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# LYSIS OF YEAST CELLS AND ERYTHROCYTES BY DIMETHYLSULFOXIDE

A. W. DE BRUIJNE and J. VAN STEVENINCK

Laboratory for Medical Chemistry, Wassenaarseweg 62, Leiden, The Netherlands

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**Abstract**—The cytolytic effects of dimethylsulfoxide on yeast cells and erythrocytes were studied in some detail. When a certain threshold concentration of the drug was exceeded, lysis increased sharply with increasing dimethylsulfoxide concentration. The lowest dimethylsulfoxide concentration causing appreciable $K^+$ leakage from the cells appeared to be 5·6 M for commercial baker's yeast, 4·2 M for the yeast strain Hansen CBS 1172, 2·8 M for human red blood cells, 5·6 M for sheep, and 7·0 M for bovine erythrocytes.

It could be shown that the dimethylsulfoxide-induced cytolysis of yeast cells should be ascribed to a direct influence of the drug on the cellular membrane. The experimental results were consistent with a drug-induced conformation change of membrane lipoproteins, presumably via a primary change of the vicinal water structure.

The differences in the kinetics and characteristics of dimethylsulfoxide-induced and surfactant-induced cytolysis are briefly discussed.

Sublytic dimethylsulfoxide concentrations appeared to have no influence on the osmotic fragility of red blood cells.

MUCH attention has been devoted in recent years to the various effects of dimethylsulfoxide (DMSO) on biological systems. This interest was stimulated by the possible applications of this drug in various fields, e.g. radioprotection,[1–3] cryobiology,[4–6] analgesia[7,8] and percutaneous absorption.[9–11] Many aspects of the effects of DMSO on biological systems are still obscure, however, on a molecular level.

In the present investigations some effects of DMSO on yeast cells and erythrocytes were studied. It appeared that relatively high concentrations of DMSO caused cytolysis. The characteristics of the process were studied in some detail, in an attempt to elucidate the physico-chemical background of the cytolysis. The results are discussed in the present communication.

## METHODS

In the present experiments two yeast strains were used: commercial baker's yeast ("Koningsgist", obtained from the Gist en Spiritusfabriek, Delft) and strain Hansen, CBS 1172. The latter yeast strain was grown aerobically as described previously.[12] Anaerobic culturing was performed as described before,[13] with addition of ergosterol to achieve maximal growth.[14]

Incubation of yeast cells with DMSO was performed by adding a chilled, concentrated yeast suspension to a DMSO solution in a thermostat bath. This was done as preliminary experiments had shown that the reverse procedure: DMSO addition to the yeast cells in diluted suspension, caused heat damage of the cells (demonstrated by a rapid initial $K^+$ loss) brought about by the heat of dilution. If not otherwise

indicated the experiments were conducted in an unbuffered medium at pH 5·5, with a final yeast concentration of 10 per cent.

Heparinized human, bovine and sheep blood was centrifuged shortly after collection, the plasma and buffy coat being discarded. The red blood cells were washed three times in buffered 1% NaCl and added to DMSO solutions in 1% NaCl. Buffered NaCl solutions were prepared according to Parpart *et al.*[15] A final erythrocyte concentration of 2·5% was utilized.

Osmotic fragility measurements following preincubation of the red cells with sublytic DMSO solutions in 1% NaCl were performed as described before,[16] with one essential modification. The hypotonic NaCl solutions were supplemented with the same DMSO concentration, utilized during preincubation.

$K^+$ determinations were carried out with a flame photometer. All data concerning $K^+$ leakage were corrected for the small and slow loss of $K^+$ observed in control experiments, without DMSO added to the medium. Assay of ninhydrin reactive substances was performed according to Moore and Stein.[17] Hemolysis was calculated from the amount of hemoglobin liberated in the supernatant, measured according to Crosby *et al.*[18]

The percentage of non-viable yeast cells was measured by selective staining of non-viable cells, as described previously.[19] Measurements of the mean cellular volume were performed by centrifugation of the cell suspension for 30 min at 3000 rev/min in Hamburger type hematocrit tubes.

