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Properties of red blood cell after multiday exposure to 31 ATA

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Shiraki K, Sagawa S, Konda N, Nakayama H, Matsuda M. Properties of red blood cell after multiday exposure to 31 ATA. Undersea Biomed Res 1983; 10(4):349–358.—Hematologic changes, osmotic fragility of red cells, and lipid contents of plasma and red blood cells were determined during the course of a 14-day dry saturation dive at 31 ATA (SEADRAGON IV). Increases in hematocrit and red blood cell count were observed during the early period at 31 ATA, which was attributed to hemoconcentration resulting from a diuresis. The osmotic fragility of red blood cells decreased slightly during the course of the experiment, but the changes were not related to the atmospheric pressure. Cholesterol and phospholipid content of the red blood cell and the plasma showed slight, insignificant daily variations independent of pressure. However, the same overall correlation was observed between the osmotic fragility and the phosphatidylcholine content of red blood cells and between the osmotic fragility and the total cholesterol level in the plasma, at both 1 ATA and 31 ATA. These correlations revealed an intimate relationship between the cell fragility and lipid composition of the cell membrane and the plasma within intra- and interindividual variations during the course of the experiment, but did not indicate any significant alteration of red blood cell function by the prolonged exposure to 31 ATA helium-oxygen environment. These results indicate that the exposure of human divers to 31 ATA for 14 days does not seem to induce any irreversible chemical changes in the red blood cell.

erythrocyte
lipids
osmotic fragility
pressure

In recent years, many dry saturation diving experiments have been conducted in which human divers are exposed to a pressure up to 70 ATA for many days. Typically, the oxygen pressure of the hyperbaric chamber gas in these dives is maintained slightly hyperoxic, 0.3 to 0.6 ATA, depending on the level of the chamber pressure. Therefore, divers are exposed not only to high hydrostatic pressure but also to high diluent gas (usually helium) and oxygen pressures during the multiday dive period.

Goldinger et al. (1) reported that both ouabain-sensitive and -insensitive Na efflux is inhibited reversibly by 40% when human erythrocytes are exposed in vitro to 30–140 ATA of hydrostatic pressure for 1–2 h. Similar results were obtained during a brief exposure of human erythrocytes

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to high helium or argon pressure (2). Although the mechanism underlying these pressure effects is unknown at present, it is possible that pressure might alter the membrane chemistry such as the lipid content, which could be detected during a prolonged exposure to high pressure. Such a change in the membrane lipid could also alter the osmotic fragility of the erythrocyte. However, the long-term effect of high pressure on the membrane lipid chemistry or the osmotic fragility of human erythrocytes, or both, is poorly understood.

The maintenance of a hyperoxic level of P_{O_2} might also result in a detrimental effect on the erythropoietic process during a prolonged dive leading to a reduction in the erythrocyte count.

The present investigation was undertaken during the course of a 14-day dry saturation dive at 31 ATA (SEADRAGON IV), which was conducted at the Japan Marine Science and Technology Center, to study in human divers 1) hematologic changes, 2) osmotic fragility, and 3) lipid (including phospholipids) content of plasma and erythrocytes. The overall results indicate that the prolonged exposure to 31 ATA He-O_2 environment does not significantly alter these functions.

METHODS

Four healthy male volunteer divers stayed in a hyperbaric chamber (7.5 m long, 2.3 m diam) in the Japan Marine Science and Technology Center, Yokosuka, Japan. The physical characteristics of subjects are shown in Table 1. The experiment was undertaken from July 30 through August 31, 1979, and the overall diver profile is shown in Fig. 1. During the predive and postdive periods the subjects breathed the chamber air, which was maintained at a temperature (T_a) of 26°C ± 0.5°C and a relative humidity (rh) of 60%. The chamber pressure was first raised to 11 ATA in 10 h with a constant rate of 10 m/h, and was maintained at this level for 14 h. Using the identical schedule the chamber pressure was further increased to 21 ATA and then to 31 ATA, and the total time for completing the compression was 58 h. The pressure was kept at 31 ATA for 14 days, after which decompression was carried out according to the standard U.S. Navy schedule. Twelve days were required to complete the decompression. The chamber temperature at 31 ATA was raised to 31.0°C ± 0.2°C. A total pressure of 31 ATA consisted of 0.4 ATA oxygen, 0.79 ATA nitrogen, less than 0.004 ATA carbon dioxide, and helium to make up the rest.

