Methane-induced haemolysis of human erythrocytes

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Human erythrocytes were exposed to high concentrations of methane and nitrogen through the application of elevated partial pressures of these gas molecules. Cell leakage (haemolysis) was measured for cells exposed to these gases under a wide range of experimental conditions. Application of methane produces haemolysis at pressures far below the hydrostatic pressures known to disrupt membrane or protein structure. The effects of changes in buffer, temperature, diffusion rate and detergents were studied. Methane acts co-operatively with detergents to produce haemolysis at much lower detergent concentration than is required in the absence of methane or in the presence of nitrogen. At sufficiently high concentrations of methane, all cells are haemolysed. Increased temperature enhances the effect. Methane produces 50 % haemolysis at a concentration of about 0.33 M compared with about 7.5 M methanol required for the same degree of haemolysis.

INTRODUCTION

Small gaseous hydrocarbons exhibit properties that are potentially advantageous for the manipulation of membrane components and detergents. At relatively low concentrations these molecules exhibit anaesthetic behaviour, presumably mediated through their interaction with membrane-associated binding sites [1,2]. At higher concentrations they disrupt the membrane structure and function. The potential for using this disruption for the preparation and processing of membrane components is largely unexplored. Although only sparingly soluble at atmospheric pressure (1 standard atmosphere is about 0.1 MPa), small hydrophobic molecules such as methane can be forced into aqueous solution at higher concentrations by application at high partial pressures. For instance, methane has a solubility of about 1.5 mM at atmospheric pressure in water, but over 0.5 M at 100 MPa [3]. As most proteins do not denature at hydrostatic pressures of less than 150–200 MPa [4–5], it is possible to expose biological molecules in their native conformation to relatively high concentrations of small hydrophobic gas molecules. The partitioning of gaseous hydrophobic molecules between aqueous solutions and the hydrophobic interiors of membrane systems is likely to result in much higher effective concentrations of the gas molecules within the membranes. For instance, the partition coefficient of methane between n-octonol and water is about 100 : 1, depending on the pressure and temperature of the system [3,6]. This makes it possible to produce very high local concentrations of methane within membrane systems at moderate pressures. It is also presumably one reason for the broad anaesthetic properties of most hydrophobic gases.

In principle, the effect of small gaseous hydrocarbons on membrane systems may be mediated via several mechanisms. First, they partition into hydrophobic compartments, such as lipid bilayers [7], potentially altering their structure and affecting their stability. Secondly, they compete for water of hydration with other hydrophobic solutes, such as detergents, and force these molecules out of solution, moving them into either micelles or membranes. Thirdly, to achieve moderate concentrations of small gaseous hydrocarbons in biological systems requires the application of high partial pressures of the gas, and the system will be affected by the pressure changes. The consequent effect of this may not solely be due to the hydrostatic pressure. For example, Dodson et al. [8] have found that the physiological effects of high hydrostatic pressure on tadpoles (*Rana pipiens*) were different from the effects produced by high-pressure helium or nitrogen, for the same pressure. Similar effects have been observed by Nelson and co-workers [9] on the growth of microbial organisms and they have used the term hydrostatic and hyperbaric to distinguish these effects.

The effect of hydrostatic pressure on biological systems has been characterized in detail [4,10,11]. For instance, hydrostatic pressures of several hundred atmospheres have been shown to affect cell function and properties such as ion transport [12], release of membrane proteins [13], receptor–protein dissociation [14] and lipid-phase transitions [15]. Mild pressure in the range of a few atmospheres does not usually affect cell function, even though it has been shown that application of pressures of the order of 0.2–0.5 MPa increases the ability of intact erythrocytes to withstand lytic actions of snake venom phospholipase A_2 [16]. In contrast with the detailed study of the hydrostatic effects, there have only been limited studies on the effects of hyperbaric pressure effects on biological systems. The majority of this work has been by those who study the hyperbaric effect of gases such as O_2, N_2 and He and high-pressure nervous syndrome or high-pressure narcosis on humans who dive under water [17].

The interactions of moderate concentrations of small gaseous hydrocarbons with membranes in both the presence and absence of detergents are assessed here by measuring the level of haemolysis induced by moderate concentrations of methane, and comparing this with the activity of nitrogen and methanol. Haemolysis is a complex process that has been used as an assay for the membrane-targeted action of many compounds [18,19]. The extent of haemolysis is readily measured and quantified, but the results are difficult to interpret in terms of a specific molecular mechanism. However, under experimental conditions in which other probes are difficult to apply, haemolysis can be used to measure the relative activity of two substances, thereby providing qualitative information not accessible by other techniques.