[$^{14}$C]DMSO was measured in a liquid scintillation counter, utilizing the liquid scintillator described by Bray.[20] Fatty acids from yeast phospholipids were analysed by gas chromatography. The lipid extraction was carried out as described by Deierkauf and Booij.[21] The phospholipids were isolated from the total lipid extract according to Zöllner and Wolfram.[22]

## RESULTS

The loss of cellular $K^+$ and ninhydrin reactive substances during incubation of commercial baker's yeast cells with DMSO at 25° is depicted in Fig. 1. Immediately after addition of the yeast cells to the DMSO solution $K^+$ leakage begins, proceeding linearly with time. Deviations from linearity occur after longer incubation periods. This $K^+$ leakage is associated with a concomitant loss of ninhydrin reactive substances to the medium, during the entire experimental period. The ninhydrin reactive substances appeared to be dialyzable, indicating that small molecules are involved. In control experiments, without DMSO added to the medium, the medium never showed a positive ninhydrin reaction.

The correlation between $K^+$ leakage and the increase of the number of non-viable cells is shown in Fig. 2. Apparently the percentage of non-viable cells, as measured by selective staining of these cells, is somewhat smaller than the % $K^+$ loss, during the entire experimental period. The microscopic appearance of the cell wall was normal, both of the stained and of the unstained yeast cells.

The influence of the DMSO concentration on $K^+$ loss is shown in Fig. 3. At drug concentrations lower than 5·6 M the cytolytic effect is very small. At higher concentrations however, the cytolytic effect increases sharply with the drug concentration, as shown in Figs. 3 and 4. Therefore 5·6 M may be described as a threshold concentra-

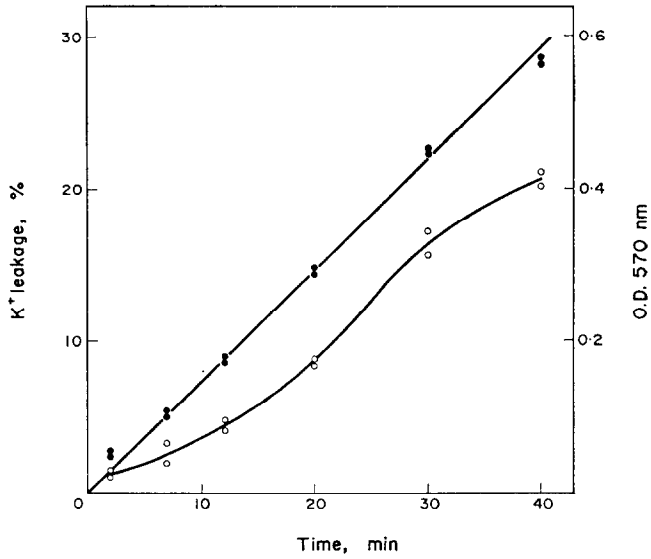


FIG. 1. The leakage of $K^+$ (●—●) and of ninhydrin reactive substances (○—○) from yeast cells at a DMSO concentration of 7·4 M at 25°. The ninhydrin reaction of the supernatant is expressed in O.D., 570 nm.

tion, defined arbitrary as the lowest drug concentration causing more than 10% $K^+$ loss in 60 min.

With the yeast strain Hansen, CBS 1172 similar results were obtained, except that these yeast cells appeared to be significantly more sensitive to DMSO. The drug threshold concentration appeared to be about 4·2 M for this yeast strain.