The average caloric intake ranged from 2800 to 3000 kcal per day throughout the dive, and the rate of water exchange and body weight of the divers were checked daily. The results of studies on the energy and fluid balance of the diver were published previously (3).

Twenty ml of venous blood was withdrawn from antecubital vein at 0630 on the dive days indicated in Fig. 1. Immediately after withdrawal the blood was mixed with ethylenediamine-

TABLE 1
PHYSICAL CHARACTERISTICS OF THE SUBJECTS

Subject	Age, yr	Height, cm	Weight, kg
KM	28	168.5	59.5
SO	31	167.7	61.6
TF	35	168.2	61.5
SM	25	180.5	73.5

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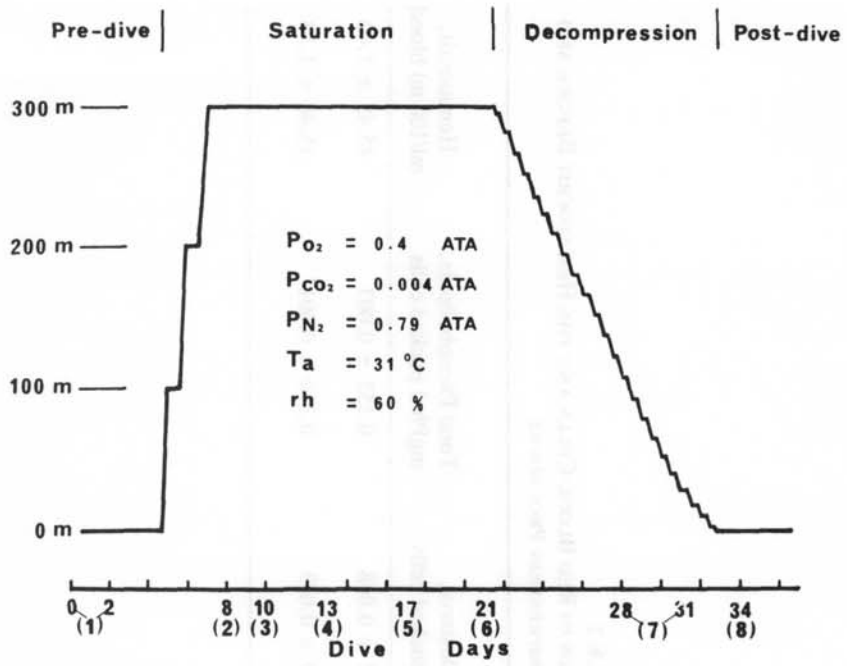

Fig. 1. Dive profile and partial pressures of chamber gas. Dive days on abscissa also indicate days of blood drawing from the divers.

tetraacetate (EDTA)-2K and was placed in ice inside a small decompression chamber (0.24 m long, 0.17 m diam), which was specially designed to transfer the sample through the medical lock to outside of the chamber. Once the small decompression chamber was locked out, it was decompressed gradually (approx 1 h) inside a refrigerator.

Possible hematologic changes attendant to the above decompression procedure were checked as follows: 20 ml of blood were withdrawn from each of 5 healthy subjects outside the chamber, and each sample was divided into 2 groups. The hematologic parameters of one group were measured immediately after the blood withdrawal, and the other group was transferred into the chamber through the medical lock and kept at 31 ATA for 30 min. Then the samples were again transferred out to 1 ATA by the same procedure, using the small decompression chamber as described above, and the same hematologic study was made. The osmotic fragility, cholesterol, total phospholipids of red cells, and the hematocrit of the blood samples obtained at 1 ATA (outside the chamber) were not significantly altered by the 30-min exposure to 31 ATA and the subsequent decompression procedure (Table 2).

