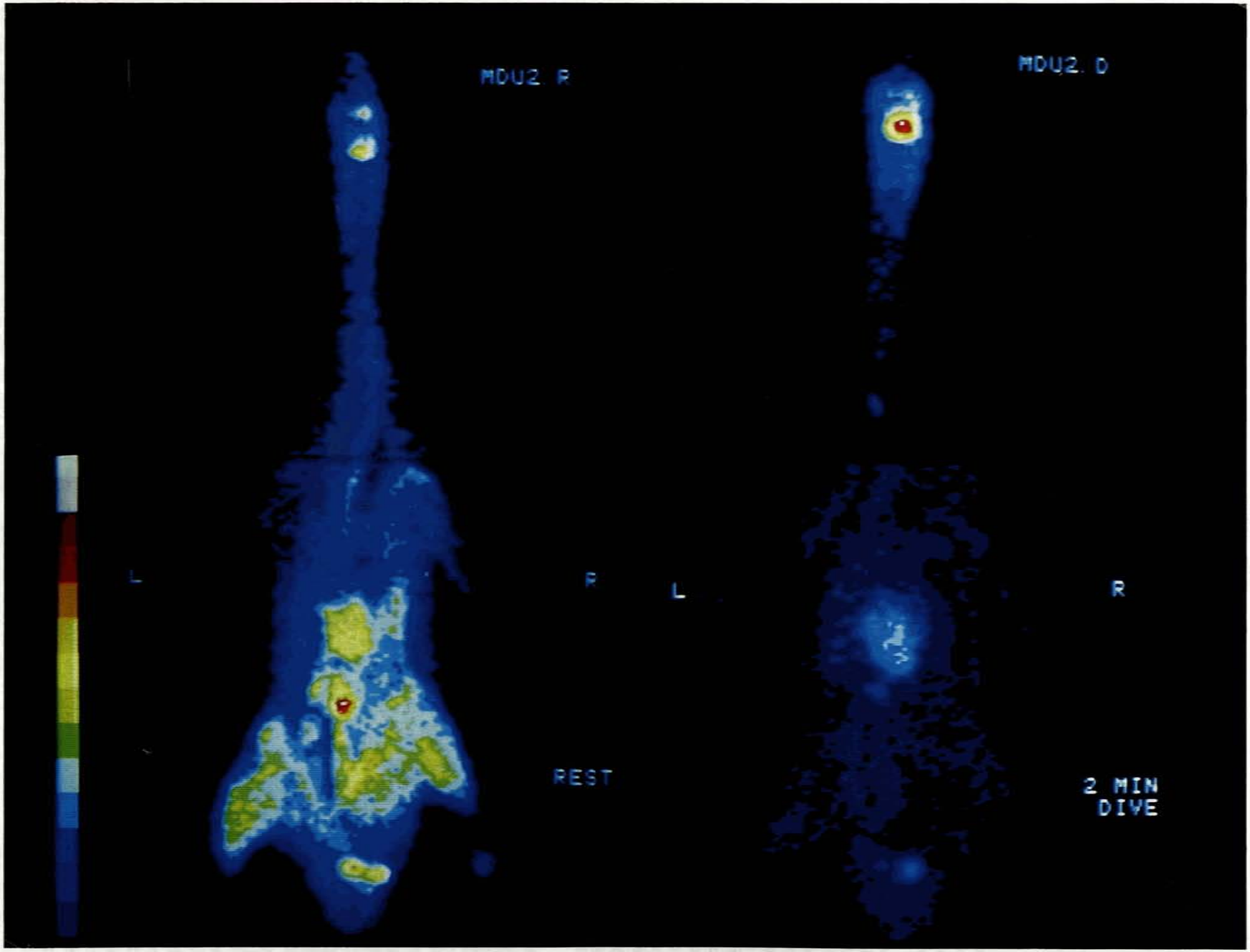

FIG. 1. Blood flow distribution in a resting (rest) and forcibly submerged duck (dive) after 2 min. The image is a composite of two scans, one for the body and one for the head. Each region was scanned for exactly the same length of time. The time between scans was just that required to reposition the duck. The colour scale indicates the relative level of blood flow (red, high flow; blue, low flow). During rest, blood flow is widely distributed, whereas during diving, it is largely restricted to the thorax and head.