Abbreviation used: DOPC, dioleoylphosphatidylcholine.
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MATERIALS AND METHODS

Materials

N-Octyl β-D-glucopyranoside (minimum purity 98 %) was obtained from Sigma (St. Louis, MO, U.S.A.); Triton X-100 (electrophoresis grade) was obtained from Bio-Rad (Richmond, CA, U.S.A.). A high-pressure cell was purchased from Ruska (Houston, TX, U.S.A.). Methane and nitrogen were produced from Airco Gases (Murray Hill, NJ, U.S.A.) in high-pressure cylinders (about 40 MPa). The purity of methane was 99 %. Typical impurities present in methane are 0.6 % N_2, 0.2 % O_2, 0.2 % CO and CO_2 and 0.1 % ethane. Nitrogen was 99.998 % pure and the impurities are 0.0005 % O_2 and 0.0001 % total hydrocarbon.

High-pressure instrumentation

A commercially available (model no. 2239-8000; Ruska) high-pressure gas cell has been modified in our laboratory and is shown schematically in Figures 1(a) and 1(b). The cell can be pressurized up to about 70 MPa at ambient temperature. The cell
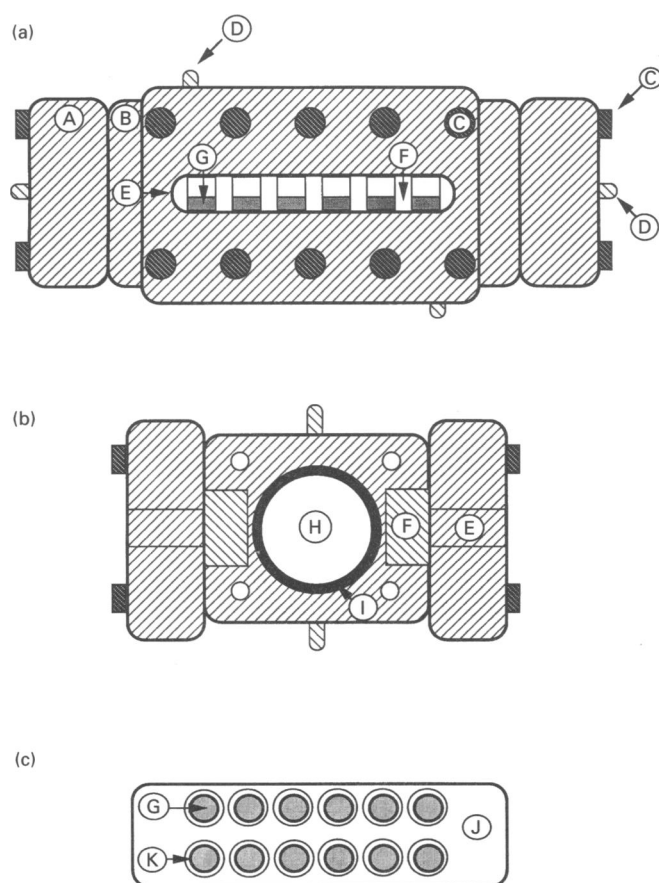
Figure 1   Diagrammatic representation of the high-pressure cell

(a) Front view of the high-pressure cell; (b) side view of the cell without the lid and the sample holder; (c) top view of sample and the sample holder. A, Lid; B, cell body; C, holding screws; D, inlet/outlet port; E, rectangular opening; F, optical window; G, sample; H, cylindrical interior cavity; I, pressure gasket; J, stainless-steel sample holder; K, Pyrex tubes containing sample. The sample holder (J) containing sample tubes (K) is inserted into the cylindrical interior cavity (H) and the lid (A) is tightened to a preset torque against the gasket (I) to ensure a leak-free operation. The entire cell is enclosed inside a safety cabinet. Inlet/outlet ports (D) are used for pressurization and depressurization.

