Hydrostatic-pressure and temperature effects on the molecular order of erythrocyte membranes from deep-, shallow-, and non-diving mammals

E.E. Williams, B.S. Stewart, C.A. Beuchat, G.N. Somero, and J.R. Hazel

Abstract: Little is known about the cellular mechanisms involved in the tolerance of deep-diving marine mammals to hydrostatic pressures that cause serious pathologies when experienced by other mammals. We compared fatty-acid composition, cholesterol content, and the effects of pressure on the molecular order of erythrocyte membranes from deep-, shallow-, and non-diving mammals to determine how these properties may be related to diving performance. Erythrocytes were collected from two deep-diving phocid pinnipeds (northern elephant seal (Mirounga angustirostris) and harbor seal (Phoca vitulina)), a relatively shallow-diving otariid pinniped (northern fur seal (Callorhinus ursinus)), and several nondiving terrestrial mammals (dog (Canis familiaris), horse (Equus caballus), and cow (Bos taurus)). Fatty-acid composition clearly distinguished the phocids from the other species. The monoene content of erythrocyte membranes was substantially lower (3 vs. 20%) whereas the lipid unsaturation indices, the ratio of \( \alpha \) to \( \gamma \)-linolenic acids, and the proportions of long-chain polyunsaturated fatty acids were substantially higher in the phocids. The cell-membrane cholesterol content was also significantly lower in erythrocytes from the deep-diving phocids (cholesterol:phospholipid ratios 0.2–0.3) than from most other mammals (1.0). Membranes from the phocids were more ordered than those from the shallow- and non-divers, and were also more sensitive to changes in pressure and temperature. The physiological significance of these differences in cell-membrane structure, which affect the order and sensitivity of cell membranes to hydrostatic pressure, is unknown, but they may be important adaptations that allow repeated and prolonged exposure to great hydrostatic pressure.

Résumé : On sait peu de choses au sujet des mécanismes cellulaires qui assurent la tolérance aux pressions hydrostatiques chez les mammifères marins qui plongent en eau profonde, pressions qui entraînent des pathologies graves chez les autres mammifères. Nous avons comparé la composition en acides gras, le contenu en cholestérol et les effets de la pression sur l’arrangement moléculaire des membranes érythrocytaires de mammifères plongeurs en eau profonde et en eau peu profonde et de mammifères non plongeurs pour déterminer comment ces propriétés peuvent être reliées à la performance à la plongée. Des érythrocytes ont été prélevés chez deux pinnipèdes plongeurs en eau profonde (l’Éléphant de mer boréal (Mirounga angustirostris) et le Phoque commun (Phoca vitulina)), chez un pinnipède otariidé plongeur en eau relativement peu profonde (l’Otarie à fourrure du Nord (Callorhinus ursinus)) et chez plusieurs mammifères terrestres non plongeurs (Chien (Canis familiaris), Cheval (Equus caballus) et Vache (Bos taurus)). La composition en acides gras établit clairement la distinction entre les phocidés et les autres espèces. Chez les phocidés, le contenu en monoènes des membranes érythrocytaires est nettement moins élevé (3 vs. 20%), alors que les coefficients d’insaturation des lipides, le rapport entre les acides linoléiques \( \alpha \) et \( \gamma \) et les proportions d’acides gras polyinsaturés à chaînes longues sont substantiellement plus élevées. Le contenu en cholestérol des membranes érythrocytaires est aussi significativement plus faible chez les phocidés qui plongent en eau profonde (rapport cholestérol : phospholipides de 0,2–0,3) que chez la plupart des autres mammifères (1,0). Les membranes des phocidés ont un arrangement moléculaire plus ordonné que celles des mammifères plongeurs en eau peu profonde et que celles des animaux non plongeurs ; elles sont aussi plus sensibles aux changements de pression et de température. L’importance physiologique de ces différences de structure dans les membranes cellulaires, qui affectent l’arrangement et la sensibilité des membranes aux pressions hydrostatiques, reste obscure, mais il s’agit peut-être là d’adaptations importantes qui permettent l’exposition répétée et prolongée à des pressions hydrostatiques élevées.