Red blood cells (RBC) were counted with a microcell counter (Toa-Microcell Counter CC-108, Kobe, Japan), while the hemoglobin concentration of the blood was determined by the cyanmethemoglobin method and the hematocrit by the capillary tube method. By using these values the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were calculated.

The osmotic fragility of RBC was measured in a series of hypotonic NaCl solutions, using a method described by Hisaoka and Shiraki (4), and the results were expressed by the concentration of NaCl giving 50% hemolysis, which was defined as the half-hemolysis rate (HHR). The HHR value was very useful to compare the shift of the hemolysis curve in a series of

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TABLE 2

OSMOTIC FRAGILITY, CHOLESTEROL, AND TOTAL PHOSPHOLIPIDS IN RED BLOOD CELLS AND THE HEMATOCRIT BEFORE AND AFTER THE 30-MIN DECOMPRESSION PROCEDURE

Time of Measurement	Osmotic Fragility (HHR), %NaCl	Cholesterol, mg/ml packed cells	Total Phospholipids, mgP/ml packed cells	Hematocrit, ml/100 ml blood
Before decompression	0.488 ± 0.014	1.872 ± 0.048	0.152 ± 0.003	45.45 ± 1.40
After decompression	0.487 ± 0.013	1.860 ± 0.043	0.150 ± 0.001	45.46 ± 1.39

Values are means ± SE of 5 subjects.

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hypotonic NaCl solutions caused by the change in osmotic fragility of red blood cells. Any possibility of a masking effect by the calcium chelator (EDTA-2K) on the change in fragility was tested by comparing the change in fragility of incubated blood samples added with EDTA-2K and heparin. The result indicated no difference in the change of osmotic fragility of the two samples by the use of our method.

A portion of the EDTA-blood mixture was centrifuged at 1500 *g* for 10 min at 5°C and then the red cells were washed 3 times with cold isotonic phosphate buffer (pH 7.4) to remove white cells and platelets. The washed red cells were resuspended in 0.9% NaCl at a density corresponding to the original hematocrit value and then were used for the measurement of the packed cell volume and the lipids content. Lipids were extracted from the red cells and the plasma with a mixture of 2 vol of chloroform and 1 vol of methyl alcohol, as described by Folch et al. (5), and portions of the extracts were used to measure total cholesterol, free cholesterol (6), and total phospholipid (7). Values for cell lipids were expressed on the basis of the volume (in ml) of packed cells.

The quantitative separation of phospholipids in the red cell and the plasma was made by using thin-layer chromatography (8).

RESULTS

Hematologic parameters during the dive are summarized in Table 3. The RBC count increased significantly on Days 8 and 10 of the dive. The hematocrit value remained elevated until Day 17 of the dive but returned to the predive level on Day 21. The hemoglobin concentration tended to increase on Days 8 and 10 of the dive, but the increase was not statistically significant. During the postdive period hematologic parameters returned to the predive levels. On the other hand, the mean corpuscular values (MCV, MCH, MCHC) did not change significantly throughout the experiment.

The change in plasma volume, as estimated by the formula of van Beaumont (9), was expressed as percent of deviation from the predive value (%ΔPV). Since van Beaumont's formula suggests the relationship between %ΔPV and changes in hematocrit is not linear, the statistical significance of %ΔPV does not always match with that of the change in the hematocrit value.

A significant reduction of plasma volume was observed in Days 8–17 of the dive (or the 1st through 9th day at 31 ATA) ranging from 3.9% to 9.3%, as shown in Fig. 2. The maximal reduction of plasma volume (hemoconcentration) was observed on Day 10 of the dive (or the 3rd day at 31 ATA), after which the plasma volume gradually returned to the predive control level. The reduction of the plasma volume was also supported by the synchronized increase in the concentration of total plasma protein.