is made of 450 series steel and has four inlet/outlet ports. There are two rectangular optical windows and two lids perpendicular to the windows. The lids and windows are secured with screws tightened against gaskets to a preset torque to ensure uniform and leak-free closure. Opening and closing of only one lid is required for all the operations described below. The centre of the cell has a cylindrical cavity with a volume of 100 ml. In order to reduce the risks of working with large amounts of gas under pressure, a sample holder was designed to fill the 100 ml working volume of the pressure cell, reducing the volume of the gas within the cell to about 25 ml. The stainless-steel sample holder used in this study (Figure 1c) has two rows of six equally spaced holes (1.25 cm diameter and 1.5 cm deep) designed to hold appropriately cut Pyrex glass tubes containing the samples. The high-pressure cell is mounted on a steel support and the support itself is fitted to the base plate of a safety cabinet made of plywood and steel. The high-pressure cell is enclosed inside the safety cabinet. The cell is connected to a gas booster (Hogan Fluid Power, Houston, TX, U.S.A.) which is in turn connected to the high-pressure cylinder through high-pressure lines containing several high-pressure valves (Swagelok, Solon, OH, U.S.A.). Gas from a high-pressure cylinder is pressurized using the gas booster to gas pressures in the range 0.1–70 MPa to an accuracy of 0.25 MPa. Once the desired pressure has been achieved, the cell can be isolated and the pressure maintained for the course of the experiment by closing the high-pressure valve.

After the sample had been introduced, the lids and the safety cabinet were securely closed. The cell was first flushed out by pumping the desired gas to about 0.5–1 MPa, maintaining this range for about 2 min, and then slowly leaking the gas to a vented hood. This procedure was repeated at least three times to remove all traces of air and moisture from the interior of the cell. After flushing, the cell was pressurized in steps of 5 MPa until the desired pressure was reached. A few minutes were allowed to lapse after each pressure step, to avoid any increase in temperature due to the compression of the gas and to minimize the stress on the high-pressure cell. At the end of the experiment, pressure was reduced gradually by equalizing the pressure inside the booster with that inside the cell and then reopening the isolation valve. Subsequently, the pressure in the cell was brought down to atmospheric over a period of 1–1.5 h by releasing the gas to the vented hood. Characterization and further details of the high-pressure cell are described elsewhere [19a].

Isolation of human erythrocytes

Whole blood (peripheral) was drawn from normal volunteers and collected in Vacutainers (Beckton-Dickinson, Rutherford, NJ, U.S.A.) containing lithium heparin as anticoagulant. Erythrocytes were isolated by standard techniques [20]: blood was centrifuged at 1000 *g* for 5 min in a clinical centrifuge and supernatant plasma was removed by Pasteur pipette. Pelleted cells were resuspended in prefiltered (0.2 μm filter) PBS (pH 7.4) and centrifuged as above. Buffy coat and supernatant were removed and the process was repeated five times to remove white blood cells and platelets. Erythrocytes were finally resuspended in PBS, refrigerated at 4 °C and used until 15 days old. For experiments, a 5 ml aliquot of erythrocyte suspension was drawn from the stock, centrifuged at 1000 *g* for 3 min, and 0.5 ml of the pellet was removed and diluted with 49.5 ml of PBS, to give a 1 % erythrocyte suspension. This 1 % erythrocyte suspension has approx. 1.2×10^8 cells/ml. Erythrocyte suspensions were also prepared by a similar process in prefiltered (0.2 μm filter) Alsever's buffer, pH 6.4 [0.42 % (w/v) NaCl, 0.8 % (w/v) tri-

sodium citrate dihydrate, 2.05% (w/v) glucose, pH adjusted with citric acid].

Application of high pressure to erythrocytes

Aliquots of a 1% erythrocyte suspension were placed at room temperature in glass tubes which were then put in the sample holder and subsequently transferred to the high-pressure cell. The high-pressure cell was then sealed. Erythrocytes were incubated against high-pressure gas as described above. A similar set of erythrocyte suspensions was kept in air at room temperature and 0.1 MPa as a control. After depressurization, all samples (control and pressurized) were centrifuged in Eppendorf tubes at 1000 g in an Eppendorf Micro 5415 Centrifuge (Brinkmann, Westbury, NY, U.S.A.) for 5 min. Supernatant was removed and the erythrocyte pellet was lysed by adding distilled water to each sample to a 1 ml final volume. The supernatant was also diluted to 1 ml. The lysed erythrocytes (ghosts) were pelleted by centrifuging in Eppendorf tubes at 15000 g for 30 min. Each sample was then diluted 10-fold.

