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THE INFLUENCE OF DIMETHYLSULFOXIDE ON THE RED CELL MEMBRANE

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Abstract—Dimethylsulfoxide protects human red blood cells against osmotic haemolysis. This protection is caused by a drug-induced increase of the critical volume of the cells. Protection is abolished, when the cells are preincubated with dimethylsulfoxide in isotonic NaCl. Dimethylsulfoxide-induced K^+ loss from red blood cells appeared to depend on the red cell volume. Presumably this phenomenon is related to volume dependent conformation changes at the cell surface, as described in recent literature. Further, dimethylsulfoxide causes a strong decrease of the red blood cell deformability, reflecting its profound influence on the physico-chemical properties of biomembranes. This effect also depends on the red cell volume. The results indicate that dimethylsulfoxide has similar effects on biomembranes as a wide variety of anesthetics. The effects of these anesthetics on biomembranes are usually attributed to a hydrophobic interaction with the membrane. It is shown, however, that a different interpretation of the results is also possible.

IN A PRECEDING paper it was shown that dimethylsulfoxide (DMSO), in relatively high concentrations, causes haemolysis of red blood cells.[1] It appeared that the DMSO effect was localized at the cytoplasmic membrane. Further, experimental evidence was presented, indicating that DMSO in sublytic concentrations did not cause increased osmotic fragility. The controversial results of Gerhards *et al.*,[2,3] showing an apparent increase of the osmotic fragility, could be attributed to differences in the experimental procedure. Gerhards *et al.* incubated red blood cells with DMSO, dissolved in isotonic NaCl, for 30 min and transferred a small volume of this suspension into a much larger volume of DMSO-free hypotonic NaCl solution. The latter solution is extremely hypotonic with respect to the DMSO containing cell content, resulting in an apparent increase of osmotic fragility. In our experiments the preincubation was identical, but the hypotonic NaCl solutions contained the same DMSO concentration as the preincubation medium. With this procedure osmotic fragility was identical with and without DMSO, within the experimental error.

In further experiments it appeared that under different conditions DMSO decreased the osmotic fragility considerably. This protection against osmotic haemolysis appeared to be coupled with a pronounced influence on other physico-chemical properties of the red cell membrane. The results of these studies are reported in the present communication.

METHODS

Heparinized human blood was centrifuged shortly after collection. Plasma and buffy coat were discarded. Subsequently the red blood cells were washed three times in a 1% buffered NaCl solution, prepared according to Parpart *et al.*[4]

Osmotic fragility was measured as described before.[5] The red blood cells were either preincubated as a 50 per cent suspension in a 1% NaCl solution supplemented with 3·2 M DMSO, or in 1% NaCl without the drug. From this suspension 0·2 ml was transferred to 10 ml hypotonic NaCl solution, or hypotonic NaCl solution supplemented with 3·2 M DMSO.

Mean cellular volumes were measured in Hamburger type haematocrit tubes, centrifuged for 20 min at 3000 rev/min. Haematocrit values were corrected for trapped medium. Trapped medium was measured by adding 2% ^{14}C-labeled dextran (mol. wt. 60,000–90,000) to the medium; 0·05 ml of cells, packed in a Hamburger type tube, were resuspended in 2 ml isotonic salt solution, or isotonic salt solution with DMSO. Radioactivity in the supernatant of this suspension was measured in a liquid scintillation counter, with the liquid scintillator described by Bray.[6]

The critical cell volume for osmotic haemolysis was measured as described before.[5] The percentage haemolysis was determined by measuring haemoglobin, liberated in the supernatant, according to Crosby *et al.*[7] K^+ in the supernatant was determined with a flame photometer. Partition coefficients of DMSO were measured as described previously.[1]

Red cell deformability was studied by two techniques. In the first place viscosity measurements were done in a Brookfield LVT cone-plate viscometer, by the technique described by Weed *et al.*[8] After preincubation the cell suspensions were centrifuged and resuspended in the same medium to a 80 per cent suspension. Viscosity measurements were done on this cell suspension at 37°.