The osmotic fragility of RBC did not change significantly during the dive, although it tended to decrease progressively during the course of the experiment (Fig. 3).

The cholesterol level, molar ratio of cholesterol to phospholipid (C/P), and total phospholipid level of RBC did not change significantly during the dive, although they showed relatively wide daily fluctuations (data not shown). The phosphatidylcholine content of RBC decreased from predive level of 4.30 ± 0.06 (mg P $\cdot 10^{-2} \cdot$ ml^{-1}) to 3.86 ± 0.09 on the 1st day at 31 ATA and remained low during the rest of the experimental periods. Figure 4 shows an inverse relationship between the osmotic fragility and the phosphatidylcholine content of RBC. In this figure, all values obtained during the periods of predive control, 31 ATA, decompression, and postdive control are included. Overall, the basic relationship between the osmotic fragility and the

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TABLE 3
HEMATOLOGIC DATA TAKEN DURING DIVE

Measurement	Unit	Dive Days						
		2 (predive control)	8	10	13 (31-ATA saturation period)	17	21	28 & 31 (decompression)	34 (postdive control)
Erythrocyte count	$10^4/mm^3$	477 ± 33	512 ± 29**	502 ± 32*	474 ± 34	477 ± 30	493 ± 35	474 ± 24	468 ± 30
Hematocrit	ml/100 ml	45.5 ± 1.8	46.8 ± 2.1	47.9 ± 2.1*	46.7 ± 1.9*	46.5 ± 1.9*	44.7 ± 2.2	44.6 ± 1.7	44.1 ± 1.8
Hemoglobin	g/100 ml	15.5 ± 0.9	16.6 ± 0.8	16.3 ± 0.9	15.4 ± 1.0	16.0 ± 0.8	15.8 ± 0.8	15.6 ± 0.6	15.5 ± 0.9
Mean corpuscular volume	μm^3	96.0 ± 3.8	91.6 ± 1.5	96.0 ± 3.1	96.1 ± 3.7	97.8 ± 2.7	90.9 ± 2.5	94.3 ± 1.9	94.6 ± 2.8
Mean corpuscular hemoglobin	pg	32.6 ± 1.1	32.5 ± 0.7	32.5 ± 1.2	32.6 ± 2.1	33.6 ± 0.5	32.1 ± 0.7	32.9 ± 0.8	33.2 ± 0.3
Mean corpuscular hemoglobin concentration	g/100 ml	34.0 ± 0.6	35.5 ± 1.0	33.9 ± 0.7	33.9 ± 1.5	34.4 ± 0.6	35.3 ± 0.2	34.9 ± 0.4	35.2 ± 1.0
Total plasma protein	g/100 ml	6.8 ± 0.3	7.2 ± 0.3*	7.2 ± 0.2*	7.2 ± 0.3*	7.1 ± 0.2*	6.9 ± 0.3	7.0 ± 0.1	7.1 ± 0.1

Values are means ± SE of 4 subjects. *$P < 0.05$, **$P < 0.01$.

