Effect of Dimethylsulfoxide Concentration on the Permeability of Neonatal Rat Stratum Corneum to Alkanols

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The effect of dimethylsulfoxide (DMSO) concentration on the permeability of neonatal rat stratum corneum to ^{14}C labeled propan-1-ol and hexan-1-ol was studied in vitro. The permeability coefficients were determined from a range of DMSO-water systems. After soaking in water overnight, the same stratum corneum was used with water as both delivery and recipient phases for the alkanols. Concentrations below 70% DMSO reduced the penetration rate as a result of the solvent effect of DMSO and the formation of a DMSO-alkanol complex. Above 70% DMSO permeability increased, with a permeability coefficient greater than that from water being achieved at concentrations in excess of 80% DMSO. The second run, with water as delivery phase, showed that the effect was reversible below 70% DMSO, but that at higher concentrations DMSO had produced an irreversible change in the permeability of stratum corneum. We hypothesize a hydrogen bond–mediated mechanism for the increased permeability. *J Invest Dermatol 89:426–429, 1987*

Early work rapidly established that dimethylsulfoxide (DMSO) was capable of increasing the permeability of the skin [1–3]. Several workers who have prepared extensive reviews of skin penetration included sections on DMSO [4–8] and also considered possible mechanisms for the effect of DMSO. Most reports have been concerned with concentrations between 70 and 100% DMSO. There are few reports on the use of lower DMSO concentrations, but where they do exist, they tend to be contradictory.

Selkirk and Douglas [9] found significant increases in the penetration rate of proflavine hemisulphate using 15% DMSO, whereas Sweeney et al [10] showed that water penetration only increased with DMSO pretreatment at greater than 60% concentration. In contrast, Allenby et al [11] showed that changes in electrical resistance of the skin, associated with altered permeability, occurred over a wide range of DMSO concentrations, but concluded that DMSO below 50% was inactive as a penetration enhancer. Similar conclusions were reached by Sekura and Scala [12] and Elfbaum and Laden [13]. The early work of Stoughton and Fritsch [1] reported increased absorption with DMSO concentrations ranging from 10–50%, and the recent work of Akhter and Barry [14] also showed increased penetration of a steroid with 20% DMSO. The recent report by Kurihara-Bergstrom et al [15] has demonstrated that the effect of DMSO varies with concentration and also with the physicochemical properties of the penetrant used.

The literature is also conflicting on the reversibility of DMSO activity. The work of Kligman [16] suggested that 90 and 100% DMSO produced only minor damage to the horny layer. Sweeney et al [10] reported that the effect of DMSO on skin permeability was permanent, however. The in vitro work of Astley and Levine [17] suggested a partial reversibility of human stratum corneum barrier capacity after treatment with DMSO. Partial reversibility of the structural changes in stratum corneum induced by 80% DMSO has been reported by Chandrasekaran [18].

There is, therefore, a need for a greater awareness of the concentration dependence of the penetration enhancing properties of DMSO before an understanding of the underlying processes can be reached. The latter are, presumably, physicochemical in nature, and so it is important in the design of experiments to control as many variables as possible so that deductions about physicochemical changes can be made. This paper reports work in which propan-1-ol and hexan-1-ol penetration rates were studied from a range of DMSO-water systems. Neonatal rat stratum corneum was used to avoid the presence of pilosebaceous glands and thereby ensure that the observed penetration had occurred via the stratum corneum and not another route. Steady state, or pseudo-steady state conditions, were achieved using an "infinite" dose of penetrant to facilitate drawing conclusions about changes in the physicochemical environment of the penetrant within the stratum corneum.

MATERIALS AND METHODS

Preparation of Stratum Corneum Samples Stratum corneum was prepared by killing rats within 12 h of birth and peeling off the skin from an incision along the abdomen. The skin was immersed in water at 60°C for 10 s, followed by digestion for 1 h at 35°C in 0.1% trypsin (in normal saline buffered to pH 7.4

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Abbreviations:

DMSO: dimethylsulfoxide

Kp: permeability coefficient

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with phosphate). The digested epidermis was gently and firmly removed using cotton wool sticks. Finally, the stratum corneum was thoroughly rinsed in distilled water before being spread on a fine stainless steel mesh to dry.

Chemicals [^{14}C]Propan-1-ol (Radiochemical Centre, Amersham, U.K.), [^{14}C]hexan-1-ol (ICN Pharmaceuticals Ltd, High Wycombe, U.K.) dimethylsulfoxide (Fisons Scientific Ltd, Loughborough, U.K.), and Instagel (Packard Instrument Co. Ltd, Pangbourne, U.K.) were used.