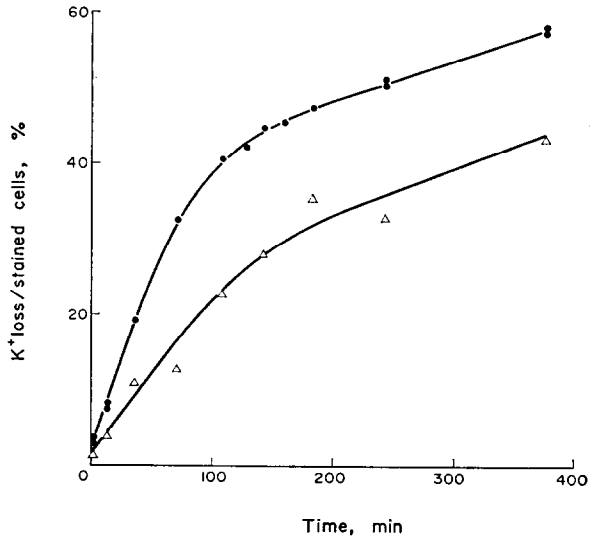


FIG. 2. The percentage of $K^+$ loss (●—●) and of stained (non-viable) cells (△—△) in the course of time at a DMSO concentration of 6·8 M, at 25°.

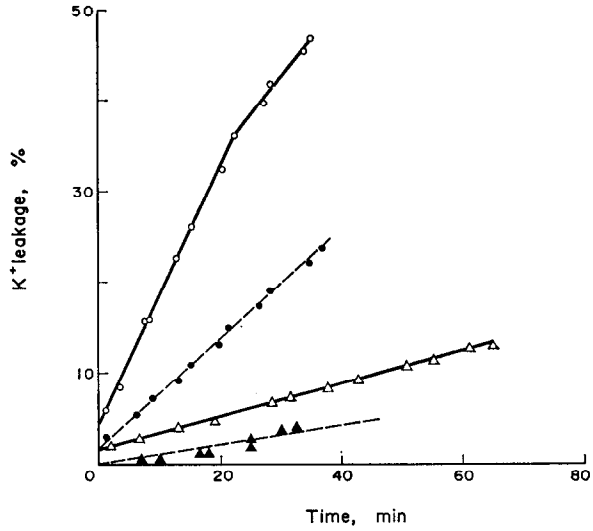


FIG. 3. The leakage of $K^+$ in the course of time at 25°, at DMSO concentrations of 7·4 M (○—○), 6·8 M (●—●), 6·2 M (△—△) and 5·5 M (▲—▲).

Control measurements of the mean cellular volume after varying incubation periods with DMSO never revealed osmotic swelling of the cells.

Studies on the influence of temperature on the cytolytic process revealed an increase of the velocity of $K^+$ loss with increasing temperature, measured over the range 0–25°. A typical experiment is shown in Fig. 5. Calculation of the velocity of $K^+$

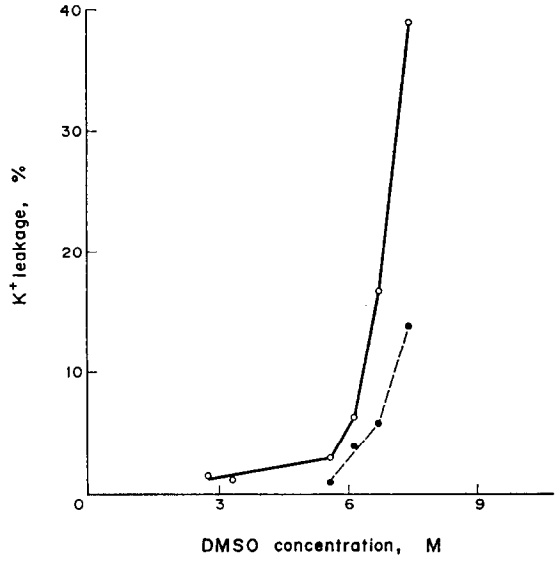


FIG. 4. The influence of DMSO concentration on $K^+$ loss at 15° (●—●) and at 25° (○—○). The $K^+$ loss was measured after an incubation period of 25 min.