Further, deformability was studied by measuring red cell filterability, as described by Miller *et al.*,[9] with minor modifications. A 1·6 per cent cell suspension was filtered through a 25 mm circular Nucleopore polycarbonate filter, with a mean pore diameter of 5 μm, at a constant flow rate of 10 ml/min. The constant flow rate was maintained by a Braun Perfusor pump. Filtration pressure was measured at regular intervals during 3–4 min.

RESULTS

In preliminary experiments it appeared that a rapid osmotic equilibration takes place when DMSO is added to the medium, DMSO entering the cells freely. In accordance sublytical DMSO concentrations, causing neither haemolysis nor K^+ loss, had no influence on the mean cellular volume, measured immediately after addition of the drug. The same conclusion was reached by Farrant.[10] Therefore the osmotic balance in studies on osmotic fragility will not be influenced by the presence of the drug.

In the first series of experiments, identical to the experiments described previously,[1] red blood cells were preincubated for 30 min in 3·2 M DMSO solved in 1% NaCl and subsequently transferred to NaCl solutions of 0–1 per cent, supplemented with the same DMSO concentration. Under these circumstances DMSO had no clear influence on the osmotic fragility of the cells, with small variations falling within the experimental error.

If the washed cells were not preincubated however, but transferred immediately to hypotonic NaCl solutions supplemented with DMSO, the results were completely different. As shown in Fig. 1 DMSO gave under these experimental conditions a strong protection against osmotic haemolysis. This protective effect was not in-

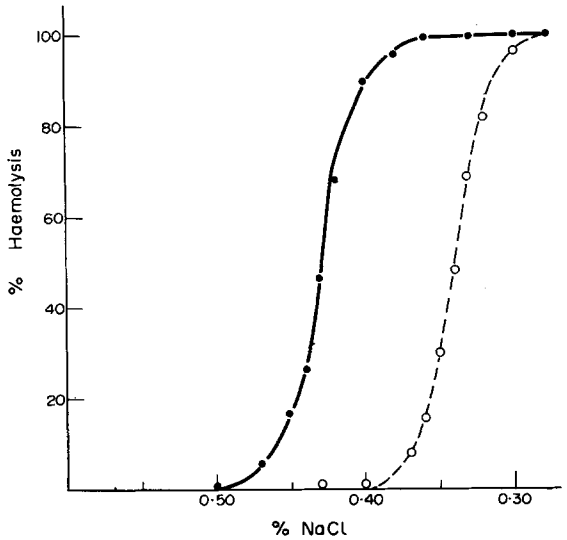

FIG. 1. Osmotic haemolysis of human erythrocytes in the absence (●——●) and in the presence (○——○) of 3·2 M DMSO, at 25°. The red blood cells were not preincubated.

fluenced by variations of the incubation period in the hypotonic NaCl–DMSO solutions from 10 to 180 min (Fig. 2). The protective effect increased with increasing DMSO concentration up to about 3·2 M. At higher concentrations DMSO has a haemolytic effect (Fig. 3).

To elucidate the background of this protection the swelling of erythrocytes in hypotonic NaCl–DMSO solutions was followed by haematocrit measurements. The haematocrit values as measured in Hamburger type haematocrit tubes were corrected for medium trapping. Trapped medium, measured with ^{14}C-dextran, appeared

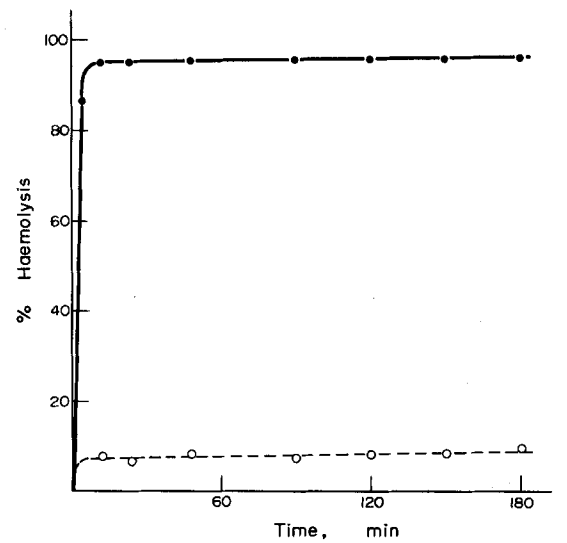

FIG. 2. Osmotic haemolysis of human erythrocytes in 0·38% NaCl at 25° in the course of time. ●——●: control; ○——○: 3·2 M DMSO.