Diffusion Experiments Penetration measurements were made in a diffusion cell, the two chambers of which had a volume of about 6 ml and were separated by the stratum corneum sample. The appropriate DMSO-water system was used as both donor and receptor phases to avoid one-way diffusion of DMSO through the stratum corneum. Both phases were continually stirred using magnetic stirrer followers. Concentrations of 3 to 4 μl/ml of alkanol were used in the donor phase. One-millliliter samples of receptor phase were removed at hourly intervals and replaced with fresh solution. Ten milliliters of the scintillator Instagel was added to each sample and the radioactivity measured using a Packard Tri-Carb Scintillation Spectrometer (Model 2425). The results were corrected to cumulative μl cm^{-3} of alkanol against time (hours) and used to calculate the permeability coefficient (Kp) using the equation: Kp = penetration rate/concentration of penetrant. Experiments were carried out at 22° ± 2°C. Results from at least four experiments were averaged.

After each penetration rate determination, the stratum corneum was stored overnight (about 16 h) in distilled water. After thorough rinsing, the penetration rates of the alkanols were redetermined from distilled water to a distilled water receptor phase using the same procedures as above.

To confirm whether hydration of the stratum corneum had an effect, the permeability coefficient from water for some experiments was determined before measurement both in the presence of and after DMSO. A further set of controls was carried out in which DMSO was included on one side only or on both sides of the stratum corneum in order to determine the effect of a DMSO concentration gradient both with and against the penetrant concentration gradient.

Infrared Spectra Infrared spectra were obtained using a Perkin Elmer 681 IR Spectrometer. A small quantity of the appropriate sample (DMSO, propanol, hexanol, or mixture) was mixed with carbon tetrachloride. A 0.01-mm NaCl cell was used.

RESULTS AND DISCUSSION

Initial checks confirmed that the passage of the alkanols was by passive diffusion and that the permeability of the stratum corneum did not vary with the side of the penetrant (hexanol Kp × 10^3 cm/h, from outer surface 10.3 ± 1.7, epidermal side 10.2 ± 1.6). Likewise, it was shown that hydration of the stratum corneum, if it occurred during an experiment, would not significantly affect permeability (hexanol Kp × 10^3 cm/h dry 10.3 ± 2.8 24 h hydrated stratum corneum 10.4 ± 1.0). This was further confirmed by using water as the donor phase before the use of DMSO solutions. There was no difference in the value of Kp obtained in such experiments from those obtained when fresh dry stratum corneum was in contact with DMSO solutions.

Table I shows the effect on Kp of hexanol of incorporating DMSO in none, either, or both donor and receptor phases. The maximum reduction in Kp was found when 70% DMSO was used on both sides of the stratum corneum. The use of water as the receptor solution increased the penetration significantly, presumably because of water diffusing through the stratum corneum and reducing the effective concentration of DMSO and its effect on Kp [17]. Conversely, the presence of DMSO in the receptor phase only still caused a reduction in Kp from water. This process can be explained by the back diffusion of DMSO through the stratum corneum, to then exert an effect on the availability of the penetrant. It follows from these results that, in any work looking at the effect of DMSO concentration on permeability, it is essential to ensure that concentration gradients, and hence the dilution of DMSO, does not occur. In this work, identical donor and receptor phases were used throughout.

Table I. Effect of Water and 70% DMSO on the Permeability Coefficient (Kp) of Hexanol Through Neonatal Rat Stratum Corneum

Donor Phase	Receptor Phase	Kp × 10^3 (cm/hr)
Water	Water	10.3 ± 2.8
70% DMSO	70% DMSO	0.81 ± 0.18
Water	70% DMSO	7.3 ± 0.5
70% DMSO	Water	1.14 ± 0.23

Figs 1 and 2 present the data obtained for the permeability coefficient of propanol and hexanol from a range of DMSO-water delivery systems and for the second run from water.