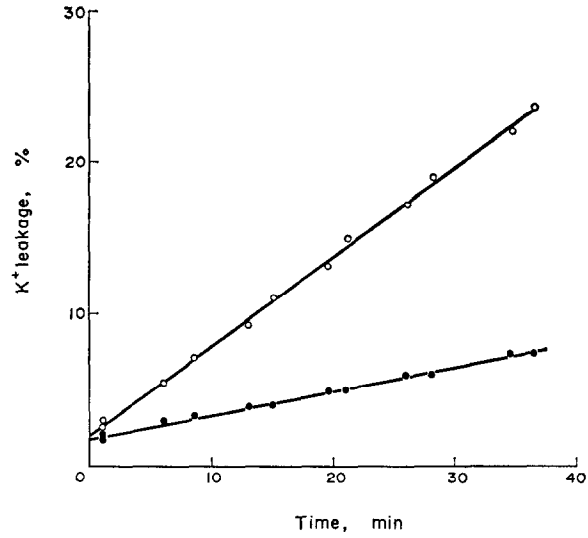


FIG. 5. $K^+$ loss from yeast cells at 25° (○—○) and at 15° (●—●) at a DMSO concentration of 6·8 M.

leakage according to the Arrhenius equation revealed an energy of activation of 22,000 cal/mole over the temperature range 15–25°.

The observed permeability increase of the cytoplasmic membrane can be caused either by a direct influence of DMSO on the membrane from the outside or by an indirect effect, following DMSO penetration into the cytoplasm. To differentiate

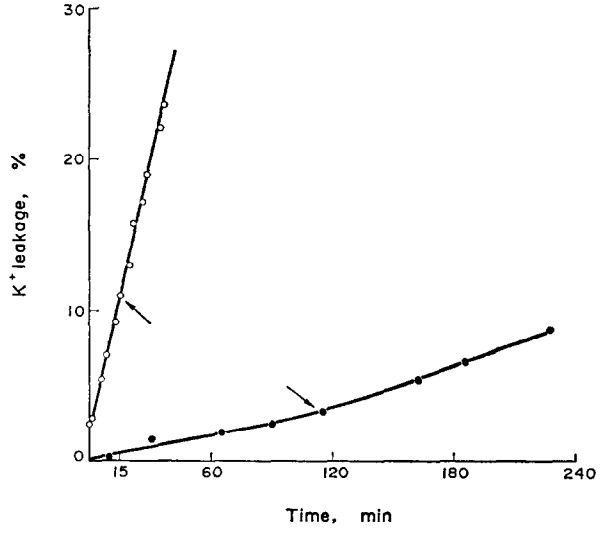


FIG. 6. $K^+$ leakage from yeast cells at DMSO concentrations of 6·8 M (○—○) and 3·4 M (●—●) at 25°. At the moments indicated by arrows, the intracellular drug concentrations are equal in the two experiments, as described in the text.

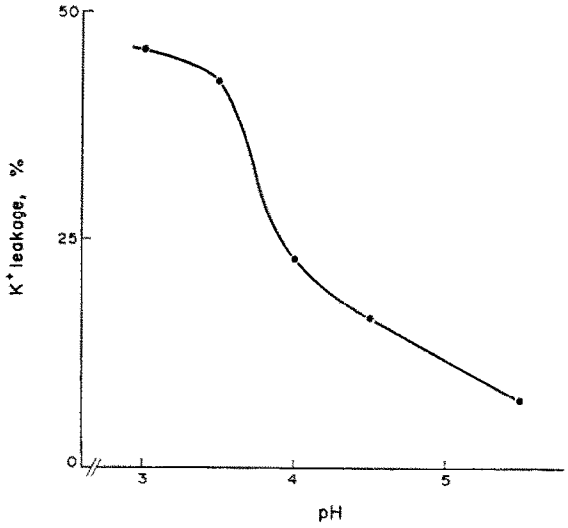


FIG. 7. The influence of pH on DMSO-induced $K^+$ leakage from yeast cells. DMSO concentration: 6·0 M. Temperature: 25°. $K^+$ leakage was measured after 30 min.

between these two possibilities $K^+$ leakage was measured at two DMSO concentrations: 6·8 and 3·4 M. The results are shown in Fig. 6. From measurements of DMSO penetration into yeast cells it is known that DMSO reaches a constant intracellular concentration in about 120 min at 25°, irrespective of the drug concentration, with a half-time of 18 min.[23] Therefore the intracellular concentration after 18 min at 6·8 M DMSO in the medium is equal to the intracellular drug concentration after 120 min at 3·4 M (arrows in Fig. 6). The velocities of $K^+$ leakage under these two conditions are quite different, however, being about 11 times larger at 6·8 M as compared to 3·4 M DMSO in the medium.