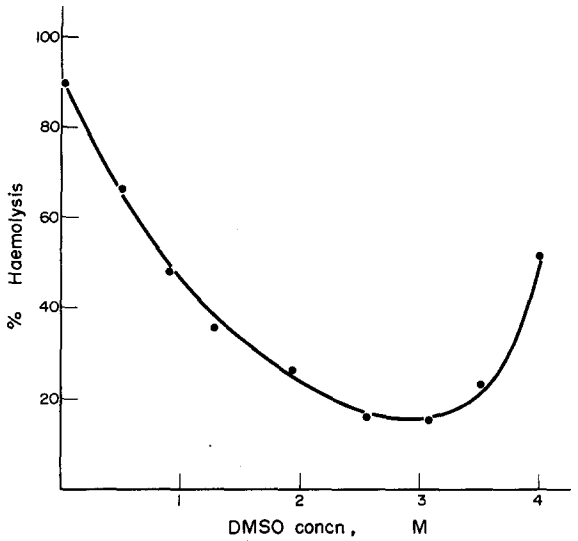

FIG. 3. The protection against osmotic haemolysis by DMSO. Haemolysis was measured after 30 min incubation at 25°, at a final NaCl concentration of 0·38%.

to be 5·7 ± 0·4 per cent in the absence and 7·5 ± 0·6 per cent in the presence of 3·2 M DMSO, over the whole NaCl concentration range from 1 to 0·4 per cent.

In Fig. 4 the corrected mean cellular volume is plotted against the reciprocal of the NaCl concentration, with and without DMSO in the medium. Apparently cell swelling in hypotonic NaCl solutions is not inhibited by DMSO.

In further experiments the critical volume at which haemolysis occurs was measured both in the absence and in the presence of DMSO. As shown in Fig. 5 the criti-

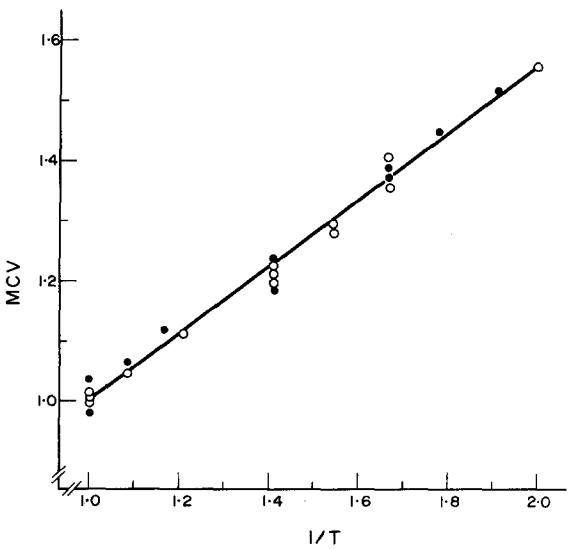

FIG. 4. The relationship between the mean cellular volume (MCV) and the reciprocal of the tonicity of the medium (1/T) in the absence (●——●) and in the presence (○——○) of 3·2 M DMSO.