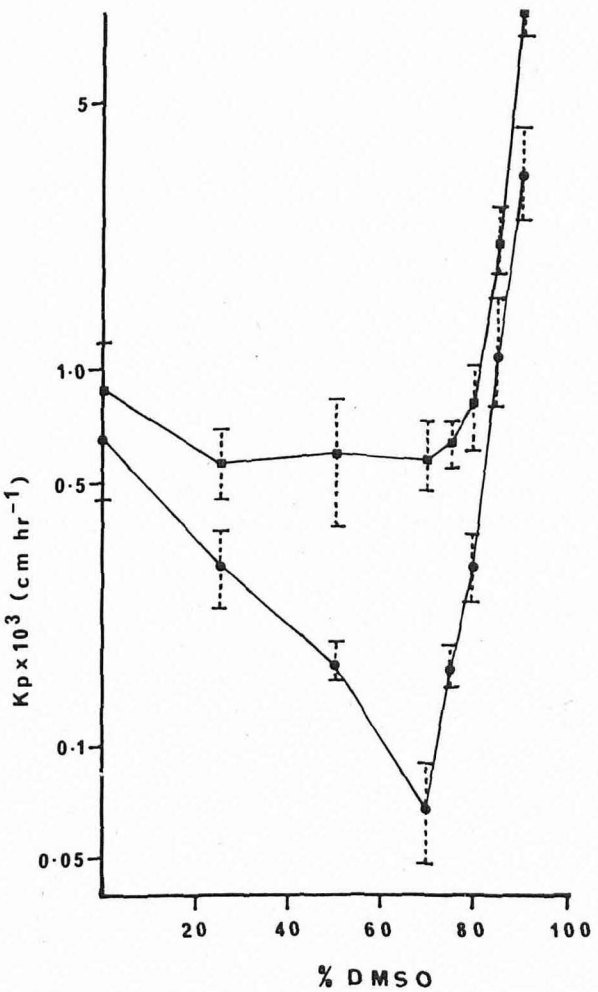

Figure 1. Graphs of the Kp of propanol in neonatal rat stratum corneum against concentration of DMSO in water at 22°C. *solid circles*, first run with DMSO-water system as both donor and receptor phase; *solid squares*, second run with water as both donor and receptor phase using the same stratum corneum, now washed, as in the first run.

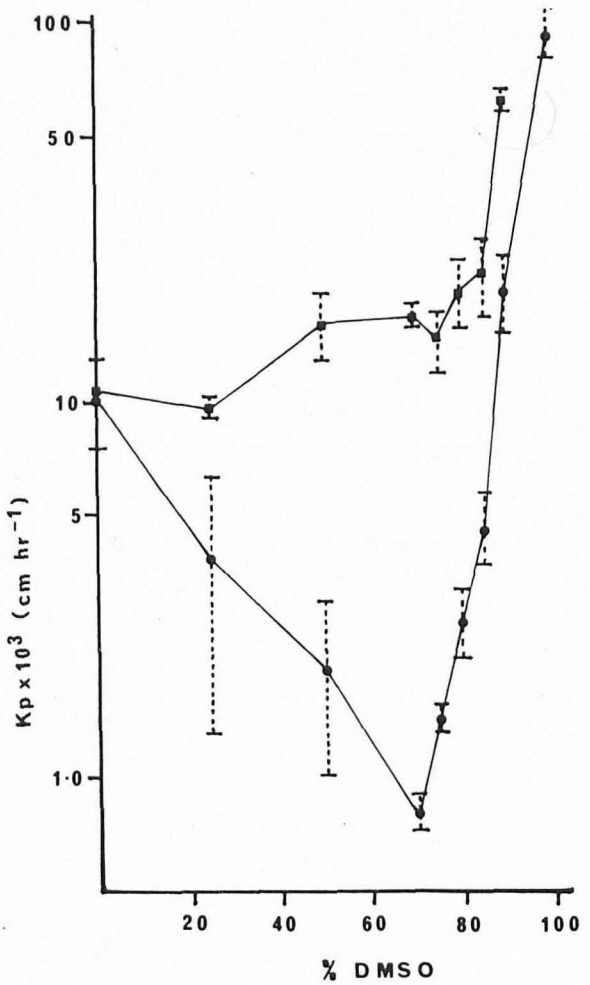

Figure 2. Graphs of the Kp of hexanol in neonatal rat stratum corneum against concentration of DMSO in water at 22°C. *solid circles,* first run with DMSO-water system as both donor and receptor phase; *solid squares,* second run with water as both donor and receptor phase using the same stratum corneum, now washed, as in the first run.

The permeability coefficient of hexanol from water was greater than the corresponding figure for propanol. This is expected because of the greater solubility of propanol in water, making it less available for partitioning into the stratum corneum [4]. As the concentration of DMSO was increased, the permeability coefficient of both alkanols was reduced, reaching a minimum in the region of 70% DMSO. Thereafter, there was a sharp increase in both permeability coefficients. A similar pattern of results has been reported recently (15) for butanol, but only when the DMSO solutions were on both sides of the stratum corneum. With the polarity of butanol being between those of propanol and hexanol, such a similarity is to be expected. The data obtained from the second runs showed that the permeability coefficient was constant up to 75% DMSO and thereafter increased. If the effect of DMSO on the stratum corneum was reversible, the second run would have given a constant value for permeability coefficient irrespective of the concentration of DMSO used on the first run. This was found to be the case up to about 75% DMSO. Above that figure, the Kp increased, indicating that the previous exposure of stratum corneum to the DMSO-water systems had caused an irreversible change in the stratum corneum, which resulted in an increased permeation rate.