TABLE 1. Total red blood cell volume during forced dives and postdive recovery

Body mass (kg)	Mixing time (min)	RBC volume (mL RBC · kg^{-1})		% circulating during dive	Aterial Hct (postdive)
		Postdive	Dive		
2.7	2	27.17	24.29	89.57	0.460
2.6	2	28.61	23.22	81.15	0.378
2.4	2	27.00	18.90	70.00	0.432
2.7	4	29.33	21.11	71.99	0.380
3.7	5	27.95	17.75	63.49	0.432
2.82 ± 0.23		28.01±0.44	21.05±1.24	75.24±4.56	0.416±0.011

NOTE: Individual values as well as means ± SE are given for five ducks.

Programmer, and Dyna Camera 4 controller (Picker, Northford, CT). Real-time image data acquisition, processing, and analysis were done with a microprocessor system, Adac DPS-2800 (Adac Laboratories, Sunnyvale, CA), interfaced to the gamma camera using Adac's Nuclear Medicine System, V2A software. The resolution of the combined detector and computer systems was 3 mm. Image size was 128 × 128 pixels × 8 bits in both MAA and labelled-RBC studies.

After labelled RBCs were injected, digital images were acquired at a rate of 1 image/s for 10 min. At the end of each scan, the computer was used to select regions of interest from a displayed digital image. The activity in the various regions of interest on each image at a given time was counted, processed, and plotted. Unfortunately, since the gamma scanning equipment yields a two-dimensional image from a three-dimensional object, it cannot provide quantitative flow data. Consequently, in an attempt to obtain quantitative blood flow data, two ducks were scanned using a three-dimensional (rotational) gamma camera, but because of the poorer effective resolution of rotational gamma cameras further studies were not pursued using this instrument. Serial photographic images of the ducks were also taken on X-ray plates, as labelled red blood cells were injected, at a rate of 2 s/image for 32 s. In the MAA studies, only a single digital and single photographic image was taken in each scan.

Results

Blood volume distribution during rest and forced submergence

The average red cell volume in five resting animals determined 20 min after diving was 28.01 ± 0.44 mL RBCs · kg body mass^{-1} (Table 1). During submergence, however, the average circulating red cell volume was measured at 21.05 ± 1.24 mL RBCs · kg^{-1}. The difference between postdive and dive values is statistically significant. Circulating red cell volume during forced submergence varied from a low of 63% to a high of 90% (with an average of 75.2 ± 4.6%) of the red cell volume circulating after recovery from the dive. Arterial haematocrit was measured in 10 ducks before and during forced dives; there were no significant differences between the dive (0.404 ± 0.007) and predive values (0.403 ± 0.006).

Relative blood flow distribution during rest and forced submergence

Relative blood flow distribution was obtained under resting and forced dive conditions in nine ducks by injecting ^{99m}Tc-labelled MAA. Composite images of a single duck are presented in Fig. 1. The arbitrary colour scale on the left indicates relative blood flow, with regions of high blood flow appearing red–white and those of low blood flow appearing blue (Fig. 1). During rest (heart rate = 276 beats/min), blood flow was widely distributed, with the lower abdominal and visceral areas having the highest relative flows (Fig. 1, rest). During forced diving, even when dive heart rate was 60 beats/min,