The influence of pH on the cytolytic effect of DMSO is shown in Fig. 7. The velocity of $K^+$ loss increases rather sharply on lowering the pH from 5·0 to 3·5. Outside this range the cytolytic response is much less sensitive to changes of the pH of the medium.

It has been suggested that DMSO might increase membrane permeability by inducing a change of the isomeric conformation of unsaturated fatty acids in the membrane phospholipids.[24] Therefore the influence of DMSO on anaerobically grown yeast was compared with the drug influence on normal, aerobically grown yeast of the strain Hansen, CBS 1172. Anaerobic growth conditions induce a major shift in the fatty acid composition of the phospholipids, comprising a change from unsaturated to saturated fatty acids (Table 1). As compared to normal yeast the anaerobically grown yeast appeared to be slightly more sensitive to DMSO. The threshold concentration decreased from about 4·2 M for normal, to about 3·5 M for anaerobically grown cells.

To determine the lipid–water solubility coefficient of DMSO, 5 ml of a 1% $^{14}C$-labelled DMSO solution in water was shaken for 18 hr with 250 ml of olive oil or hexadecane. After separation of the phases both the water and the olive oil or hexadecane layer were analysed for radioactivity. The solubility coefficients could be calculated as smaller than $10^{-4}$ in the hexadecane–water system, and between $2 \times$

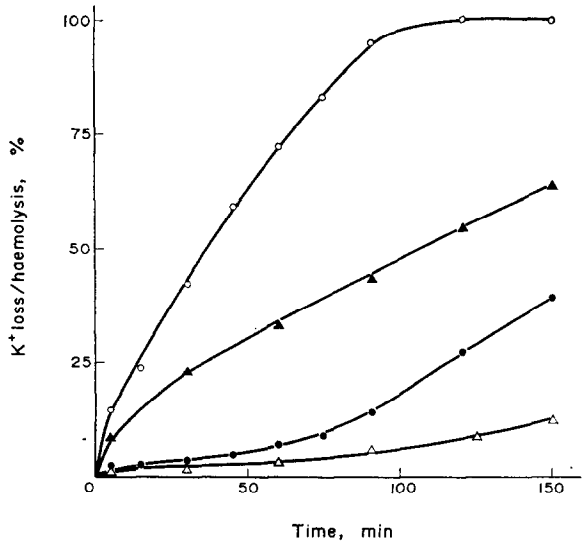


FIG. 8. $K^+$ leakage from, and hemolysis of bovine red blood cells at 25°, caused by DMSO. ○—○: $K^+$ leakage at a DMSO concentration of 7·4 M; ●—●: hemolysis at a DMSO concentration of 7·4 M; ▲—▲: $K^+$ leakage at a DMSO concentration of 7·1 M; △—△: hemolysis at a DMSO concentration of 7·1 M.

$10^{-4}$ and $4 \times 10^{-4}$ in the olive oil–water system. A more accurate calculation was impossible, because of the extreme small quantities of DMSO entering the organic phase.

In further experiments it appeared that DMSO also caused $K^+$ loss and hemolysis of red blood cells. As shown in Fig. 8 actual hemolysis was preceded by an almost complete $K^+$ loss from the cells. In the prelytic period no significant changes in the hematocrit values were found, indicating that the $K^+$ loss was counterbalanced by a simultaneous, equal $Na^+$ gain. Erythrocytes from different species exhibited significant differences in sensitivity towards DMSO. The threshold concentration was about 2·8 M for human red blood cells, 5·6 M for sheep, and 7·0 M for bovine erythrocytes.

Sublytic concentrations of DMSO (3·5–4·2 M for bovine erythrocytes) did not induce a change of the osmotic fragility of the red blood cells, if measured as described under methods.