Determination of standard conditions

UV spectra of haemoglobin released from erythrocytes after exposure to 64 MPa of methane or nitrogen for 24 h were identical with that of the control. This indicated that these exposures did not irreversibly alter haemoglobin structure. No information about the structure of haemoglobin at high gas pressures was obtained in these studies. However, this result demonstrated that absorbance at 414 nm could be used as a direct measure of haemoglobin concentration. Haemoglobin released from erythrocytes was measured to quantify percentage cell survival. Early experiments were designed to determine under what conditions the effect of methane and nitrogen could best be studied. The effect of sample volume was measured to determine the effect of diffusion time of gases through the aqueous solutions in the absence of a facility to stir the specimens in the pressure cell. Sample temperature, buffer and incubation time were also varied to determine appropriate conditions for subsequent experiments.

Percentage erythrocyte survival (or percentage haemolysis) was calculated for each 1 ml sample measurement of absorbance at 414 nm using a Hewlett-Packard diode array spectrophotometer HP 8451A (Hewlett-Packard, Corvallis, OR, U.S.A.). Percentage haemolysis and percentage erythrocyte survival were calculated using the following formula for each sample. Percentage haemolysis = $100S/(S+P)$ and percentage erythrocyte survival = 100 – percentage haemolysis, where S is total supernatant absorption and P is total pellet absorption. To measure the effect of the diffusion of gas through the aqueous samples, a wide range of specimen volumes was studied. Erythrocyte suspensions (1%) in PBS and Alsever's buffer were used to make aliquots of increasing volume (0.05, 0.1–1.0 ml in 0.1 ml steps). Experiments were carried out with increasing erythrocyte suspension volume for various incubation periods (5–25 h). The effect of high-pressure gas on detergent-induced haemolysis was studied by adding detergent to 1% erythrocyte suspensions and subjecting them to high gas pressures. N-Octyl β-D-glucopyranoside was used over the concentration range 0–0.45% (w/v) and Triton X-100 over the range 0–0.008% (w/v). Experiments on these samples were performed at gas pressures of 20, 41, 50 and 64 MPa for methane and nitrogen. Appropriate control specimens with identical detergent concentrations were left at 0.1 MPa.

RESULTS AND DISCUSSION

Effect of methane and nitrogen

The effect of methane and nitrogen on erythrocyte survival was measured as a function of the pressure of the applied gas. Cell suspensions (0.3 ml in PBS) were incubated for 24 h in the presence of 20, 41, 50 and 64 MPa methane and in the presence of identical pressures of nitrogen. After the incubation, the solutions were brought to atmospheric pressure and the extent of haemolysis was determined as described in the Materials and methods section. Both the nitrogen- and methane-incubated cell suspensions contained large amounts of gas bubbles in the solution (see below). Figure 2 is a plot of percentage haemolysis as a function of pressure for nitrogen and methane. A methane pressure of 64 MPa for 24 h haemolyses 90% of cells. Nitrogen-induced haemolysis on the other hand is constant at all pressures at a low value of 7%. This implies that, with increasing pressure of methane, increased methane-induced haemolysis occurs. Nitrogen has little effect on haemolysis as a function of pressure. The solubility of methane in aqueous solvent is 2.1 times that of nitrogen. Therefore at a given pressure the aqueous concentration of methane is more than twice that of nitrogen. This difference does not, however, explain the great difference seen in the haemolysis induced by the two gases.

Another explanation is that the cells saturated with the gaseous solutes collapsed during the decompression because of formation of intracellular bubbles leading to leakage of haemoglobin. Experiments carried out by Hemmingsen et al. [21] rule out this possibility. They showed that intact human erythrocytes and ghosts (loaded with fluorescent markers) incubated with either nitrogen or argon at pressures of 30 MPa and decompressed rapidly (about 1 s) are not haemolysed despite the presence of profuse bubbles in the medium. They have attributed this ability of erythrocytes to withstand gas supersaturation to the absence of intracellular bubble formation, in contrast with *Tetrahymena pyriformis* cells (containing food vacuoles) which were ruptured as a result of intracellular bubble formation [22]. Even though we have carried out experiments at higher pressures than Hemmingsen et al. [21], our decompression times are at least three orders of magnitude longer and our results for nitrogen are in agreement with those of Hemmingsen et al.
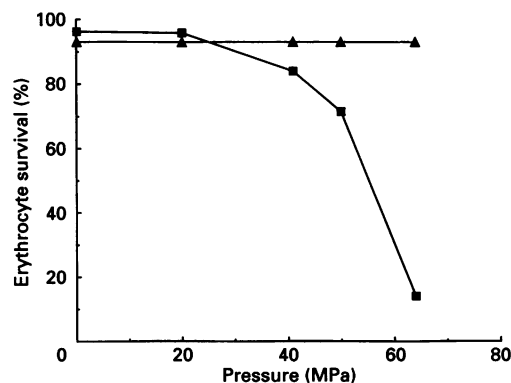