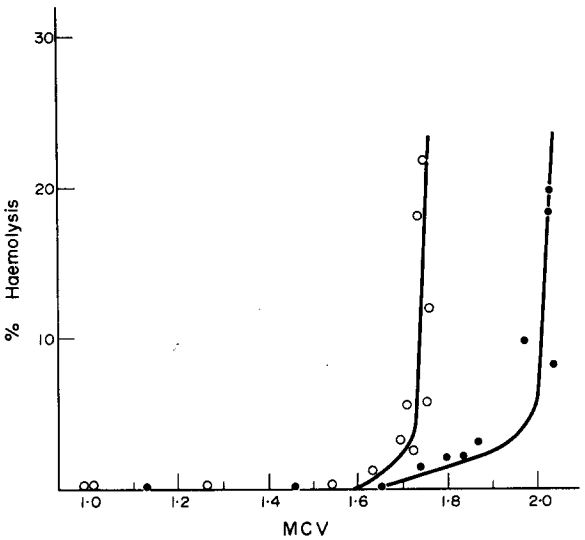

FIG. 5. The relationship between the mean cellular volume (MCV) and osmotic haemolysis at 25°, in progressively lower NaCl concentrations. O——O: control; ●——●: 3·2 M DMSO.

cal volume is significantly higher in the presence of DMSO compared with the critical volume in a drug-free medium. An arbitrary value of 100 was attributed to the critical volume of erythrocytes in the absence of DMSO. Statistical treatment of the experimental results revealed a critical volume in a drug-free medium of 100 ± 1·7 (mean ± S.D., n = 16) and of 112·5 ± 4·7 (mean ± S.D., n = 11) in the presence of 3·2 M DMSO.

It is known that DMSO changes the apparent pH of a buffer solution.[11] The apparent pH of the buffered NaCl solutions utilized in our experiments shifted from 7·3 without DMSO to 7·7 after addition of the drug. To exclude the possibility that this apparent pH shift would cause the observed phenomena, control experiments were carried out in NaCl solutions buffered at pH 7·7, in the absence of DMSO. It appeared that the osmotic fragility was slightly decreased at higher pH, but without any influence on the critical cell volume. The same conclusion was reached by Murphy.[12]

The K^+ loss from red blood cells at varying medium tonicities with and without DMSO is shown in Figs. 6 and 7. The K^+ leakage was slightly progressive in the course of time, but most K^+ loss occurred within 1 hr at 25°. Apparently DMSO provoked a strong K^+ loss in isotonic NaCl solutions, but the drug-induced leakage decreased strongly with decreasing NaCl concentrations. At NaCl concentrations of about 0·4 per cent the K^+ loss in the presence of DMSO was even significantly lower than in the absence of the drug. K^+ loss up to about 75 per cent was not associated with a change of the mean cellular volume, indicating that the K^+ leakage was osmotically compensated by an equal influx of Na^+ ions.

To elucidate whether the ionic strength, the osmolarity of the medium or the cellular volume is the determining factor in this phenomenon, further experiments were conducted in isosmotic media, in which NaCl was partly replaced by an osmotically equivalent concentration of the non-penetrating sucrose or the rapidly penetrating

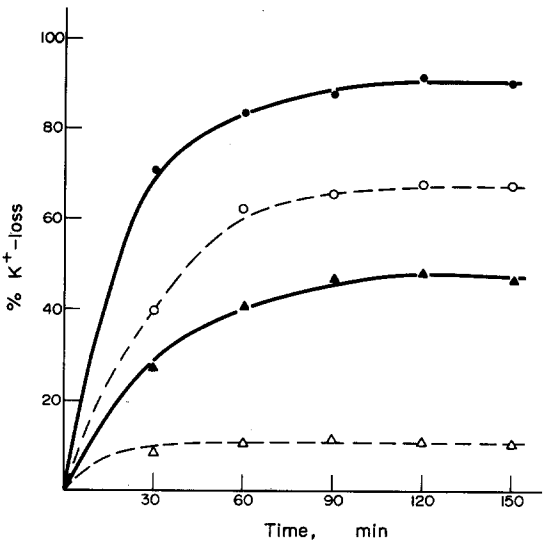

FIG. 6. K^+ loss from red blood cells at 25°, induced by 3·2 M DMSO, in the course of time at different NaCl concentrations in the medium. ●——●: 1·2% NaCl; ○——○: 1% NaCl; ▲——▲: 0·8% NaCl; △——△: 0·6% NaCl.

urea. As can be seen from Fig. 7 the cellular volume is of crucial importance; in isosmotic NaCl–sucrose mixtures the K^+ leakage was the same as in isotonic NaCl, whereas in NaCl–urea mixtures the rapidly penetrating urea had no influence on the experimental results.