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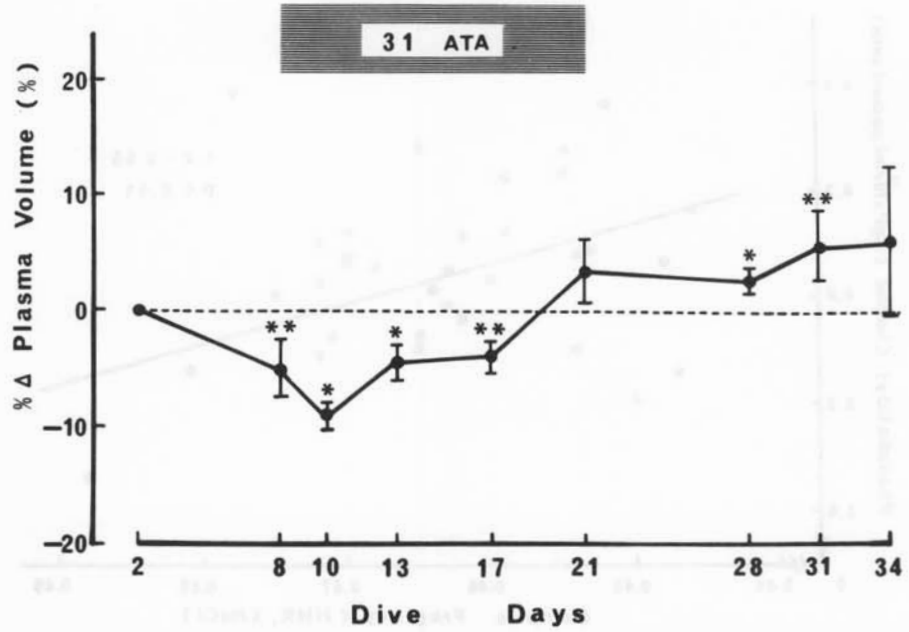

Fig. 2. Change in plasma volume. Change in plasma volume (PV) was expressed as percentage of deviation from the predive (%ΔPV). *Points* and *vertical bars* are means ± SE of 4 subjects. $^{*}P < 0.05$ and $^{**}P < 0.01$.

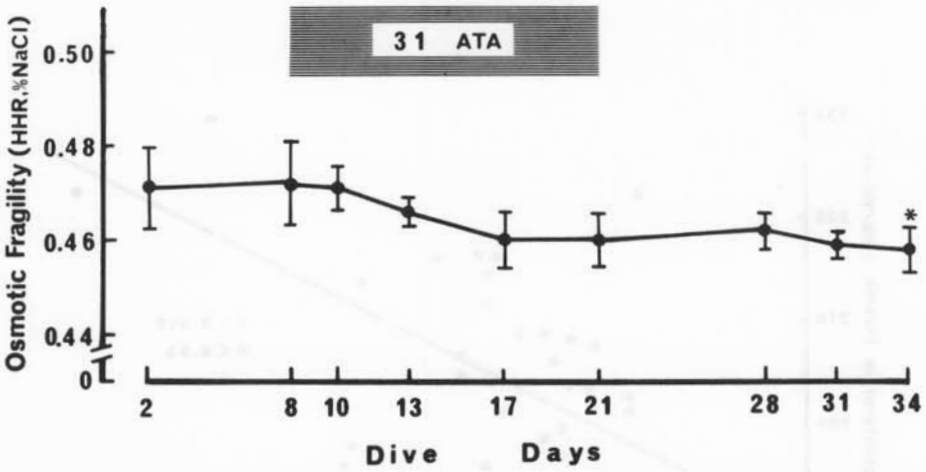

Fig. 3. Change in osmotic fragility of red blood cells during course of dive. *Points* and *vertical bars* are means ± SE of 4 subjects. HHR on ordinate is the concentration of NaCl to give 50% hemolysis; for details see text. $^{*}P < 0.05$.

phosphatidylcholine content of RBC was maintained regardless of the chamber pressure, as indicated by the single regression line.

The total phospholipid and cholesterol levels in plasma also remained unchanged throughout the dive (data not shown). However, the same positive correlation between the total cholesterol level in the plasma and the osmotic fragility of RBC was also maintained regardless of pressure (Fig. 5).