Figure 2   Effect of methane (■) and nitrogen (▲) on the degree of haemolysis of erythrocyte suspension

Aliquots of 0.3 ml of erythrocyte suspension in PBS buffer were exposed to various methane or nitrogen pressures at 23 °C for 24 h, and percentage erythrocyte survival was estimated as described in the Materials and methods section.
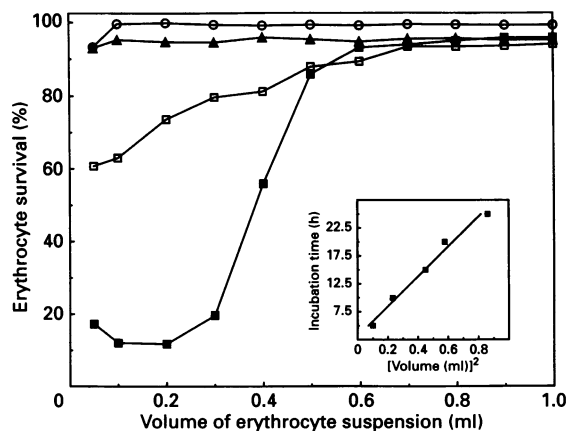
Figure 3 Effect of sample volume on erythrocyte survival in PBS and Alsever's buffers in the presence of 64 MPa of methane or 64 MPa of nitrogen for 10 h at 23 °C

The diffusion of gases in unstirred samples with volumes greater than 0.4 ml is sufficiently slow to lower the degree of haemolysis observed for experiments of 10 h duration. The diffusion time for the transport of methane through a height h (in cm) is given by $h^2 = \frac{1}{2} Dt$, where $t =$ time (in s) and $D =$ diffusion constant. As the volume of the sample is $V = hA$ where A is the cross-sectional area and is essentially a constant in these experiments, V^2 should be proportional to Dt for a diffusion-limited process. The inset shows a plot of time required to achieve 50% haemolysis against V^2, which is proportional to the square of the height of the solution (as area is held constant). This relationship is linear, supporting the proposition that methane-induced haemolysis is a diffusion-limited process. $\bigcirc$, 0.1 MPa in PBS (control); $\square$, 64 MPa methane (Alsevers); $\blacktriangle$, 64 MPa nitrogen (PBS); $\blacksquare$, 64 MPa methane (PBS).
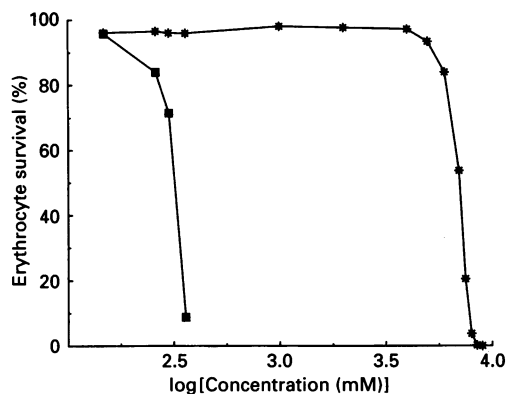

Figure 4 Comparison of haemolysis induced by methane ($\blacksquare$) and methanol (*) as a function of concentrations of these molecules

The horizontal axis is log concentration to allow the substantially different activities of methane and methanol to be plotted on the same curve. Note that at these high pressures of methane, Henry's law is not well obeyed [3].

Earlier work of Gerth and Hemmingsen [23] had shown that for nitrogen and methane the gas supersaturation tension (in atmospheres), i.e. the difference between the gas equilibration pressure and the pressure at which bubbles appear at the glass/water interface, is not significantly different for gas equilibration pressures of 30–57 MPa. Therefore the haemolysis observed in methane-incubated solutions is probably not due to bubble formation in the external medium.