The influence of DMSO on cell filterability is shown in Fig. 8. Both the initial filtering pressure and the slope of the pressure–time curve are increased by DMSO.

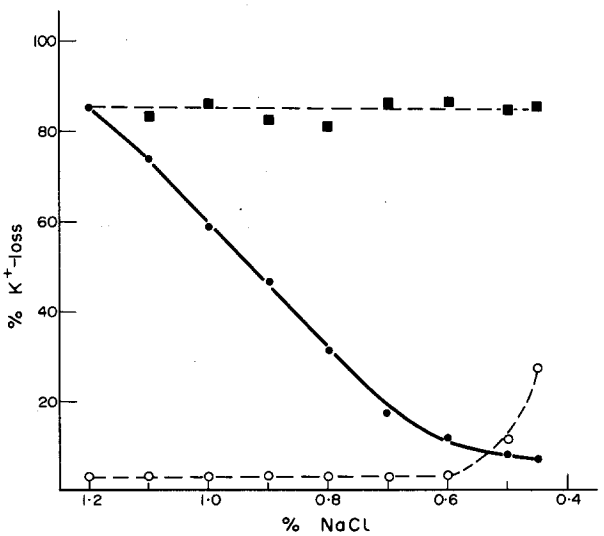

FIG. 7. The relationship between K^+ loss from red blood cells at 25°, measured after an incubation period of 60 min, and the NaCl concentration in the medium. ○——○: control, in the absence of DMSO; ●——●: 3·2 M DMSO, with and without osmotic compensation by urea; ■——■: 3·2 M DMSO, with osmotic compensation by sucrose.

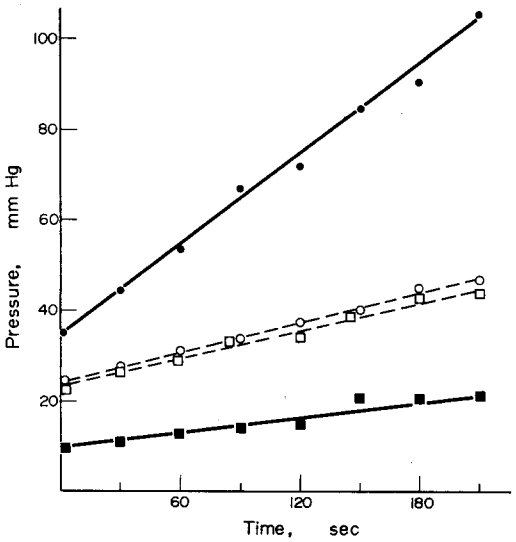

FIG. 8. Pressure–time curves for 1·6% red blood cell suspensions in 1% NaCl (■——■), 1% NaCl–3·2 M DMSO (●——●), 0·5% NaCl (□——□) and 0·5% NaCl–3·2 M DMSO (○——○).

This indicates a decreased deformability of the cells and a higher rate of plugging of the filter pores. When the same experiments were repeated in a 0·5% NaCl medium, DMSO had no significant influence on the experimental parameters (Fig. 8). Mean values of a number of experiments are shown in Table 1. In the absence of DMSO variations of the medium pH over the range 7·2–8·4 had no significant influence on cell filterability.

The influence of DMSO on the rheological properties of red blood cells is shown in Figs. 9 and 10. Apparently DMSO increases the viscosity of the cell suspension at all shear rates. In 0·5% NaCl the influence of DMSO on the viscosity of the cell suspension is much lower. In paired experiments it appeared that the influence of DMSO on the deformability of erythrocytes in a medium of 150 mosM NaCl + 150 mosM urea is identical to the influence of DMSO on red cells in a 150 mosM NaCl medium, whereas the drug effect on cells suspended in 150 mosM NaCl + 150 mosM sucrose is exactly equal to the effect on cells suspended in 300 mosM NaCl. Thus, again the cellular volume, rather than the ionic strength or the osmolarity of the medium is the determining factor.