[Traduit par la Rédaction]
Introduction

Many species of marine mammals dive to substantial depths, and phocid pinnipeds are among the deepest divers (e.g., see reviews by Boyd 1997; Butler and Jones 1997). Indeed, elephant seals (Mirounga angustirostris and Mirounga leonina) dive continuously to depths up to 1600 m while at sea for 8–10 months each year (DeLong and Stewart 1991; Hindell et al. 1991; Stewart and DeLong 1995). The dives of otariid pinnipeds are generally shallower though still considerable. For example, northern fur seals (Callorhinus ursinus) may dive to 200 m depth, and California sea lions (Zalophus californianus) and Hooker’s sea lions (Phocarctos hookeri) to 300–500 m depth (Feldkamp et al. 1989; Gales and Mattlin 1997). In contrast, well-trained humans attempting to set a world record can reach only 100 m (Hong 1988). Pinnipeds can reach and forage at these depths, owing largely to adaptations for storing large amounts of oxygen in blood and tissue, efficient locomotion, and reduced metabolism in most tissues when submerged, which allow the seals to go for long periods without breathing (e.g., Boyd 1997; Butler and Jones 1997; Ponganis et al. 1997; Cabanac 2000). However, the cellular mechanisms that permit repeated and rapid compression and decompression and prolonged exposure to great hydrostatic pressure are less clear.

When terrestrial and shallow-diving vertebrates are exposed to elevated hydrostatic pressure, the result is a suite of neurological symptoms, collectively referred to as the high-pressure neurological syndrome (HPNS; cf. Butler and Jones 1997). HPNS is characterized by motor spasms, seizures, and ultimately death (Brauer et al. 1979; Halsey 1982; Bowser-Riley 1984; Brauer 1984). Diving pinnipeds are often exposed to hydrostatic pressures far greater than those known to cause HPNS in those mammals.

Elevated hydrostatic pressure causes HPNS, in part, by altering the molecular order of cellular membranes (Bowser-Riley 1984; Brauer 1984; Hazel and Williams 1990). Pressure-induced changes in the molecular order of cellular membranes adversely influence a number of membrane-associated cell functions, including nerve conduction and excitability (Doubt and Hogan 1979) and active and passive transport of sodium (Goldinger et al. 1980). The fatty-acid and cholesterol composition of membranes has a strong influence on membrane molecular order (Hazel and Williams 1990). Consequently, the apparent insensitivity of deep-diving marine mammals to profound changes in hydrostatic pressure may be due to adaptive variation in the molecular composition of cell membranes.

Here we compare the fatty-acid and cholesterol composition of erythrocyte membranes among deep- and shallow-diving pinnipeds and some nondiving terrestrial mammals and experimentally evaluate the effects of hydrostatic pressure and temperature on the molecular order of these membranes to determine if observed differences in diving performance may be related to taxonomic differences in cell-membrane qualities.

Material and methods

We studied the composition of the cell membranes of erythrocytes of three pinniped species (northern fur seal; northern elephant seal, M. angustirostris; harbor seal, Phoca vitulina) that differ in diving performance and three nondiving terrestrial animals (dog, Canis familiaris; horse, Equus caballus; cow, Bos taurus). Our objectives were to investigate structural cellular mechanisms that may allow marine mammals to repeatedly dive to substantial depths without suffering the pathology that occurs in most mammals exposed to unusual hydrostatic pressure. We used erythrocyte membranes for our studies because mammalian erythrocytes lack internal organelles and, consequently, preparations from these cells are enriched with a single membrane type (plasma membrane) uncontaminated by the membranes of other organelles. Moreover, erythrocytes of adult mammals cannot synthesize cholesterol, fatty acids, or phospholipids (Mulder and Van Deenen 1965; Popp-Snijders et al. 1984), therefore they are unable to respond to changes in pressure or temperature by de novo synthesis of membrane components, so the lipids that we extracted from erythrocyte membranes should be identical with those found in cells in vivo.