## DISCUSSION

The DMSO-induced $K^+$ loss in yeast cells and in erythrocytes indicates a change in the permeability characteristics of these cells. Such leakage can be caused either by an all-or-none response of individual cells, having suffered major damage of the permeability barrier, or by a gradual $K^+$ loss from all cells simultaneously.[25,26] With an all-or-none response a close correlation between the % $K^+$ loss and loss of viability should be expected. In yeast suspension incubated with DMSO $K^+$ loss, leakage of ninhydrin reactive substances and increase of the number of non-viable cells occur simultaneously, without appreciable lag time, although the % $K^+$ loss was always somewhat larger than the percentage non-viable cells. Apparently this process is characterized by an initially increased permeability for cations and small molecules,

followed after a short time by an all-or-none loss of viability, indicated by the staining of the cells. In red blood cells, however, hemolysis only occurred after a preceding $K^+$ loss of about 80 per cent. Apparently the increased cation permeability is here decisive, colioid osmotic hemolysis following as a secondary process.

The experimental results clearly demonstrate that the DMSO effect must be localized at the outside of the yeast cell. The velocity of $K^+$ loss is constant during at least the first 20 min, whereas the intracellular drug concentration increases during this interval from zero to half-saturation, as discussed above. Moreover, the experiment shown in Fig. 6 demonstrates unequivocally that there is no relationship between the velocity of $K^+$ loss and the intracellular drug concentration. Apparently the only decisive factor is the DMSO concentration in the medium. Finally, the cytolytic response depended on the pH of the medium (Fig. 7). As changing the pH of the medium from 5·0 to 3·5 does not induce an appreciable intracellular pH shift,[27] this indicates again a drug influence at the cell surface.

Theoretically this DMSO effect could be ascribed either to structural disintegration of the cell wall or to an influence on the cytoplasmic membrane. In the case of disintegration of the cell wall osmotic swelling of the yeast cells should be expected, conform the reaction of yeast protoplasts. This was never observed experimentally. Moreover microscopic control never revealed evidence of protoplast formation or structural impairment of the cell wall. Further, in the case of resolution of the cell wall, a lag time between addition of DMSO and the onset of $K^+$ loss should be expected, especially at the lowest effective drug concentrations. As a lag time was never found experimentally, the kinetics of $K^+$ leakage seem to be incompatible with a disintegration of the cell wall. Therefore it may be concluded that DMSO interferes with the functional integrity of the cytoplasmic membrane.

The possibility should be considered that DMSO would affect the lipid components of the membrane. Puig Muset and Martin-Esteve have suggested that DMSO could induce a change in the isomeric conformation of unsaturated fatty acids.[23] A similar conformation change would also be induced by peroxidation of unsaturated fatty acids. It should be emphasized that these authors did find a corresponding change in the i.r.-spectra of unsaturated fatty acids, but that these experiments were performed in pure, waterfree DMSO. In anaerobically grown yeast the amount of unsaturated fatty acids was much less, as compared to normal yeast (Table 1). These results are

TABLE 1. FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS OF YEAST CELLS CULTURED UNDER AEROBIC AND ANAEROBIC CONDITIONS, IN PER CENT OF THE TOTAL FATTY ACIDS

| Fatty acid | Aerobic growth | Anaerobic growth |
|---|---|---|
| $C_{10}$–$C_{13}$ | 0·9 | 5·6 |
| $C_{14}$ | 1·4 | 9·3 |
| $C_{14:1}$ | 1·1 | — |
| $C_{15}$ | — | — |
| $C_{16}$ | 10·6 | 56·7 |
| $C_{16:1}$ | 56·3 | 13·8 |
| $C_{17}$ | — | — |
| $C_{18}$ | — | 7·5 |
| $C_{18:1}$ | 29·8 | 7·0 |
| $C_{18:2} + C_{18:3}$ | — | — |