In connection with the influence of the medium tonicity on the DMSO effects, the influence of NaCl on the octanol/water partition coefficient of DMSO was studied.

TABLE 1. THE INITIAL PRESSURE AND THE RATE OF PRESSURE RISE (mm Hg) OF 1·6% RED BLOOD CELL SUSPENSIONS FILTERED THROUGH 5 μm POLYCARBONATE SIEVES

Medium	Initial pressure	Rate of pressure rise	n
1% NaCl	10·1 ± 3·1	3·2 ± 1·3	62
1% NaCl–3·2 M DMSO	35·0 ± 18·0	20·5 ± 9·0	14
0·5% NaCl	23·0 ± 8·8	5·6 ± 1·1	18
0·5% NaCl–3·2 M DMSO	23·7 ± 11·2	6·2 ± 3·8	14

The results are expressed as mean ± S.D., n = number of experiments.

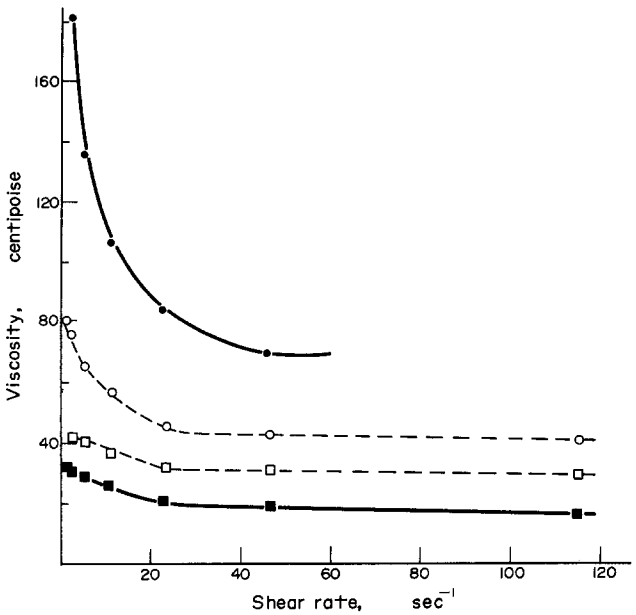

FIG. 9. The relationship between the viscosity and the shear rate of 80% red cell suspensions in 1% NaCl (■——■), 1% NaCl–3·2 M DMSO (●——●), 0·5% NaCl (□——□), and 0·5% NaCl–3·2 M DMSO (○——○), measured at 37°.

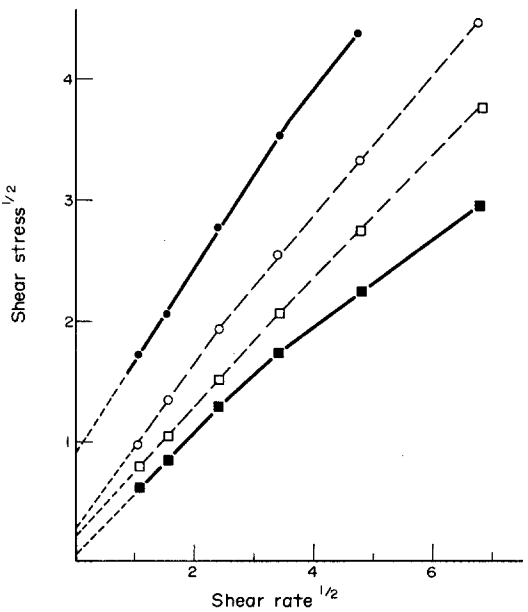

FIG. 10. Casson plot of viscosity of 80% red cell suspensions. Experimental conditions: see legend to Fig. 9.