Preparation of erythrocyte membranes

We collected blood samples from the extradural vein, the interdigital vein of a rear flipper, or the caudal–gluteal sinus (cf. Sweeney 1993) of adult pinnipeds at San Nicolas and San Miguel islands in southern California. We chemically immobilized (with 2–4 mg/kg ketamine hydrochloride) elephant seals prior to sampling, but only physically restrained harbor and northern fur seals and the terrestrial mammals while collecting blood samples. The blood samples were then stored at ~80°C in heparin-treated glass vacuum vials and later thawed and diluted with 4.8 volumes of buffer A (5 mM phosphate buffer, pH 8.00, at 23°C) and subjected to four additional freeze–thaw cycles (~80°C to room temperature). Further dilution with 2.4 volumes of buffer A was followed by centrifugation for 30 min at 150 000 × g. The membrane pellet (predominantly erythrocyte membranes but also containing leukocyte membranes) was washed three times by resuspension in 7.2 volumes of buffer A and centrifugation for 30 min at 150 000 × g. The membrane preparations were resuspended in 1.0–1.5 mL buffer A and stored at ~80°C until use.

Fatty acid methyl ester, cholesterol, and phospholipid phosphorus analyses

Fatty acid methyl esters were prepared from total lipid extracts (Bligh and Dyer 1959) of purified membrane fractions by acid-catalyzed transesterification in methanolic HCl (Christie 1982). The methyl esters were extracted with hexane, then separated by capillary gas chromatography, at 190°C on a Omegawax 320 column (30 m) with a Hewlett Packard 5840A chromatograph and peak integrator. Triplicate analyses were carried out for each membrane preparation. Membrane cholesterol was assayed by means of a cholesterol oxidase-based fluorometric method (Crockett and Hazel 1995) and a Perkin–Elmer MFP-44A fluorescence spectrophotometer. Commercially available cholesterol was used as the standard. Membrane phospholipid content was assessed by measuring the total phospholipid phosphorus content (Chen et al. 1956) of the chloroform–methanol extracts of the erythrocyte membrane preparations.

Determination of membrane order

We measured membrane anisotropy, which is directly proportional to membrane molecular order (Shinitzky and Barenholz 1978), in a high-pressure optical vessel (Mustafa et al. 1971). Measurements were made at 37°C to simulate body-core temperature and at 27°C to simulate the conditions experienced by cells in the peripheral circulation (Irving and Hart 1957). The lower temperature allowed a comparison of pressure and temperature sensitivities of the membranes. The sample chamber was stirred by a motorized magnet situated below it and a small Teflon-coated stir bar located within it. The temperature of the sample chamber was maintained to within 0.1°C of the desired value by a water-circulating temperature-
regulated water bath. Pressure in the vessel was increased in 1.7-
MPa increments at a rate of 3.4 MPa/min by means of a hand-
operated hydraulic pump. We then made three measurements at
each of seven chamber pressures (0.1, 4.1, 6.9, 11.0, 13.7, 17.9,
and 20.7 MPa). The greatest experimental pressure used was equal
to a dive to 2072 m depth, slightly more than the deepest dives yet
recorded for free-ranging elephant seals.

Erythrocyte-membrane suspensions were thawed on ice and diluted
to an absorbance of less than 0.150 absorbance units at 364 nm
with buffer A, after which 2 mM 1,6-diphenyl-1,3,5-hexatriene
(DPH) in N,N-dimethylformamide was added (3 mL/mL diluted
membrane suspension) and the mixture was slowly stirred in the
dark for 1 h. Membrane anisotropy was measured using the steady-
state fluorescence depolarization of DPH with a Perkin–Elmer
model MPF-44A fluorescence spectrophotometer. Excitation was
at 364 nm and emission was measured at 430 nm, both using slit
widths of 5 nm. The excitation polarizing filter was successively
oriented at angles of 0° and 90°, and emission was measured parallel
and perpendicular to these orientations. There was no emission
from light-scattering. Depolarization due to polarizer inefficiency
and window birefringence at high pressure was corrected (Paladini
1981) using both (i) the intensity of the signal arising from DPH in
stable planar lamellae vesicles of dipalmitoylphosphatidylincholine (pre-
pared by the ether-evaporation method of Grunner et al. 1985),
which exhibits a well-defined phase transition at 41°C and (ii) DPH
and fluorescein in glycerol at 0°C. The anisotropy parameter (in ar-
bitrary units) was calculated from the corrected polarization (P)
data as

\[
[1] \quad \left(\frac{(3r_0 - Pr_0)}{2P}\right) - 1
\]

(Shinitzky and Barenholz 1978), where the intrinsic anisotropy (r_0)
dephospholipids of cellular membranes.