Effect of sample volume and buffer

The effect of diffusion time was studied by measuring erythrocyte survival at 64 MPa of methane and nitrogen for different volumes of cell suspension in order to confirm the results shown in Figure 2. Results of these experiments carried out in PBS as well as Alsever's buffer are shown in Figure 3. Except for the smallest sample volume, less than 3 % of the control erythrocytes (in PBS) were haemolysed at 0.1 MPa in the 10 h of this experiment. The 0.05 ml sample at 0.1 MPa exhibited 7 % haemolysis, presumably because of disruption of erythrocytes at the air/water interface or to the errors involved in handling small volumes. In Alsever's buffer, pH 6.4, erythrocyte survival at 0.1 MPa was similar to that in BPS (results not shown). At low sample volumes (0.1–0.4 ml) erythrocyte survival for 10 h at 64 MPa methane in PBS is about 20 % and in Alsever's buffer about 60 %. Cell survival increases rapidly with increasing volume for both the buffers. In the case of 64 MPa nitrogen pressure, even for the smallest volume, erythrocyte survival was more than 90 %.

The diffusion constant D of methane in water is 1.904×10^{-5} cm²/s [24], making it unlikely that methane diffused completely to the sedimented erythrocytes at the bottom of the sample tubes over the course of these experiments. For example, given this value of D and using the expression for diffusion in one dimension over a height h as, $1/2\, Dt = h^2$, we obtain $t \sim 8$ h, for $h = 0.5$ cm. To substantiate further the idea that haemolysis is diffusion-limited in the absence of stirring, the time required to produce 50 % haemolysis (PBS buffer) was plotted as a function of the square of the volume since volume $(V) = hA$. For a diffusion-limited process, this should produce a straight line, as seen in the inset of Figure 3. These experiments validated the use of sample volumes of 0.3 ml and incubation times of more than 12 h. Decreasing this time led to incomplete diffusion of the applied gas into the specimen. Increased temperature also enhanced the effect of methane on haemolysis (results not shown). However, owing to problems in maintaining the pressure cell at a constant uniform temperature, most experiments were carried out at room temperature.

Comparison with methanol-induced haemolysis

Figure 4 shows the percentage haemolysis induced by methane and methanol as a function of their molar concentrations. Methane is over 20 times as efficient as methanol in erythrocyte haemolysis. Small aliphatic alcohols are considered to be potent haemolysing agents and believed to destabilize membrane by pore formation [25]. Methane is much more effective at haemolysing erythrocytes but whether it acts via the same mechanism or a different one is not known. The partition coefficient of methane between octonol and an aqueous solution is more than 70 times higher than for methanol for the same interface [26] (the difference is much higher if the partition coefficients are considered for hexadecane and an aqueous solution [26]) and this may contribute to the greater potency of methane for haemolysis. Chi et al. [27] found that the correlation between the observed haemolysis and concentration of small-chain alcohols improved when they used the membrane concentration (calculated using the partition coefficient) rather than the aqueous concentration. A related explanation may involve the region of the membrane at which the n-alcohols and n-alkanes become partitioned (see below).

Effect of gases on detergent-induced haemolysis

Erythrocyte suspensions containing increasing concentrations of n-octyl β-D-glucopyranoside (0–0.45 %) were incubated under
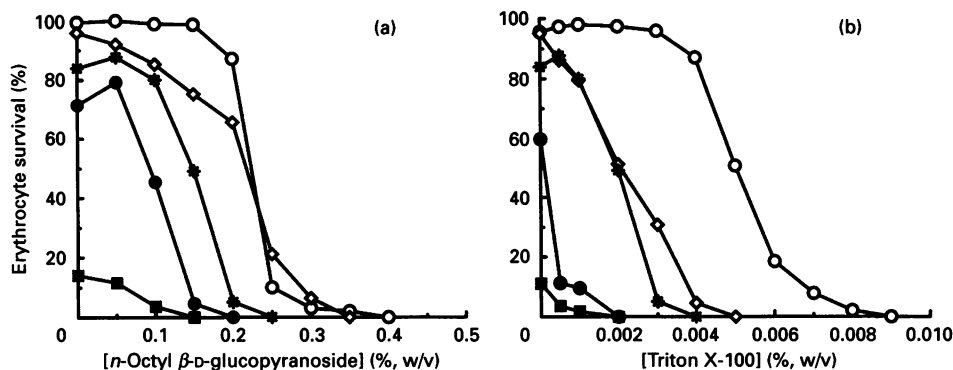
Figure 5   Effect of methane on detergent-induced haemolysis of erythrocytes in PBS buffer

(a) Effect of methane on haemolysis induced by n-octyl β-D-glucopyranoside and (b) effect of methane on haemolysis induced by Triton X-100. ○, 0.1 MPa air (control); ◇, 20 MPa methane; ✳, 41 MPa methane; ●, 50 MPa methane; ■, 64 MPa methane.