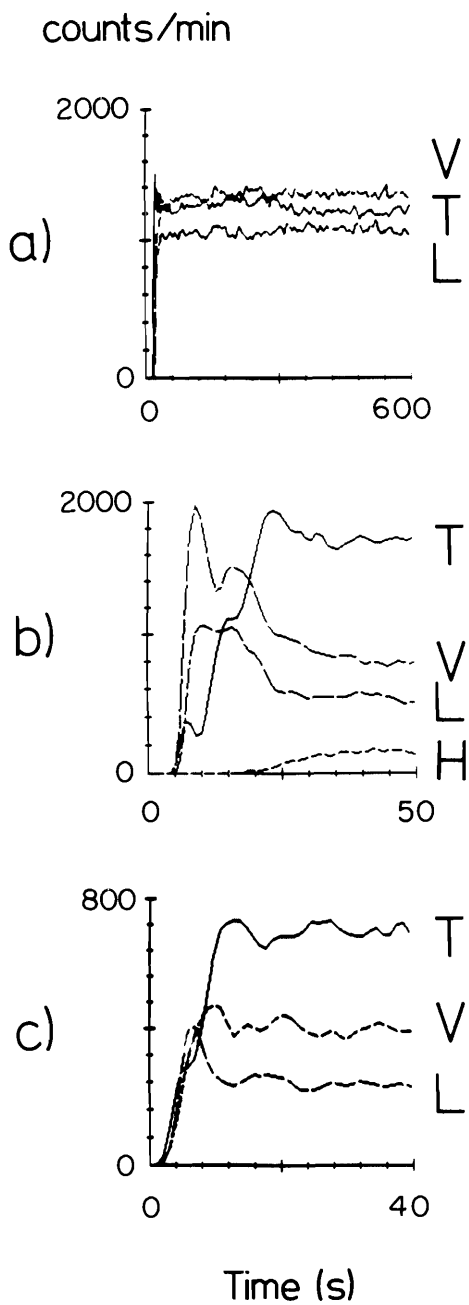

FIG. 2. Time–activity curves of a bolus injection of ^{99m}Tc-labelled red blood cells into three ducks during rest (each graph represents one duck). In Fig. 1*a*, the time base is compressed whereas in Figs. 1*b* and 1*c*, it is expanded. T, thorax; V, viscera; L, legs; H, head.