Materials
DPH was obtained from Molecular Probes (Eugene, Oreg.). N,N-
-dimethylformamide, fluorescein, cholesterol, and dipalmitoylphos-
pholipidylcholine were obtained from Sigma Chemical Co. (St.
Louis, Mo.). Protein was determined using the microprotocol of
Pierce’s (Rockford, Ill.) Coomassie Plus assay with buffer A as dil-
uent and bovine serum albumin as standard.

Statistics
Multiple comparisons were made using one-way ANOVA with
unequal sample sizes followed by Scheffe’s post-hoc test. Slopes
and intercepts were calculated by linear regression and significance
was judged at \( p \leq 0.05 \). All percentage data were arcsine-
transformed before statistical analysis (Sokal and Rohlf 1981).
Values are reported as means ± 1 SE.

Results
Lipid composition of erythrocyte membranes
The fatty-acid composition of erythrocyte membranes var-
ied significantly among the six mammal species (Table 1).
The saturated fatty acids palmitic acid (16:0) and stearic
acid (18:0) contributed substantially (up to 36%) to the total
fatty-acid content in each species. The monoenes palmitoleic
acid (16:1) and oleic acid (18:1) and the two isomers of
linolenic acid (18:3n-6 and 18:3n-3) varied among species
but were nonetheless represented still. The monoenes (18:1
18:1, and 20:1 contributed less (≤16%) overall to the total
fatty-acid groups in all the pinnipeds than in the terrestrial
species (\( p = 0.03 \); Table 1). Indeed, monoenes contributed
less than 4% to the fatty acids in the erythrocyte membranes
of the two phocid pinnipeds. The monoene content of the
otariid pinniped (northern fur seal) was closest to that of the
terrestrial mammals (Table 1). Diminished proportions of
18:1 in the membranes of elephant and harbor seals ac-
counted for most of the observed difference in monoene
content (Table 1).

In the phocids, the ratio of \( \alpha \)-linolenic acid (18:3n-3) to \( \gamma 
-linolenic acid (18:3n-6) was substantially greater than unity
(averaging 19 in the northern elephant seal and 39 in the
harbor seal) compared with less than unity in the fur seal
and the terrestrial mammals (Table 1). The erythrocyte
membranes of phocids also had notably higher quantities of long-
chain, particularly long chain polyunsaturated, fatty acids
(Table 1) (together, 22- and 24-carbon fatty acids repre-
sented 28% of the total in both the elephant and the harbor
seal) than the fur seal (16%) and the terrestrial mammals
(16% for dog, 8% for horse, and 12% for cow).

The unsaturation index (calculated as the average number
of double bonds per 100 fatty-acid chains) varied among
species (Table 1). It was greater in the two deepest diving
species than in the other species, though the difference was
not significant (\( p = 0.064 \)). It should be noted that both the
cis–trans configuration and the number and position of dou-
ble bonds in a fatty acid impact the molecular order of the
phospholipids of cellular membranes.

The ratio of cholesterol to phospholipids (mol/mol), an
index of the relative amount of cholesterol in the erythrocyte
membrane, was 0.21 ± 0.04 (\( N = 3 \)) in elephant seals compared
with 0.26 ± 0.04 in harbor seals (\( N = 3 \)). This difference was
not significant, though both values are considerably lower
than those reported for the erythrocytes of terrestrial mam-
nals (i.e., those for dog, horse, cow, pig, ox, sheep, rabbit,
rat, and human range from 0.8 to 1.1; Van Deenen and De
Gier 1964; Nelson 1967). The tissue available from the
northern fur seal was not adequate for quantitative measure-
ment of this ratio.