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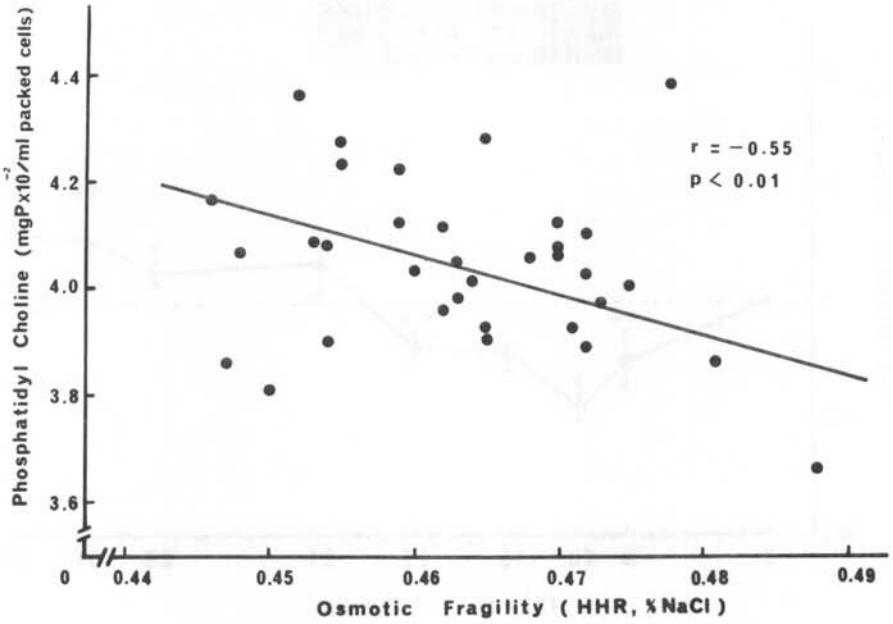

Fig. 4. Relationship between osmotic fragility and phosphatidylcholine content of RBC. All values obtained during predive control, 31-ATA, decompression, and postdive control periods are included in figure. Regression line was calculated by least-squares method.

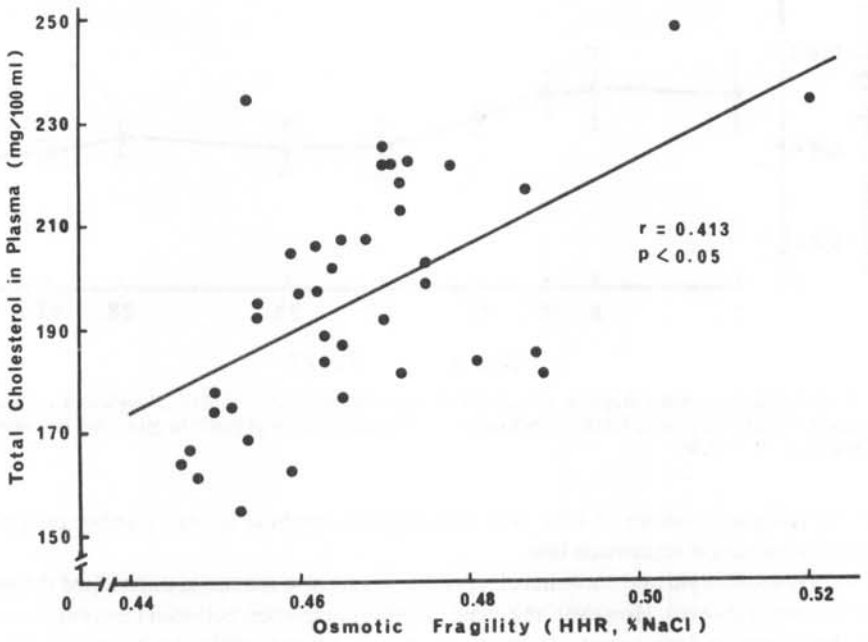

Fig. 5. Relationship between osmotic fragility of RBC and total cholesterol level in the plasma. All values obtained during predive control, 31-ATA, decompression and postdive control periods are included in the figure. Regression line was calculated by least-squares method.

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DISCUSSION

In the present experiment, 4 divers were exposed to a thermoneutral 31-ATA He-O_2 environment for 14 days. Although the O_2 pressure of the chamber gas was slightly hyperoxic (0.4 ATA) at 31 ATA, the results indicate that the erythropoietic activity appears to be well maintained. Actually, the erythrocyte count increased slightly during the early phase at 31 ATA (Table 3), which is most likely due to a mild dehydration. A large diuresis that was accompanied by a reduction of the body weight (by 0.7 kg) was observed at 31 ATA, especially during the early phase (3), strongly suggesting a net loss of body fluid. The magnitude of decrease in plasma volume was estimated to be approximately 10% on Day 10 of the dive (Fig. 2).