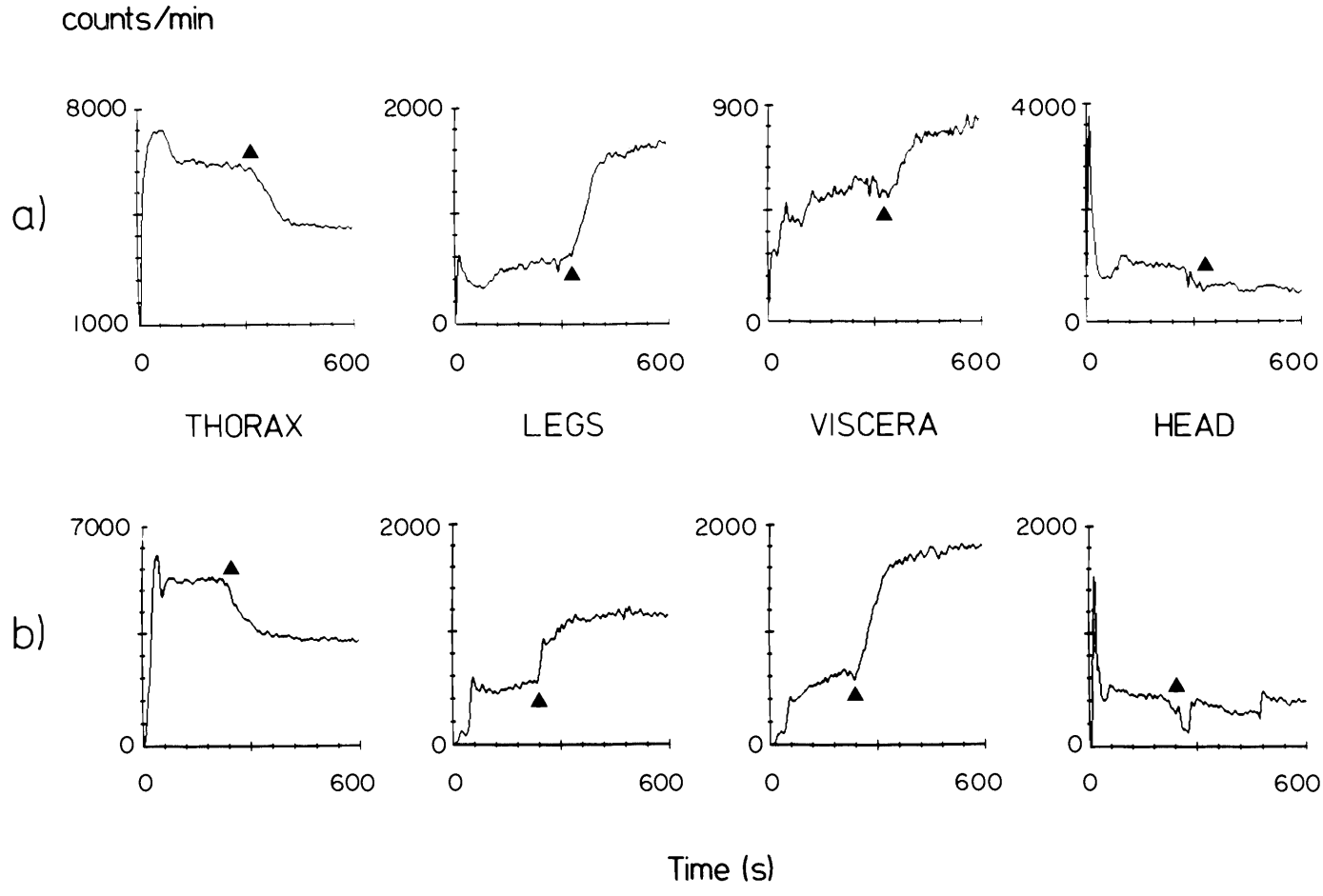

FIG. 3. Time–activity curves of a bolus injection of ^{99m}Tc-labelled red blood cells into two ducks 2 min after the start of a forced dive (Figs. 3*a* and 3*b* each represent one duck). ▲, end of dive.

blood flow was restricted largely to the thoracic and head areas (Fig. 1, dive).

Time–activity curves of bolus-injected ^{99m}Tc-labelled RCBs during rest and forced submergence

Ducks were scanned while ^{99m}Tc-labelled red blood cells were injected arterially during rest and diving (Figs. 2 and 3). In resting animals, labelled blood cells were distributed throughout the animal within 30 s as indicated by the activity reaching a stable level in the head, leg, thoracic, and visceral areas within this time (Figs. 2*a*, 2*b*, 2*c*). The arterial distribution of the bolus was variable and was dependent on the rate and smoothness of injection and how fast the cannula was flushed. Activity would be expected to appear in the central cardiovascular area first since the labelled cells enter via a cannula in this area, but even with a higher resolution of the time base this was not usually obvious (Figs. 2*b* and 2*c*). However, in the one animal in which activity in the head region was monitored, appearance of activity in the head region was delayed for 10–15 s, although this might not be a typical result (Fig. 2*b*).

A bolus of ^{99m}Tc-labelled RBCs injected 2 min after the start of a dive gave simultaneous increases in activity in all of the observed regions (Fig. 3). Peak activity occurred first in the head area, with peak activity in the thoracic area being reached as the bolus returned from peripheral areas. In the head and chest regions, the activity equilibrated within 2 min (Figs. 3*a* and 3*b*, head and thorax). However, activity continued to increase in the visceral tissue areas throughout the dive, although activity in the legs appeared to reach a stable level some 3–4 min after injection (Figs. 3*a* and 3*b*, legs and viscera). Immediately after the ducks surfaced, activity in the thoracic area fell, and a second and final equilibrium plateau was reached (Fig. 3), while activity increased in the leg and visceral regions. Activity in the head region appeared to fall postdive, but this cannot be confirmed with the present data because the head had to be moved to terminate the dive, which caused an abrupt drop in activity. Repositioning of the head after emergence was probably not exact, making comparisons of postdive and dive levels inaccurate.