Erythrocyte-membrane molecular order
Membrane anisotropy (i.e., membrane molecular order)
increased in response to both elevation in hydrostatic pres-
sure and reduction in temperature in each species (Fig. 1; for
comparison, the anisotropy parameter for artificial mem-
branes composed entirely of dipalmitoylphosphatidylincholine
at 20°C was 5.0). Erythrocyte membranes from elephant and
harbor seals were significantly more sensitive to changes in
both temperature (the differences in height of the two lines
in each panel of Fig. 1) and pressure (the slopes of those
lines) than were those of the fur seal and the terrestrial
mammals (Fig. 1). Erythrocyte membranes from elephant
and harbor seals were much more ordered than those of the
terrestrial mammals even at normal body-core temperature
(37°C) and sea-level pressure (0.1 MPa). Membrane order,
as indexed by the change in the value of the anisotropy pa-
rameter, increased more (0.27°C for the elephant seal and
0.11°C for the harbor seal) as temperature declined in the
phocids than in the fur seal (0.06°C) and the terrestrial
mammals (0.03–0.05°C; all \( p < 0.01 \)). Similarly, erythro-
cytes of elephant and harbor seals were more sensitive to
changes in pressure (anisotropy increase of 627 × 10^{-4}/MPa
in the elephant seal and 561 × 10^{-4}/MPa in the harbor seal)
than were those of the fur seal (142 × 10⁻⁴/MPa) and the terrestrial mammals (188 × 10⁻⁴ to 153 × 10⁻⁴/MPa; p < 0.01). Anisotropy-parameter values for the erythrocyte membranes of elephant and harbor seals deviated substantially at the highest pressures and lowest temperature tested from the linear pattern characteristic at lower pressure and higher temperatures (Fig. 1).

### Table 1. The fatty acid composition (mass percent of total fatty acids) of erythrocyte membranes from diving and terrestrial mammals.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Elephant seal ((N = 5))</th>
<th>Harbor seal ((N = 4))</th>
<th>Northern fur seal ((N = 3))</th>
<th>Dog ((N = 3))</th>
<th>Horse ((N = 3))</th>
<th>Cow ((N = 1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>17.3 (3.5)</td>
<td>16.8 (1.3)</td>
<td>15.1 (1.0)</td>
<td>16.9 (0.4)</td>
<td>14.1 (0.4)</td>
<td>14.4</td>
</tr>
<tr>
<td>16:1</td>
<td>0.9abc (0.2)</td>
<td>0.9def (0.1)</td>
<td>4.3ad (0.1)</td>
<td>4.9be (0.4)</td>
<td>4.0ef (0.9)</td>
<td>7.4</td>
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<tr>
<td>16:2</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>1.6 (0.0)</td>
<td>2.2 (0.2)</td>
<td>1.9 (0.2)</td>
<td>3.0</td>
</tr>
<tr>
<td>16:3</td>
<td>0.7a (0.1)</td>
<td>0.4b (0.1)</td>
<td>1.4bc (0.1)</td>
<td>1.1 (0.1)</td>
<td>1.9ac (0.3)</td>
<td>3.2</td>
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<td>13.4 (0.9)</td>
<td>15.8 (0.9)</td>
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<td>0.9 (0.9)</td>
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<td>0.3def (0.1)</td>
<td>3.6adgh (0.3)</td>
<td>8.5begi (0.6)</td>
<td>23.2cfhi (1.2)</td>
<td>6.8</td>
</tr>
<tr>
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<td>11.7def (1.3)</td>
<td>1.3d (0.2)</td>
<td>1.4e (0.2)</td>
<td>2.0f (0.4)</td>
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<td>1.4bd (0.3)</td>
<td>1.3ce (0.2)</td>
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<td>0.9de (0.1)</td>
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<td>8.9bd (0.6)</td>
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<th>3.4</th>
<th>3.4</th>
<th>15.5</th>
<th>17.5</th>
<th>25.9</th>
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<td>Ratio of α-linolenic acid to γ-linolenic acid</td>
<td>18.67</td>
<td>39.00</td>
<td>0.36</td>
<td>0.16</td>
<td>0.09</td>
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<tr>
<td>Long chain polyunsaturated fatty acid (≥22 carbons) content (%)</td>
<td>25.9</td>
<td>26.1</td>
<td>8.8</td>
<td>7.9</td>
<td>4.8</td>
<td>5.8</td>
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<td>Unsaturation indexa</td>
<td>189.2</td>
<td>162.9</td>
<td>157.3</td>
<td>142.8</td>
<td>157.5</td>
<td>143.5</td>
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</tbody>
</table>

Note: Values are given as the mean ± SEM (in parentheses) of the indicated number of individuals (N). In each row, values followed by the same letter are significantly different; nd, not detected.

