Volume capacity and contraction control of the seal spleen

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A rise in the red blood cell concentration has been observed during diving in the Weddell seal Leptonychotes weddelli (9, 23, 25, 30) and in the elephant seal Mirounga angustirostris (8). This rise in red blood cell concentration is believed to contribute toward extended submersion. While the source of this hematocrit elevation is undocumented in seals, several authors have suggested that the spleen is the place of origin (4, 7, 23, 29, 30). This is based on the fact that several terrestrial mammals have been shown to have a spleen that may act as a red blood cell reservoir (for review, see Ref. 31). Recently, in vivo experiments on Weddell seals, Hurford et al. (23) found that changes in splenic size (length and thickness) were accompanied by changes in arterial hematocrit levels. However, marine mammals also possess huge venous sinuses (5) in which blood flow may fluctuate substantially and at times even be stagnant (15, 22). It is, therefore, reasonable to assume that the large veins can also, by simple sedimentation of blood cells, be an origin of variations in systemic hematocrit in pinnipeds.

Several attempts have been made to estimate the volume capacity of the pinniped spleen (3, 19, 26), but most of these studies were based on postmortem weighing of the organ, which yields little real information on the size of the organ in vivo. Castellini and Castellini (7) estimated the mass of the spleen of three species of phocid seals to represent 4–14% of body mass by back calculations based on the rise in hematocrit observed during diving. In so doing, they had to assume, of course, that all the red blood cells originated from the spleen. Ponganis and co-workers (29) attempted to measure the maximal splenic volume in anesthetized harbor seals (Phoca vitulina) and sea lions (Zalophus californianus) by using computerized tomography. These animals were reported to have a spleen mass that varied between 0.8 and 3.0% of body mass.

The purpose of the present study was to test whether the seal spleen can contract and expel its contents in a way similar to that previously described for some terrestrial mammals. By using an in vitro technique, we have administered various drugs (adrenergic and cholinergic) to the spleens of hooded seals (Cystophora cristata), which are able to dive for >52 min and reach depths in excess of 968 m (18). We also recorded maximal splenic capacity and the hematocrit of the arterial blood and of the splenic venous outflow of these animals. Some measurements were also made on spleens from the harp seal (Phoca groenlandica), which is a not-so-capable diver (Ref. 17; Folkow and Blix, unpublished observations).

MATERIALS AND METHODS

Experimental Animals

A total of 12 hooded seals and 4 harp seals of both genders were caught or shot on the sea ice off the east coast of Greenland (71°N, 18°W) and then were used in this study. All animals were collected under license from the Norwegian Government, and the experiments were carried out under permit from the Norwegian Committee on Ethics in Animal Experimentation. Nine of the animals, including all the harp seals, were shot and used immediately onboard a research vessel equipped with proper laboratories while three others were brought alive to the Department of Arctic Biology at the University of Tromsø, Norway, and kept indoors in freshwater tanks under simulated natural-light conditions (70°N). The latter were fed capelin (Mallotus villosus) twice a day. In preparation for an experiment, the captive seals were killed by use of a “hakapik” according to Norwegian sealing regulations, without any use of anesthetics (2). An incision on the left ventral side of the animal provided easy access to the spleen. The splenic artery and vein were immediately clamped and ligated. The spleen was then carefully freed from all mesenteric connections with the surrounding organs, the short gastric and the epiploic vessels were clamped and cut, and finally the splenic artery and the splenic vein were cut and the spleen was removed.

Pharmacological Experiments

For the pharmacological experiments, one subadult hooded seal weighing 138 kg and two 7-mo-old hooded seals, weighing 52.4 and 66.8 kg, were used. In <30 min postmortem, the splenic artery was cannulated and the spleen was perfused with 37°C oxygenated saline. The splenic volume was measured plethysmographically. After removal of the spleen and start of perfusion, the spleen was placed in a bath made of two transparent Plexiglas cylinders (Fig. 1). The internal smaller cylinder, the organ

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bath (diameter: 0.25 m, length: 0.5 m), was filled with saline, and the larger cylinder surrounding the small one was circulated with thermostatically controlled water at 37°C. The composition of the physiological saline was (in mM) 119.0 NaCl, 25.0 NaHCO₃, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄, 5.5 glucose, and 0.027 EDTA. It was kept at 37°C and was oxygenated with a mixture of 95% O₂-5% CO₂.