A similar pattern of labelled RBC distribution was seen when RBCs were injected via the veno-venous shunt, although the appearance of activity in peripheral regions was somewhat delayed (Fig. 4) compared with arterial injection (Fig. 3). This is probably due to the slow injection of the labelled RBCs (1 min). In the thoracic area, peak activity was reached approximately 30 s after the last of the ^{99m}Tc-labelled RBCs had been injected. Activity subsequently fell and reached a steady level 2 min after the peak. Activity decreased in the veno-venous shunt (Fig. 4*c*), while it continued to increase in the visceral and leg areas (Fig. 4*b*) during the dive. Upon termination of the forced dive, activity in the thoracic region fell, while the activity in the visceral and leg regions increased. These changes were similar to those observed in Fig. 3. There

was no discernible change in activity in the extracorporeal shunt postdive (Fig. 4*c*) because background activity levels were higher than any radioactivity contained within the shunt.

Vascular volume changes during forced submergence

In seven ducks, ^{99m}Tc-labelled RBCs were injected 10–20 min before submergence. During a 4-min dive, the activity in the abdominal and leg areas decreased, indicating a reduction in the number of labelled RBCs in these regions (Fig. 5), while the activity in the thoracic area increased, indicating an increase in labelled RBCs in this area (Fig. 5). At the start of the forced dive, activity levels usually dropped faster in the visceral than in the leg area (Fig. 5). At the termination of the dive, counts in all three areas returned to resting levels although activity in the leg region appeared to return to resting levels faster than in the other two regions (Fig. 5). More than one dive was performed with each duck and, except for a predicted reduction in overall levels of activity due to radioactive decay, qualitatively similar results were obtained on subsequent dives (Figs. 5*b* and 5*d*).

Discussion

Blood flow distribution monitored by organ trapping of macroaggrated albumin has confirmed the pattern of blood flow distribution during rest and forced submergence in ducks determined by other methods (Johansen 1964; Jones et al. 1979). However, measurements of red cell volume during, compared with after, diving showed that about three quarters of the total red cell volume was circulating, which attests to a much wider flow distribution than was evident from MAA studies. In fact, blood flow studies using labelled red blood cells showed that considerable peripheral circulation of the blood also occurred in forced dives. It is this peripheral flow, combined with a redistribution of the total blood volume towards the central cardiovascular area, that ensures maximum utilization of the blood oxygen store.

Gamma scans of radioisotope-labelled red blood cells, injected either arterially or venously 2 min into a forced dive, showed that blood flow continued to the leg and visceral areas since radioactivity increased in those regions. If flow had ceased to those areas during the dive, the radioactivity would not have increased. Microsphere studies by Jones et al. (1979) indicated that blood flow to muscle was arrested during forced submergence in ducks. This was the same result that Djojosugito et al. (1969) found, but the latter authors also showed that blood flow to the webs was maintained. As a consequence of this maintained web flow, the distal venous blood store will be circulated while the skeletal muscle beds are bypassed.

During the dive, labelled RBCs that had been injected and mixed before the start of the dive decreased in the visceral and leg areas but increased in the thoracic area (Fig. 5). Radioactivity can increase or decrease (other than by radioactive decay) by a change in either flow rate or the number of red cells in the area. It is unlikely that there was an increase in overall blood flow rate in the central cardiovascular area because cardiac output during forced submergence is greatly decreased while myocardial flow is largely unchanged (Jones and Holeton 1972; Jones et al. 1979). Therefore, the only way radioactivity could rise is by an increase in the number of red blood cells in the region. In other words, the blood volume must be shifted from the peripheral to the central venous area during diving. Djojosugito et al. (1969) reported that blood was actively expulsed from skeletal muscle by venoconstriction, while Langille (1983) measured a marked increase in venous tone during forced submersion. The effect of these actions would be to reduce the vascular space in the peripheral veins and to shift the blood to the central venous compartment allowing most of the blood pool to be available to the heart and brain in dives.