The average number of double bonds per 100 fatty acids.

than were those of the fur seal (142 × 10⁻⁴/MPa) and the terrestrial mammals (188 × 10⁻⁴ to 153 × 10⁻⁴/MPa; p < 0.01). Anisotropy-parameter values for the erythrocyte membranes of elephant and harbor seals deviated substantially at the highest pressures and lowest temperature tested from the linear pattern characteristic at lower pressure and higher temperatures (Fig. 1).

### Discussion

The performance of deep-diving pinnipeds, documented by numerous researchers, demonstrates clearly that these mammals can tolerate exposure to relatively great hydrostatic pressure without suffering ill effects, though it is also clear that in other mammals, substantially lower hydrostatic pressures adversely affect membrane molecular order and function and can trigger serious pathology (i.e., HPNS; Doubt and Hogan 1979; Brauer et al. 1979; Doubt and Hogan 1980; Goldinger et al. 1980; Halsey 1982; Bowser-Riley 1984; Hazel and Williams 1990). It is also known that when the animals are confronted with a change in pressure (or temperature as a thermodynamic counterpart), membrane fatty-acid composition and cholesterol content may be altered in some species to preserve membrane molecular order and function (Sinensky 1974; Hazel and Williams 1990). The fatty-acid composition and cholesterol content, and the effects of hydrostatic pressure and temperature on the molecular order of erythrocyte membranes, differed fundamentally between the two deep-diving phocids and all others. Levels of monoenoic fatty acids and cholesterol were significantly lower in erythrocyte membranes of phocid pinnipeds and ratios of α- to γ-linolenic acids and content of long-chain polyunsaturated fatty acids were substantially higher. Nonetheless, the molecular order of phocid erythrocyte...
Consequently, some of the differences in fatty-acid composition between the deep-diving pinnipeds and the terrestrial mammals we studied may better explain the evident differences in membrane lipid composition, which counteracts the membrane-ordering effects of elevated pressure and reduced temperature. Yet other differences that we observed in the elephant and harbor seal, such as the substantially reduced levels of 18:1, are less easy to reconcile with homeoviscous theory because monoene fatty acids (especially 18:1) decrease membrane molecular order to almost the same degree as polyunsaturated fatty acids do (Coolbear et al. 1983; Holte et al. 1985). Moreover, pinnipeds experience continuous and rapid changes in pressure when diving, which differs fundamentally from conditions of constant high pressure experienced by abyssal animals. If membranes fashioned to function properly at the surface are dysfunctional at depth, it is also likely that membranes constructed to function at depth are dysfunctional at the surface. To maintain membrane molecular order relatively constant under the cyclic patterns of exposure to hydrostatic pressure experienced by diving pinnipeds, homeoviscous restructuring would have to occur within seconds, or a few minutes at most. Membrane restructuring on the time scale of hours to a few minutes has been reported in some species (Carey and Hazel 1989; Dey and Farkas 1992; Williams and Hazel 1994a, 1995; Williams and Somero 1996), and it is possible that cells from other species may respond more quickly. Though erythrocytes lack the ability to synthesize membrane lipids de novo (Mulder and Van Deenan 1965; Popp-Snijders et al. 1984), rearrangement of phospholipid components to produce new lipid structures, perhaps resulting in continuous restructuring, is possible (Lynch and Thompson 1984; Williams and Hazel 1994b). However, it would likely be inefficient and metabolically very costly at the temporal and pressure scales faced by diving pinnipeds. Consequently, we think that continuous restructuring of the membrane is not a likely explanation for the apparent cellular tolerance of pinnipeds to hydrostatic pressure.

Alternatively, the cell membranes of deep-diving pinnipeds might have a unique composition that makes them less sensitive to changes in pressure than the membranes of other species, which allows membrane molecular order to be maintained over a large range of pressures. Such a mechanism would be instantaneous and energetically efficient. But this alternative is clearly not supported by our findings. The observed differences in membrane lipid composition did not lower the pressure sensitivity of the membranes from the deep divers (Fig. 1). Indeed, membranes from the deep-diving phocids were the most sensitive to pressure and temperature (Fig. 1).