The organ bath communicated with the outside through a small pipe. The cylinders were tilted to have a weak slope. Any change in the splenic volume was directly reflected in the size of a bubble of air at the top of the organ bath, measured by use of a ruler fixed to the bath. The size of the bubble was finally calibrated against volume (by using an equation correlating the volume withdrawn from the organ bath into a graduated cylinder to the corresponding value read simultaneously from the ruler). Within the bath, the spleen was perfused through its cannulated splenic artery while the perfusate left through the cannulated splenic vein, without being recirculated. On the arterial side, a peristaltic pump was used to maintain the arterial pressure at 96–110 mmHg, as directly read on a water column manometer connected close to the splenic artery. The minute volume of the pump was changed to maintain pressure despite any change in the splenic vascular resistance. On the venous side, the catheter in the splenic vein was raised to maintain a steady venous pressure of 6–8 mmHg.

Experiment 1. Epinephrine was administered in doses of 0.005, 0.01, 0.5, 1.0, or 5.0 µg/kg. Sufficient time (10–45 min) was allowed for the spleen to recover to its preinjection size before another dose was given.

Experiment 2. In separate series of experiments, epinephrine (1.0 µg/kg) and norepinephrine (1.0 µg/kg) were administered before and 4 and 9 min after injection of the α-adrenoceptor-blocker phentolamine (100 µg/kg).

Experiment 3. In separate series of experiments, acetylcholine was administered in doses of 0.1, 1.0, and 5.0 µg/kg and the β-adrenoceptor-agonist isoprenaline in doses of 0.1 and 1.0 µg/kg.

Experiment 4. In separate series of experiments, acetylcholine (5.0 µg/kg) and isoprenaline (1.0 µg/kg) were given 2–3 min after epinephrine (5.0 µg/kg).

Splenic Volumes

The maximal volume capacity of the spleen was measured in four hooded seals (270 kg, adult; 120 kg, adult; 56 kg, subadult; and 52 kg, juvenile) and in two adult harp seals (102 and 121 kg). In these animals, the excised spleens were first dilated by arterial perfusion with saline at a pressure of 96–110 mmHg, with a venous pressure of 12 mmHg, in the plethysmograph, after which they were removed to be weighed before and after injection of a high dose of epinephrine (1.0 or 5.0 µg/kg) into the splenic artery for determination of the maximal volume the spleen can expel.

Hematocrit Levels

The hematocrit was determined immediately after death in splenic venous and/or aortic blood samples from six hooded seals. The spleens of three of these seals (120 kg, adult; 148 kg, adult; and 52 kg, juvenile) were found to be dilated, whereas the spleens of the other three (214 kg, adult; 25 kg, 1 wk old; and 25 kg, 1 wk old) were contracted. Data were also collected from one adult harp seal and one 1-wk-old harp seal pup, both with dilated spleens. Hematocrit levels were determined by use of heparinized microhematocrit tubes after centrifugation at 13,000 revolutions/min for 5 min in a microcentrifuge.
Statistics

When appropriate, results were meaned for each animal before group means were calculated to avoid nesting effect. Multiple dose responses were compared and tested with nonparametric Kruskal-Wallis one-way analysis of variance. Other data were tested with Student's t-test when parametric and with Kolmogorov-Smirnov one-sample or \( \chi^2 \) tests when nonparametric.

RESULTS

Pharmacological Experiments

In vitro experiments were carried out for up to 7–9 h without apparent deterioration of the organ, as indicated by the capacity of the spleens to respond in a reproducible way to various drug injections.