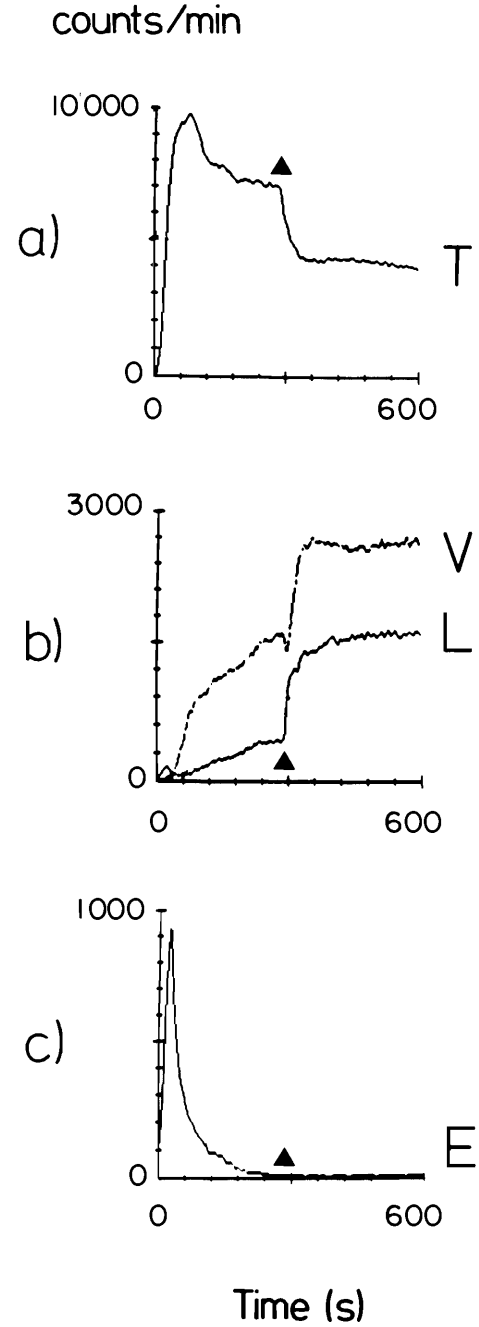

FIG. 4. Time–activity curves of labelled red blood cells infused through a veno-venous shunt: (*a*) activity in the thorax (T), (*b*) activity in the viscera (V) and legs (L), and (*c*) activity in the extracorporeal shunt (E). ▲, end of dive.

The dynamic tracing of radioactively labelled red blood cells injected during a dive showed that once initial mixing had taken place, little further mixing occurred with any isolated portion of the blood volume because activity levels in the central and even peripheral cardiovascular areas reached a plateau. This is contrary to what was suggested by Murphy et al.