methane pressures of 20, 41, 50 and 64 MPa for 12 h at 22 °C. Similar experiments were performed in the presence of Triton X-100 at concentrations of 0–0.01 %. The results of representative experiments are shown in Figure 5. The curves in Figures 5(a) and 5(b) correspond to experiments with n-octyl β-D-gluco-pyranoside and Triton X-100 respectively. These results indicate that methane promotes the destabilization of membranes by detergents. Nitrogen does not significantly increase haemolysis as a function of detergent concentration. For example, erythrocytes incubated for 12 h at 22 °C under 64 MPa nitrogen in the presence of 0.225 % (w/v) n-octyl β-D-glucopyranoside show 50 % haemolysis compared with 48.4 % haemolysis exhibited by the unpressurized control sample. Detergent-induced haemolysis occurs close to the critical micellar concentration of the detergents, especially for non-ionic detergents [28,29]. However, there is not always a simple correlation between the critical micellar concentration and haemolysis [29]. There have, however, been suggestions that detergent-induced haemolysis may involve intra-bilayer non-bilayer phase [30,31].

The mechanism by which methane enhances detergent-induced haemolysis is not immediately clear. By competing with detergent molecules for the water of hydration, methane will drive detergent molecules out of solution, either into micelles or into the membrane. This enhances the effect of any given concentration of a detergent on the membrane by increasing the number of the detergent molecules that are interacting with the membrane. The natural aggregated form of a detergent is a micelle; detergents have too large an area-to-volume ratio to form a bilayer [32]. When they interact with a membrane, the lipid structure will be disrupted in a way that will be consistent with an increase in the area-to-volume ratio of the hydrophobic phase. Small hydrophobic molecules such as methane would be expected to produce the opposite effect on the area-to-volume ratio, presumably increasing the volume of the hydrophobic phase with little or no effect on its area. Consequently, these two opposing effects might be expected to cancel out one another; however, such a cancelling out is not observed and therefore other effects must contribute to methane-induced haemolysis.

King et al. [33] have studied the interaction of n-hexane with dioleoylphosphatidylcholine (DOPC) bilayer and reported that the partial molar volume of hexane in bilayer is close to zero. According to these authors, the thickness of the hydrocarbon region ($\mathscr{D}_h$) in DOPC does not change even when one molecule of hexane is added per lipid molecule. This observation is in contrast with earlier reports and the widely held belief that added hexane should increase the bilayer thickness. Whether the very small volume change seen for n-hexane in pure DOPC bilayer is universal and applicable to small gaseous alkanes in hetero-geneous membrane lipids is not known. If the near-zero volume change seen for n-hexane is true for methane also, then it can partly explain the co-operative effect seen in the case of methane-induced haemolysis by detergents. White et al. [34] have also shown that partitioned hexane is predominantly present in the hydrocarbon core of the bilayer (the middle). From the structure of fluid DOPC bilayer determined by the combined use of X-ray and neutron diffraction by White and co-workers [34,35], one can infer the possible partitioning of n-alcohols in the interfacial region of the bilayer as opposed to the predominant partition at the hydrocarbon core for the n-alkanes. This may partly explain the difference between methane and methanol, the latter being likely to be present mostly in the interfacial region and hence requiring a much higher concentration in order to be partitioned further into the membrane to produce haemolysis. However, detailed structural information is required for the further understanding of methane-induced haemolysis in both the presence and absence of detergents.

Although the molecular mechanism of methane-induced haemolysis is unclear, these results suggest that the strong interactions of methane with membranes may have clear advantages for a number of biochemical procedures. First, methane may make possible the use of smaller detergents for the solubilization of membrane components. This could be advantageous for the isolation, purification and crystallization of membrane proteins. Second, methane could be used to improve the efficiency of current isolations or to solubilize detergent-resistant components under relatively mild conditions. Finally, methane can be removed completely from any biological specimen through the release of pressure at the end of an experiment, making it a completely clean reagent for processing of products for human usage.

The results presented here demonstrate that methane is highly interactive with membranes, and its application can result in the complete haemolysis of erythrocytes in suspension at hydrostatic pressures that have little effect on protein structure. Methane is much more effective than methanol at producing these effects whereas nitrogen has little effect. Methane also enhances the destabilization of the erythrocyte membrane by the action of detergents.