All the spleens contracted within 1–3 min regardless of the doses of epinephrine. The contraction started at the apex and at the margins of the spleen and progressed toward the venous hilus. The time taken to return to the dilated stage was more or less proportional to the magnitude of the contraction (see Fig. 2), as shown by use of the Kruskal-Wallis test on the grouped data from two hooded seals (138 and 52.4 kg; \( K = 1.84, P < 0.25 \)). The dose-dependent response of the spleen of the 52.4-kg juvenile hooded seal to epinephrine is illustrated in Fig. 2.

Epinephrine injections in doses of 0.005 µg/kg caused minor responses. When 0.01 µg/kg epinephrine was injected, the spleen responded weakly and expelled only 750 ml. A dose of 1.0 µg/kg caused a stronger contraction with expulsion of 1,550 ml. Injection of 5.0 µg/kg of epinephrine in pilot experiments proved to cause extremely prolonged contraction periods and was avoided because of the potential risk of disrupting the preparation. Furthermore, this dose was found to increase the expelled volume by only ~20%, and, consequently, a concentration of 1.0 µg/kg was chosen as a submaximal dose for further experiments.

The spleens reacted similarly to epinephrine and norepinephrine, and the subsequent rates of dilation were also similar (Fig. 3). The mean (±SD) volume of fluid expelled from the spleens after an epinephrine dose of 1.0 µg/kg was 751 ± 693 ml (\( n = 3 \)) compared with 579 ± 519 ml (\( n = 3 \)) for norepinephrine in the same dose. These differences were not significantly different according to a paired Student's t-test (\( t = 1.47, P = 0.28 \)). In two hooded seals, injections of the general \( \alpha \)-adrenoceptor blocker phentolamine before epinephrine and norepinephrine largely abolished their effects (the degree of splenic emptying was reduced by 99.3 and 99.9%, respectively, for epinephrine, and by 99.1 and 92.7%, respectively, for norepinephrine, compared with the volume expulsion when epinephrine and norepinephrine were injected alone in doses of 1.0 µg/kg).

The \( \beta \)-adrenoceptor-agonist isoprenaline, given to dilated spleens (\( n = 3 \)) at doses of 0.1 and 1.0 µg/kg (experiment 3), did not cause any measurable volume effects. However, it was noticeable during the experiments that the color of the venous outflow became dark red after the injection of isoprenaline, an effect that lasted for ~5 min.

Injections of acetylcholine at concentrations of 0.1, 1.0, and 5.0 µg/kg (experiment 3) did not produce any measurable effects on the organ. Furthermore, when acetylcholine or isoprenaline was given in doses of 5.0 and 1.0 µg/kg, respectively, to spleens that were first made to contract by use of standard doses (1.0 µg/kg) of epinephrine (experiment 4), no obvious effect could be seen with regard to either the contraction state or the time taken by the spleen to return to its precontraction volume.

Fig. 2. Rate of contraction and subsequent dilation in a hooded seal spleen when subjected to a graded stimulation with epinephrine: 0.005 µg/kg (●), 0.01 µg/kg (○), 0.5 µg/kg (■), and 1.0 µg/kg (□).

Fig. 3. Rate of contraction and subsequent dilation in a hooded seal spleen when subjected to epinephrine (1.0 µg/kg; ●) and norepinephrine (1.0 µg/kg; ○).
Splenic Volumes

Figure 4 illustrates the relationship between the mass of the maximally dilated spleens and the respective body mass of seven hooded seals. The relationship can be described by a linear function: \( SM = 17.5M + 1085 \) \((r^2 = 0.92; n = 7)\), where \( SM \) is spleen mass (g) and \( M \) is body mass (kg).