The low cholesterol:phospholipid ratios that we found in erythrocyte membranes from deep-diving pinnipeds compared with terrestrial mammals suggest that alterations in this ratio may be important in deep divers. Cholesterol clearly reduces the temperature sensitivity of membrane order in the intestinal epithelium of trout (Crockett and Hazel 1997). This is consistent with our finding that the sensitivity of erythrocyte membranes to pressure was less in the species whose mem-

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branes were richer in cholesterol. If membrane cholesterol content is indeed related to diving performance, then we predict that cholesterol:phospholipid ratios in erythrocyte membranes of shallow-diving marine mammals would range between 0.7 and 0.3, i.e., somewhere between the values for terrestrial species and those we found for deep-diving species. The cholesterol:phospholipid ratios in membranes of blood platelets range from 0.378 in ringed seals (Phoca hispida) to 0.435 in harp seals (Phoca groenlandica) and 0.458 in grey seals (Halichoerus grypus; Ahmed et al. 1989).

From what is known of the diving performance of these relatively shallow divers (cf. Lydersen and Hammill 1993; Lydersen and Kovač 1993; Lydersen et al. 1994), the cholesterol:phospholipid ratios of the blood platelets are within the predicted range. Several other physical properties of cell membranes are greatly affected by cholesterol, including intrinsic membrane curvature stress, membrane microdomain formation, and membrane fusibility (Williams 1998), which may also explain some of the variability among marine mammals in their diving performance.

We think that, overall, our data and observations support the argument that increased membrane order is an adaptation for deep diving. Homeoviscous adaptation does not appear to preserve membrane order under pressure in erythrocytes of deep-diving pinnipeds. Membranes from elephant and harbor seals have the greatest molecular order and are more sensitive to pressure and temperature than the other species we studied. And membranes from elephant and harbor seals have substantially smaller proportions of 18:1 (resulting in increased membrane order) and cholesterol (resulting in increased sensitivity of the membranes to pressure) than the other species (Butler and Jones 1997).

Though the cellular mechanisms underlying hypometabolism, an important adaptation for long-duration diving in marine mammals (e.g., Butler and Jones 1997), are as yet unknown, we think that increased membrane order may have an important role in facilitating them. It is known that membrane lipid bilayer permeability to Na⁺ and the activity of the Na⁺,K⁺-ATPase in the gills of teleost fish decrease with increased sensitivity of the membranes to pressure) than the other species (Butler and Jones 1997).

In conclusion, erythrocyte membranes from a selected group of shallow-diving marine mammals and nondiving terrestrial mammals are similar in fatty acid chain composition and cholesterol content. Membranes from these species also exhibit similar sensitivity of membrane molecular order to changes in pressure and temperature. In contrast, membranes from two deep-diving phocid pinnipeds stand out as being significantly different in fatty acid chain composition and a much lower cholesterol content. The erythrocyte-cell membranes of these deep divers also differ by being more ordered and more sensitive to changes in pressure and temperature. These differences in cell-membrane structure and sensitivity to hydrostatic pressure may be important adaptations that allow marine mammals to regularly descend to great depths to exploit productive biological communities without suffering pathologies that nondiving mammals do when exposed to increased hydrostatic pressure.

Acknowledgments

We thank P. Yochem and M.A. Castellini for comments on the manuscript, E.L. Crockett for advice on the use of the cholesterol assay, and P. Yochem for advice on handling pinniped tissues. We thank S. Lovelady for providing blood samples from the terrestrial mammals. The comments and suggestions of two anonymous reviewers improved this paper and are much appreciated. Blood samples from pinnipeds were collected under the authorization of Marine Mammal Protection Act Scientific Research Permits Nos. 579 and 827 to B.S.S. This work was supported by grants from the National Science Foundation (IBN 92-06660, IBN 95-07226, and IBN 93-07024) and from Hubbs–SeaWorld Research Institute. The work of B.S.S. was partially supported under contract to Space and Missile Systems, U.S. Air Force.

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