REFERENCES

1   Franks, N. P. and Lieb, W. R. (1994) Nature (London) **367**, 607–614
2   Evers, A. S., Berkowitz, B. A. and d'Avignon, D. A. (1987) Nature (London) **328**, 157–160
3   Clever, H. L. and Young, C. L. (1987) Methane: Solubility Data Series, vol. 27/28, Pergamon Press, New York
4   Heremans, K. (1982) Annu. Rev. Biophys. Bioeng. **11**, 1–21
5   Kundrot, C. E. and Richards, F. M. (1987) J. Mol. Biol. **193**, 157–170
6   Wilcock, R. J., Battino, R., Danforth, W. F. and Wilhelm, E. (1978) J. Chem. Thermodyn. **10**, 817–822
7   Miller, K. W., Hammond, L. and Porter, E. G. (1977) Chem. Phys. Lipids **20**, 229–241
8   Dodson, B. A., Furmaniuk, Jr., Z. W. and Miller, K. W. (1985) J. Physiol. (London) **362**, 233–244
9   Nelson, C. M., Schuppenhauer, M. R. and Clark, D. S. (1992) Appl. Environ. Microbiol. **58**, 1789–1993
10 Jannasch, H. W., Marquis, R. E. and Zimmerman, A. M. (1987) Current Perspectives in High-Pressure Biology, Academic Press, London
11 Weber, G. and Drickamer, H. G. (1983) Q. Rev. Biophys. **16**, 89–112
12 Hall, A. C., Ellory, J. C. and Klein, R. A. (1982) J. Membr. Biol. **68**, 47–56
13 Deckmann, M., Haimovitz, R. and Shinitzky, M. (1985) Biochim. Biophys. Acta **821**, 334–340
14 Royer, C. A., Weber, G., Daly, T. J. and Matthews, K. S. (1986) Biochemistry **25**, 8308–8315
15 Braganza, L. F. and Worcester, D. L. (1986) Biochemistry **25**, 2591–2596
16 Halle, D. and Yedgar, S. (1988) Biophys. J. **54**, 393–396
17 Bennett, P. B. and Marquis, R. E. (1994) Basic and Applied High Pressure Biology, University of Rochester Press, Rochester, NY
18 Portlock, S. H., Clague, M. J. and Cherry, R. J. (1990) Biochim. Biophys. Acta **1030**, 1–10
19 Lieber, M. R., Lange, Y., Weinstein, R. S. and Steck, T. L. (1984) J. Biol. Chem. **259**, 9225–9234
19a Somasundaram, T. and Makowski, L. (1995) Rev. Sci. Instrum., in the press
20 Dodge, J. T., Mitchell, C. D. and Hanahan, D. J. (1963) Arch. Biochim. Biophys. **100**, 119–130
21 Hemmingsen, B. A., Steinberg, N. A. and Hemmingsen, E. A. (1985) Biophys. J. **47**, 491–496
22 Hemmingsen, B. A. and Hemmingsen, E. A. (1983) J. Protozool. **30**, 608–612
23 Gerth, W. A. and Hemmingsen, E. A. (1976) Z. Naturforsch. **31a**, 1711–1716
24 Hille, B. (1984) Ionic Channels of Excitable Membranes, p. 172, Sinauer, Sunderland, MA
25 Chi, L.-M. and Wu, W. (1991) Biochim. Biophys. Acta **1062**, 46–50
26 Stein, W. D. and Lieb, W. R. (1986) Transport and Diffusion across Cell Membranes, pp. 89–90, Academic Press, New York
27 Chi, L.-M., Wu, W., Sung, K.-L. P. and Chien, S. (1990) Biochim. Biophys. Acta **1027**, 163–171
28 Trägner, D. and Csordas, A. (1987) Biochem. J. **244**, 605–609
29 Zaslavsky, B. Y., Ossipov, N. N., Krivich, V. S., Baholdina, L. P. and Rogozhin, S. V. (1978) Biochim. Biophys. Acta **507**, 1–7
30 Isomaa, B., Hägerstrand, H., Paatero, G. and Engblom, A. C. (1986) Biochim. Biophys. Acta **860**, 510–524
31 Isomaa, B., Hägerstrand, H. and Paatero, G. (1987) Biochim. Biophys. Acta **899**, 93–103
32 Tanford, C. (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, John Wiley, New York
33 King, G. I., Jacobs, R. E. and White, S. H. (1985) Biochemistry **24**, 4637–4645
34 White, S. H., King, G. I. and Cain, J. E. (1981) Nature (London) **290**, 161–163
35 White, S. H. and Wimley, W. C. (1994) Curr. Opin. Struc. Biol. **4**, 79–86