The mass of the spleens of four hooded seals was reduced to 18, 22, 27, and 38%, respectively, of the mass of the dilated organs, after contraction by injection of a high dose (1.0 µg/kg) of epinephrine (Fig. 4). The size of the seals did not seem to influence this response (Kruskal-Wallis test; \( K = 3.0, P > 0.05 \)). In two harp seals, splenic mass was reduced to 16 and 14% of the mass of the dilated organs (1,514 and 1,888 g, respectively) after a similar treatment. The reductions in splenic mass of the two species were not significantly different (Kolmogorov-Smirnov test; \( K = 1.0, P = 0.12 \)). Figure 4 also illustrates the mass of the contracted spleens (\( n = 4 \)) plotted against body mass, which followed this relationship: \( SM = 5.3M + 255 \) \((r^2 = 0.83; n = 4)\). The maximal volume that could be expelled (V; ml) was estimated from the difference between the masses of dilated and contracted spleens and related to body mass according to the equation \( V = 12.0M + 910 \) \((r^2 = 0.96; n = 4)\). Thus a hooded seal of 112 kg should be able to expel 2,200 ml of blood by splenic contraction. In comparison, the maximum expelled volumes of two harp seals were 1,270 and 1,622 ml for the 102- and 121-kg animals, respectively. The average maximum expelled volume of these seals, which had a mean body mass of 112 kg, was 1,446 ml, which is significantly lower than the predicted value for a similar-sized hooded seal \( (\chi^2 \text{ test}; \chi^2 = 8.69, P < 0.05) \).

Hematocrit Levels

The hematocrit values obtained from the splenic veins of dilated spleens from adult hooded seals were 88, 90, and 93%, respectively, while the values for aortic blood from the first two were 51.5 and 57.5%, respectively, the sample from the last being lost. A hooded seal pup, moreover, had a hematocrit of 88% in the splenic venous blood and 59.5% in the aorta. In contrast, an adult hooded seal with a contracted spleen had a hematocrit of 57.5% in the splenic vein and 62% in the aorta. Two harp seals, one adult and one pup, had hematocrits of 83 and 88%, respectively, in the splenic venous blood.

DISCUSSION

The present study has shown that the spleens of pinnipeds contract very strongly when stimulated with catecholamines. The spleen of the hooded seal responds to arterial epinephrine injections with a dose-dependent decrease in its volume. Because the effects of epinephrine are not abolished when the \( \beta \)-adrenergic receptors were first blocked with phentolamine, there is reason to assume that the contraction is mediated mainly through activation of \( \alpha \)-adrenoceptors. This result is in accordance with recent observations by Hurford et al. (23) in Weddell seals in which epinephrine injections caused the spleen to contract. It also corresponds well with those obtained in various terrestrial mammals, e.g., guinea pigs (13) and dogs (27).

The possible role of \( \beta \)-adrenoceptors in the seal spleen contraction is at present obscure. Isoprenaline did not affect the quantity of fluid expelled from the spleens, suggesting that activation of \( \beta \)-adrenoceptors is not involved. Yet, the fact that the color of the venous effluent became darker red after isoprenaline injections suggests that \( \beta \)-adrenoceptors do exist and somehow may be involved in the process of releasing red blood cells from the spleen. In this context it is interesting to note that injection of small doses of isoproterenol (i.e., isoprenaline) in cats inhibited the red blood cell-concentrating mechanism (20), which implies that reten-

Cholinergic-like transmitters have histochemically been identified in the spleens of Weddell seals (L. weddelli), crabeater seals (Lobodon carcinophagus), and fur seals (Arctocephalus gazella) (32). Still, injections of acetylcholine in doses of 0.1, 1.0, or 5.0 µg/kg into the spleens of our hooded seals did not affect their volume capacity. This result is in accordance with observations in cats, mice, and humans, where splenic cholinergic innervation is not present (31). In dogs, however, Daly and Scott (12) found that low doses of acetylcholine may dilate the spleen, whereas higher doses caused contraction.

The high hematocrit values that we have recorded in splenic venous blood of hooded seals (88–93%) and harp seals (82–88%) with dilated spleens suggest that seals have the same ability to concentrate red blood cells as

Fig. 4. Mass of filled and fully dilated spleens (n = 7; ○) and residual mass after epinephrine-induced contraction (n = 4; ●) in hooded seals of different body masses. Relationships can be described by the following equations: dilated spleen mass = 17.5 × body mass + 1085 \((r^2 = 0.92; n = 7)\); contracted spleen mass = 5.3 × body mass + 255 \((r^2 = 0.83; n = 4)\).
do terrestrial mammals (1, 20). Similarly, the ability of seals to expel ~80% of the splenic volume is also in accordance with data for some terrestrial mammals, such as sheep (33) and dogs (6).