Although in vitro studies showed that the Na-K pump activity as well as the ionic permeability of the RBC membrane are inhibited by high pressure (1, 2), these effects may not necessarily change the cell volume. The latter view is supported by the lack of appearance of any significant change in the mean corpuscular volume throughout the dive (Table 3). Moreover, the osmotic fragility of RBC remained unchanged at pressure (Fig. 3), a finding that is also consistent with the view that the cell volume is unchanged at 31 ATA. Since the osmotic fragility is in part dependent on the age and size of the cell, the results also suggest that there are no changes in the rate of turnover of aged cells (with larger volume) nor in the rate of production of new cells (with smaller volume) during a prolonged exposure to high pressure. However, a direct evidence is required to correlate the above-mentioned view with the erythropoietic activity in this experiment.

The osmotic fragility of RBC could also be altered if chemical composition, especially the lipid content, changes (10). The finding of no significant and pressure-dependent change in the lipid (including phospholipids) content of RBC and plasma indicates that the cell membrane appears to maintain a normal chemical structure at 31 ATA.

A consistent decrease in the phosphatidylcholine content of RBC was observed, but the decrease was not related with the pressure, because the level remained low during the rest of the experimental periods; no recovery was seen at decompression and postdive periods. The reason why there was no observed restoration of the value is out of speculation at the moment.

However, the same overall correlation between the osmotic fragility and the phosphatidylcholine content of RBC or total cholesterol level in the plasma was observed at both 1 ATA and 31 ATA (Figs. 4 and 5). These significant correlations have been reported in relation to nutritional changes (10–12), thermal stress (13, 14), and changes in the splenic function (10, 15). The present observation supported the correlation of osmotic fragility and lipids within intra- and inter-individual fluctuations during the course of the dive, but they did not indicate any pressure-dependent change of the osmotic fragility of the cell membrane.

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Shiraki K, Sagawa S, Konda N, Nakayama H, Matsuda M. Propriétés de l'érythrocyte après une exposition de plusieurs jours à 31 ATA. Undersea Biomed Res 1983; 10(4):349–358.—Les changements hématologiques, la fragilité osmotique des globules rouges et les contenus lipidiques du plasma et des érythrocytes furent déterminés au cours d'une plongée fictive à saturation de 14 jours à 31 ATA (SEADRAGON IV). Des augmentations de l'hématocrite et de la numération érythrocytaire furent observées durant la période initiale à 31 ATA, laquelle a été attribuée à l'hémoconcentration causée par une diurèse. La fragilité osmotique des hématies diminua légèrement au cour de l'expérience, mais les changements n'étaient pas reliés à la pression atmosphérique. Les contenus en cholestérol et en phospholipide des globules rouges et du plasma montrèrent des variations

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quotidiennes légères et insignifiantes indépendantes de la pression. Cependant, la même corrélation globale fut observée entre la fragilité osmotique et la teneur en phosphatidylcholine des érythrocytes, ainsi qu'entre la fragilité osmotique et le niveau de cholestérol total du plasma à 1 ATA et à 31 ATA. Ces corrélations révélèrent une relation étroite entre la fragilité de la cellule et la composition lipidique de la membrane cellulaire et du plasma à l'intérieur des variations intra- et inter-individuelles au cour de l'expérience, mais ne dévoilèrent aucune modification significative de la fonction érythrocytaire par l'exposition prolongée dans un milieu ambient d'hélium-oxygène à 31 ATA. Ces résultats indiquent que l'exposition des plongeurs à 31 ATA pendant 14 jours ne semble pas causer de changements chimiques irréversibles dans le globule rouge.

érythrocyte
lipides
fragilité osmotique
pression

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