in good agreement with similar data of Suomalainen and Nurminen.[28] Notwithstanding the smaller percentage of unsaturated fatty acids, anaerobically grown yeast appeared to be more sensitive to DMSO, as discussed above. Moreover DMSO does not catalyse peroxidation of unsaturated fatty acids, as will be discussed in a forthcoming paper. Finally a direct influence of DMSO on membrane lipids appeared unlikely *a priori*. According to the lipoid theory for anesthesia a variety of anesthetics is effective at a drug concentration of 0·033–0·065 M in the lipid phase of the membrane, calculating this concentration by multiplying the medium concentration by the olive oil–water solubility coefficient of the drug.[29] Considering the olive oil–water solubility coefficient of DMSO ($2–4 \times 10^{-4}$) and the threshold concentration of 4·2 M in the yeast strain Hansen, CBS 1172 and 5·6 M in commercial baker's yeast, the effective drug concentration in the hydrophobic regions of the membrane would be about 0·0012–0·0017 M, much lower than the pharmacologically effective concentration of a variety of anesthetics. Any direct influence of DMSO in this low concentration on lipids seems very unlikely.

It is known that DMSO has a pronounced influence on the conformation of many proteins,[30, 31] presumably by affecting the structure of the surrounding water. Recent studies have emphasized the importance of the structure of vicinal water in biological membranes, for stabilizing the functional and structural integrity.[32–34] As DMSO tends to disrupt water structure[33] it seems probable that this drug can provoke a conformation change of membrane lipoproteins via a primary change of the vicinal water structure. The high energy of activation of the cytolytic process (22,000 cal/mole) is consistent with the concept of a conformation change of membrane constituents.

It is worthwhile to compare the described cytolytic process with the cytolytic effect of cationic surfactants on yeast cells.[26,35,36] The latter differs from the DMSO-induced cytolysis by the much lower effective concentration of the surfactants, the much closer all-or-none response with no prelytic $K^+$ loss, the immediate response after surfactant addition with no progression of the lytic effect during further incubation and the lack of pH and temperature influence. This indicates a difference in the physico-chemical background of these two processes.

Sublytic DMSO concentrations did not affect the osmotic resistance of red blood cells. These results seem to contradict other studies, described in recent literature.[37, 38] The discrepancy can be attributed, however, to differences in the experimental procedure. Gerhards *et al.* incubated red blood cells with DMSO for 30 min at 38°, subsequently transferring a small volume of the suspension into a much larger volume of DMSO-free NaCl solution. In the present experiments the cells were transferred to a NaCl solution, containing the same DMSO concentration as the initial incubation medium. Only by this procedure the real osmotic fragility can be measured. If DMSO-incubated erythrocytes are transferred to a DMSO-free NaCl solution, this medium will be extremely hypotonic with respect to the DMSO containing cell content and an apparent increase of osmotic fragility will take place. This also explains why hemolysis, provoked by diluting DMSO-incubated erythrocyte suspensions with hypotonic NaCl solutions, decreased strongly if the NaCl solution was added slowly, over a period of about 10 min, instead of quickly.[39] The slow procedure allows the intracellular DMSO to permeate back to the medium on dilution, thus preventing osmotic swelling and hemolysis.