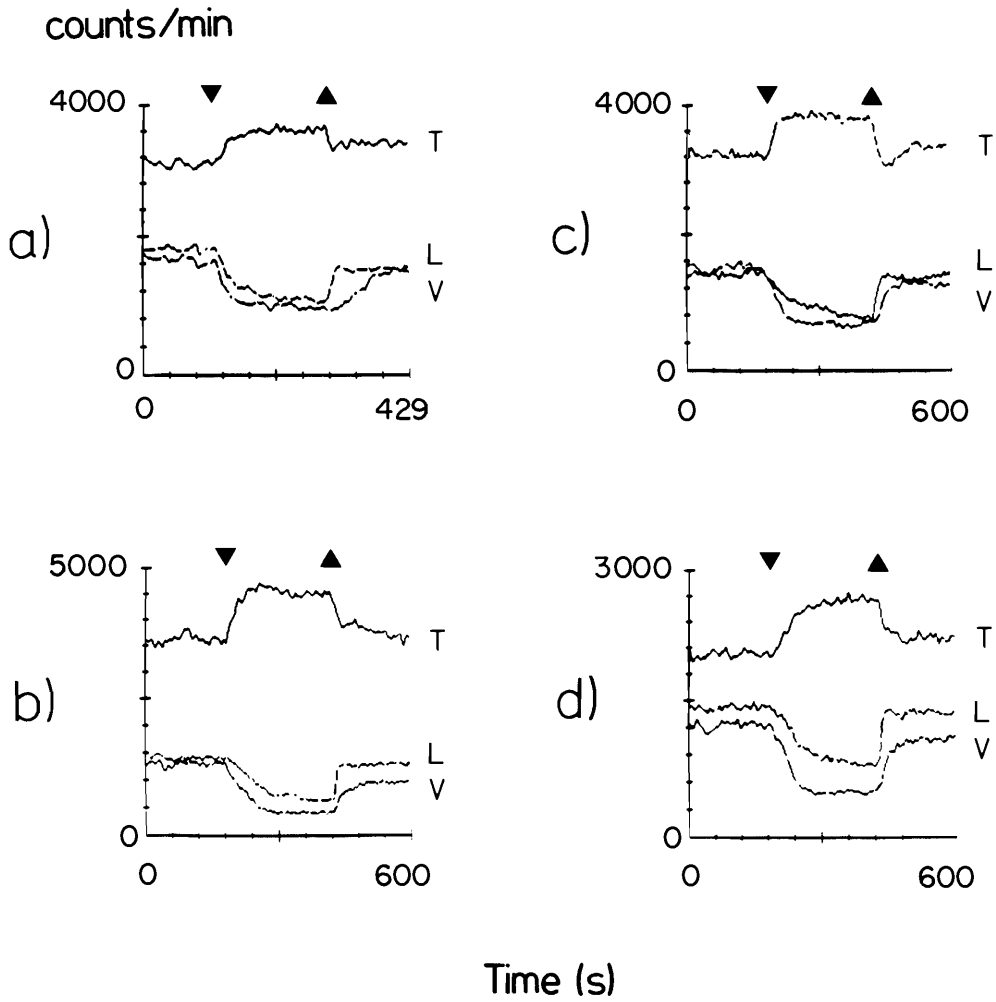

FIG. 5. Vascular volume changes during 4-min forced dives determined from the distribution of labelled RBCs. Labelled red blood cells were injected some 20 min or more before the start of the scan. Scans in Figs. 5*a*, 5*b*, and 5*c* are for different ducks, while those in Figs. 5*b* and 5*d* are scans from the same duck, 45 min apart. T, thorax; V, viscera; L, legs; ▼, start of dive; ▲, end of dive.

(1980) for seals. In ducks, there is a large central blood pool containing about 75% of the total blood volume (TBV) which mixes only slightly with the 25% of TBV located in the hypoperfused tissues during a dive. During recovery, activity increased in the peripheral regions while it fell in the central cardiovascular area. These changes represent not only flow and volume redistribution in the postdive period, but also a dilution factor due to the increase in total circulating blood volume. Furthermore, arterial haematocrit did not change during dives, in contrast to results reported for some seals (Qvist et al. 1986). This observation was not unexpected because in ducks the spleen is extremely small (Nickel et al. 1977) and therefore cannot function as a reserve of oxygenated red blood cells during diving.

Time–activity curves of labelled RBC tracers injected either arterially or venously revealed that the time to reach an equilibrium during diving was at least 4 to 10 times longer for central and peripheral regions, respectively, than when RBCs were injected during rest. In ducks, this is a reflection of the reduction in cardiac output during diving rather than mixing with peripherally located pools of blood. The time taken to attain equilibrium during diving has important implications with regard to the use of tracers and metabolites in diving studies. Most tracer studies require that the tracer be equilibrated throughout all of the animal's cardiovascular compartments before any statement can be made as to its distribution, metabolism, or disappearance. Consequently, results of such studies must be regarded with suspicion in the absence of an accurate measure of flow pattern and mixing time during diving (Murphy et al. 1980; Castellini et al. 1985; Guppy et al. 1986).

Acknowledgements

D.R.J. is grateful to the Natural Sciences and Engineering Research Council of Canada and the British Columbia Heart Foundation for operating and equipment grants which supported this study. We are grateful to Dr. W. Amman for allowing us to use the Nuclear Medicine facilities in the University of British Columbia Acute Care Hospital; Dr. D. Lyster, Department of Nuclear Medicine, Vancouver General Hospital, for discussions and assistance with nuclear medicine technology; R. Singer, Department of Nuclear Medicine, British Columbia Institute of Technology, who first suggested the use of this technology in diving studies and helped in initiating this project; and T. McLintoch, who gave considerable practical assistance.

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