It appeared that varying the NaCl concentration from 0–1 per cent did not affect the partition coefficient. In all experiments a value of about 10^{-2} was found, in agreement with the results of Leo *et al.*[14] Considering the relationship between the octanol/water partition coefficient and the medium/membrane partition,[15] this makes it unlikely that different NaCl concentrations in the described experiments are correlated with different DMSO concentrations in the membrane phase of the cells.

DISCUSSION

Apparently DMSO protects red blood cells against osmotic haemolysis when the cells are suspended directly in drug-containing hypotonic NaCl solutions. However, if the cells are preincubated in isotonic NaCl in the presence of 3·2 M DMSO no protection is observed. This is attributed to processes taking place during this preincubation. As shown e.g. in Figs. 6 and 7 the influence of DMSO on intact cells depends strongly on the cellular volume, and thus on the NaCl concentration. It is conceivable that the DMSO influence during preincubation in isotonic NaCl (leading to a substantial K^+ loss and a concomitant influx of Na^+) changes the cells in such a way, that the cells are subsequently no longer protected by the drug against osmotic haemolysis.

The strong protection by DMSO against osmotic haemolysis when the cells are not preincubated, is attributed to an increase of the critical haemolytic volume of the cells, as shown in Fig. 5. The increased critical volume is caused by a direct influence of DMSO and not indirectly, by a DMSO-induced shift of the apparent pH.

DMSO causes a pronounced decrease of the deformability of red blood cells in isotonic NaCl. This was demonstrated both by the filtration experiments and by the viscosity measurements. As pointed out by Miller *et al.*[9] the increased filtration pressure should be considered as a reflection of decreased deformability. As shown by Burton[13] the viscosity of red blood cell suspensions with a haematocrit over 60 also depends on cell deformability, increasing with decreasing deformability. The influence of DMSO on cell deformability in hypotonic (0·5%) NaCl solutions and in NaCl–urea mixtures is much less pronounced (Figs. 8 and 9). This indicates again that the influence of DMSO on the red blood cell strongly depends on the cellular volume. As shown in the Casson plot (Fig. 10) the yield stress of cell suspensions in isotonic NaCl is increased considerably by DMSO. A similar increase of the yield stress is caused by several anesthetics, as discussed previously.[16] A broad variety of chemically unrelated compounds, including a.o. tranquilizers, alcohols and detergents, share a number of common effects on biomembranes,[15, 16] viz.: (1) anesthesia, (2) protection of erythrocytes against osmotic haemolysis, (3) decreased deformability of red blood cells,[16] (4) cell lysis, at higher concentrations. Apparently DMSO exhibits similar effects on biomembranes. The lytic properties of DMSO have been described previously.[1] The anesthetic properties of DMSO have been described by several authors.[17–19] As shown in this paper the other two effects are also produced by DMSO. The analogy between the influence of DMSO and other anesthetics on biomembranes is further emphasized by recent electron-microscopic observations of Seeman[15] and Kirk and Tosteson.[20] Seeman described the rearrangement and aggregation of membrane globules, induced by phenothiazine tranquilizers. Kirk and Tosteson described an apparently similar reversible decrease and aggregation of membrane globules induced by DMSO. Possibly this indicates that the large, hetero-

geneous group of anesthetics, including DMSO, not only share a number of biochemical effects on biomembranes, but also provoke a similar change in membrane morphology.