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## REFERENCES

1. M. J. ASHWOOD-SMITH, *Ann. N.Y. Acad. Sci.* **141,** 45 (1967).
2. B. L. KAUL, *Rad. Botany* **10,** 69 (1970).
3. W. L. LAPPENBUSCH and D. L. WILLIS, *Int. J. radiat. Biol.* **18,** 217 (1970).
4. D. GREIFF and P. SEIFERT, *Cryobiol.* **4,** 295 (1968).
5. F. M. GUTTMAN, A. KHALESSI and G. BERDNIKOFF, *Cryobiol.* **6,** 339 (1970).
6. D. GREIFF and S. MACKEY, *Cryobiol.* **7,** 9 (1970).
7. D. P. BECKER, H. F. YOUNG, F. E. NULSEN and J. A. JANE, *Exp. Neurol.* **24,** 272 (1969).
8. D. S. PENROD, B. BACHARACH and J. Y. TEMPLETON, *Ann. N.Y. Acad. Sci.* **141,** 493 (1967).
9. S. G. ELFBAUM and K. LADEN, *J. Soc. Cosmetic Chemists* **19,** 119 (1968).
10. S. G. ELFBAUM and K. LADEN, *J. Soc. Cosmetic Chemists* **19,** 163 (1968).
11. S. G. ELFBAUM and K. LADEN, *J. Soc. Cosmetic Chemists* **19,** 841 (1968).
12. J. VAN STEVENINCK and H. L. BOOIJ, *J. gen. Physiol.* **48,** 43 (1964).
13. A. W. DE BRUIJNE and J. VAN STEVENINCK, *Biochim. biophys. Acta* **196,** 45 (1970).
14. A. A. ANDREASSEN and T. J. B. STIER, *J. cell. Comp. Physiol.* **41,** 23 (1953).
15. A. K. PARPART, P. B. LORENZ, E. R. PARPART, J. R. GREGG and A. M. CHASE, *J. clin. Invest.* **26,** 636 (1947).
16. J. VAN STEVENINCK, W. K. GJÖSUND and H. L. BOOIJ, *Biochem. Pharmac.* **16,** 837 (1967).
17. S. MOORE and W. H. STEIN, *J. biol. Chem.* **176,** 367 (1948).
18. W. H. CROSBY, J. I. MUNN and F. W. FURTH, *U.S. arm. Forces med. J.* **5,** 693 (1954).
19. M. MAAS and J. VAN STEVENINCK, *Experientia* **23,** 405 (1967).
20. G. A. BRAY, *Analyt. Biochem.* **1,** 279 (1960).
21. F. A. DEIERKAUF and H. L. BOOIJ, *Biochim. biophys. Acta* **150,** 214 (1968).
22. N. ZÖLLNER and G. WOLFRAM, *Klin. Wschr.* **40,** 1101 (1962).
23. A. W. DE BRUIJNE and J. VAN STEVENINCK, *Biochim. biophys. Acta* **211,** 555 (1970).
24. P. PUIG MUSET and J. MARTIN-ESTEVE, *Experientia* **21,** 649 (1965).
25. H. PASSOW, A. ROTHSTEIN and T. W. CLARKSON, *Pharmac. Rev.* **13,** 185 (1961).
26. M. MAAS, Cytolysis of yeast by surfactants, Thesis Leiden (1969).
27. A. KOTYK, *Fol. microbiol.* **8,** 27 (1963).
28. H. SUOMALAINEN and A. J. A. KERÄNEN, *Suomen Kemistilehti* 36 B, 88 (1963).
29. E. J. ARIENS, A. M. SIMONIS and J. M. VAN ROSSUM, in *Molecular Pharmacology* (Ed. E. J. ARIENS) p. 287. Academic Press, New York (1964),
30. D. H. RAMMLER and A. ZAFFARONI, *Ann. N.Y. Acad. sci.* **141,** 13 (1967).
31. K. HAMAGUCHI, *J. Biochem.* **56,** 441 (1964).
32. O. HECHTER, *Fedn Proc.* **24,** S-91 (1965).
33. W. DROST-HANSEN, in *Chemistry of the Cell Interface* (Ed. H. D. BROWN). AcademicPress, New York, in press.
34. M. F. COLDMAN and W. GOOD, *Biochim. biophys. Acta* **150,** 194 (1968).
35. J. C. RIEMERSMA, *J. Pharm. Pharmac.* **18,** 602 (1966).
36. J. C. RIEMERSMA, *J. Pharm. Pharmac.* **18,** 657 (1966).
37. E. GERHARDS, H. GIBIAN and G. RASPÉ, *Arzneim. ittel. Forsch.* **15,** 1295 (1965).
38. E. GERHARDS and H. GIBIAN, *Ann. N.Y. Acad. Sci.* **141,** 65 (1967).
39. I. HOPPE, *Bibl. haemat.* **32,** 217 (1969).