The maximal splenic expulsion during in vitro contraction in hooded and harp seals may be compared with the estimated total blood volume in the two species. If we assume this volume to be 12% of body mass (14), an adult 250-kg hooded seal would be able to expel ~13% of its blood volume (Fig. 4) while the corresponding value for an adult 112-kg harp seal is ~11%. Despite the fact that the hooded seal equals the Weddell seal in diving ability (18), this is dwarfed by the estimated splenic volume value of 30–40% for the latter (23, 30), which indicates that large species differences may exist among various phocid species. However, the Weddell seal estimates were mainly based on the assumption that the observed increase in the hematocrit during a dive was caused by splenic contraction alone, a notion that deserves to be reexamined.

The mass of the maximally dilated hooded seal spleen represents 2–4% of body mass (Fig. 4) and that of the adult harp seal 1.5% of body mass. These values are substantially lower than the 13.9% for the Weddell seal, 7.3–10.5% for the northern elephant seal, and 4.3% for the harbor seal, as estimated by Castellini and Castellini (7). But, again, their indirect estimates were based on the assumption that the spleen is the only origin of red blood cells. Ponganis and co-workers (29) have up to this time provided the only in vivo determination of seal splenic volume by use of computed tomodraphy in harbor seals. Their study indicates a splenic mass of 0.8–3.0% of body mass, which is comparable with our data. Thus the low value of the spleen-to-body mass ratio in the only two studies where the volume of the spleen was measured reinforces the assumption that some red blood cell sequestration may also occur elsewhere in the body, possibly in the inferior vena cava and the hepatic sinuses. Such venous sequestration of red blood cells has previously been shown to take place, e.g., in dogs (6).

Our data on maximal splenic volume expulsion and hematocrit values in hooded and harp seals may be used to estimate how much splenic contraction may theoretically contribute to their aerobic diving limits [ADLs (25)]. According to our data, a 250-kg hooded seal will be able to expel 3.9 liters of blood with a hematocrit of 90%. Hooded seal blood with a hematocrit of 63% has a hemoglobin (Hb) concentration of 264 g/l (11). Blood with a hematocrit value of 90% will then have a Hb concentration of 1.43 × 264 g/l, and the O2-binding capacity of Hb is 1.34 ml O2/g Hb (24). Finally, if one assumes that the diving metabolic rate of the hooded seal is similar to that of the Weddell seal, i.e., 4.5 ml O2·kg⁻¹·min⁻¹ (10, 28) and that splenic blood is 100% saturated with O2, it follows that the estimated ADL of our 250-kg hooded seal will only increase at most by ~105 s on splenic contraction, while the value for a 112-kg harp seal is only ~80 s. This minor increase in ADL for two expert divers like the harp seal, and, in particular, the hooded seal, suggests that splenic contraction is not primarily a means to extend submersion time, as proposed by Hochachka (21) and Zapol (34) but more likely is a means to increase the O2-carrying capacity of blood and, hence, reduce surface time during repeated diving, as suggested by Castellini and co-workers (9) and Ponganis and colleagues (29). Another way of looking at it is that splenic red blood cell concentration takes place to reduce blood viscosity while the animal is at rest, as suggested by Elsner and Mieselman (16).

In conclusion, this study has shown that the spleen of some arctic phocids is capable of storing substantial blood volumes, which are released by rapid and forceful contraction of the spleen on α-adrenergic receptor activation, whereas β-adrenergic and cholinergic receptors have little effect. The hematocrit of harp and hooded seal splenic venous blood may reach levels of 90% or more, and maximal splenic contraction causes expulsion of a volume (ml) that relates to body mass (kg) according to the relationship $V = 12.0M + 910$ in hooded seals. This increase in the circulating blood volume does not seem to cause any substantial increase of the ADL of hooded seals but will, of course, increase the O2-carrying capacity of the blood, and, conversely, the blood viscosity will be reduced on splenic dilation.

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