Many investigators have tried to clarify the biochemical background of the anesthetic action. Most authors postulate a hydrophobic interaction of the anesthetic with the membrane. In connection with a modified version of the Meyer–Overton rule of anesthesia Seeman has pointed out that the product of the drug concentration in the membrane (C_{membr}) and the van der Waals volume of the drug molecule (V_{mol}) is the most constant parameter for a wide variety of anesthetics at the effective concentration, falling within the range 1·0–8·5 ml/kg dry membrane.[15] According to Seeman, C_{membr} can be estimated from the octanol/water partition coefficient, with the equation: $C_{membr} = 0{\cdot}2 \times$ octanol/water partition coefficient $\times$ effective concentration.[15] Considering that the effective DMSO concentration is 3·2 M, the octanol/water partition coefficient is 10^{-2} [14] and the van der Waals volume to be about 51,[21] the volume occupation ($C_{membr} \cdot V_{mol}$) will be about 0·33. Considering the approximate character of this calculation, this value is not too far outside the range calculated by Seeman. This again supports the modified Meyer–Overton rule of anesthesia. However, the significance of this rule on a molecular basis is not clear yet. The results may be interpreted as indicating a hydrophobic anesthetic–membrane interaction, but a different interpretation is also possible.[15] It should be realized that both water-saturated octanol and the biological membrane are heterogeneous systems with hydrophobic and hydrophilic regions. The octanol/water partition coefficient of water is 7×10^{-2}, 7 times higher than the DMSO partition coefficient.[14] This means that 1 litre of water-saturated octanol contains about 6·3 moles of octanol and about 3·85 moles of water and that the DMSO/water ratio in the bulk phase of water is 7 times higher than the DMSO/water ratio in the octanol phase. Apparently the affinity of DMSO for water in the bulk phase is much greater than its affinity for water in the octanol phase. It seems obvious that a strongly hydrophilic substance like DMSO, present in the octanol phase, will accumulate at the hydrophilic, water-containing regions, just as a strongly hydrophobic substance will accumulate at the hydrophobic regions within the octanol phase.

Transferring these considerations to the biological membrane this means that the constancy of $C_{membr} \cdot V_{mol}$ at the effective concentration of all anesthetics does not indicate a similar localization of the different anesthetic molecules in the membrane. It seems inevitable to conclude that the rather constant total volume occupation of anesthetics in the membrane concerns mainly the hydrophobic core in the case of a hydrophobic anesthetic and mainly the hydrophilic regions in the membrane in the case of e.g. DMSO. Therefore the modified Meyer–Overton rule does not prove irrefutably a hydrophobic anesthetic–membrane interaction; other possibilities should be considered as well.

An alternative explanation for anesthetic action has been proposed by Pauling[22] and Miller.[23] These authors suggested that a change in the membrane water structure would be the primary effect of anesthetics. This would be in agreement with the conclusions in a previous paper,[1] in which arguments were presented indicating that DMSO affects the membrane via a disruption of water structures at the cell interface. DMSO is actually a potent breaker of water structures.[24] In this connection it is relevant that organic non-electrolytes can be dissolved in structured water, resulting in

the formation of clathrates.[24] Recently Smolen and Hagman have presented experimental evidence indicating that the solubility of these compounds in structured water, their biological permeability and their permeability through artificial layers of structured water increase with increasing olive oil/water partition coefficients.[25] According to these results a correlation between the biological effect and partition coefficients would not decide between the two possible modes of action: interaction with the hydrophobic regions of the cellular membrane or perturbation of the water structure at the cell interface.

A third possibility is a different primary action of different anesthetics, leading to a similar final result. Many anesthetics may attack the membrane via a hydrophobic interaction, whereas e.g. DMSO may affect the membrane via a disruption of water structures. According to the fluid mosaic model of membranes[26] a perturbation of the membrane may subsequently be followed by an effect on the conformation, distribution and function of membrane components, not involved in the initial interaction. There are no pertinent observations yet, allowing a decision between these possibilities.

A surprizing observation was the influence of the cellular volume on the DMSO effects. As shown in the results section both the DMSO-induced K^+ loss and the effect on red cell deformability decrease with increasing cellular volume. A possible explanation for these observations is given by recent investigations concerning the effect of red cell volume on cation fluxes.[27–31] It appeared that the red cell volume had a pronounced influence on cation fluxes. According to Kregenow[29,30] this phenomenon is involved in the volume-controlling mechanism of red cells. Poznansky and Solomon suggested, based on experimental observations, that the volume dependence of cation fluxes is caused by a conformational change in receptor sites at the cell surface.[31] If membrane proteins do occur in different conformations depending on the cellular volume, it is conceivable that the effect of DMSO will depend on the actual conformation of the proteins at the moment of drug addition. Further experiments will be necessary to investigate if the effect of other drugs, particularly anesthetics, is also affected by cellular volume.

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