Figures 1 and 2 show that the reduction in permeability coefficient between 0 and 70% DMSO is similar for both alkanols. For example, the ratios of the Kp at 0% DMSO to that at 70% DMSO were 10.1 and 12.7 for propanol and hexanol, respectively. This similarity suggests a similar mechanism for the reduction of permeability of both alkanols. Dugard and Embery [19] have attributed reduced penetration rates of alkanols from 25 to 50% DMSO to the effect of partition coefficient. Because of the solvent properties of DMSO, as the concentration was increased, so the thermodynamic activity of the penetrant was reduced. Using an experimental design similar to that in the present work, a close correlation between observed permeability and thermodynamic activity of the penetrant in DMSO concentrations up to 50% DMSO has been clearly demonstrated [15] for methanol, butanol, and octanol. This behavior, therefore, is not related to the nature of the penetrant, but to the solvent effect of the DMSO delivery phase, thereby confirming the explanation for the observations made in this study.

In addition to this solvent effect, infrared investigation indicated that hydrogen bonding of the alkanols to DMSO also occurred. Hexanol alone showed one sharp band at 3640 cm^{-1} and one broad band at 3350 cm^{-1}, indicating free and bound hydroxyl groups. The addition of an equal volume of DMSO produced a large reduction in the free hydroxyl band at 3640 cm^{-1}, which was almost eliminated at 2 vol DMSO to 1 vol hexanol. There was a corresponding increase in size of the broad band at 3350 cm^{-1}, which also shifted to 3420 cm^{-1}. Similar results were obtained with propanol. This confirms the report of Szmant [20] that a hydrogen-bonded complexation occurs between DMSO and alkanols. It follows, therefore, that both propanol and hexanol will have a greater affinity for DMSO solutions, confirming that the reduction in Kp up to 70% arises from reduced availability of the penetrant.

On the second run, when water was the delivery phase, complete reversibility of the reduction in Kp was observed. This indicates that the presence of up to 70% DMSO had left no residual effect on the stratum corneum and further supports the view that a solvent effect was responsible for the reduced permeability coefficient in the presence of low DMSO concentrations.

Over the concentration range 75 to 90% DMSO, the flux of both alkanols increased with concentration (compared with the flux from 70% DMSO). This suggests that there has been a reduction in the diffusional resistance of the stratum corneum barrier. An approximate indication of the effect of DMSO on skin permeability may be obtained by comparing the Kp at any concentration with the lowest Kp observed. Thus, the ratio of Kp at 90% DMSO to the lowest (70% DMSO) shows a 47-fold and 23-fold increase for propanol and hexanol, respectively. (These ratios assume a similar thermodynamic activity of the alkanols in both DMSO solutions. This is unlikely to be the case, and, because the thermodynamic activity at 90% DMSO is likely to be lower, the actual increase in stratum corneum permeability is probably greater than indicated by these ratios).

Figures 1 and 2, therefore, show two opposed effects of DMSO, the penetrant-vehicle interaction discussed above and a skin-vehicle interaction leading to a decreased diffusional resistance. To what extent these effects overlap each other cannot be enumerated from the present data. Consequently, it was not possible to determine the concentration of DMSO at which increased stratum corneum permeability was shown. However, it is worth noting that the reduction in thermodynamic activity continues to increase up to 100% DMSO for butanol and octanol [15]. From this information, it is reasonable to assume that propanol and hexanol behave in a similar way. It follows that the penetration-enhancing effect of DMSO is actually greater than the Kp data suggests and that it may have started at a slightly lower concentration than Figs 1 and 2 suggest.

On the second run, with water as donor phase, there was no significant change in Kp up to pre-treatment with 80% DMSO. Thereafter a sharp increase in permeability was observed. Thus, the effect of DMSO was reversible below and irreversible above about 80% DMSO. Although the literature contains no report of work on alkanols covering such a range of DMSO concen-

trations, the results reported here are in general agreement with the published work, although it disagrees with Kligman [16], who reported that the effect of 90% and 100% DMSO on stratum corneum was reversible.

It has been suggested [19,21] that DMSO increases skin permeability by increasing skin hydration and causing swelling. The possibility of this was studied by comparing the penetration of hexanol through stratum corneum after a 6-h treatment with 90% DMSO, with stratum corneum treated identically but dried under room conditions before penetration measurements. The latter gave a Kp of $69.5 \pm 3.9 \times 10^{-3}$ cm/hr, the former a Kp of $62.2 \pm 3.9 \times 10^{-3}$ cm/hr. This strongly indicates that the irreversible effect of 90% DMSO is not due to hydration or swelling of stratum corneum. Thus, the increasing permeability of stratum corneum found with concentrations in excess of 70% DMSO is unlikely to be due to a simple hydration effect. It may therefore indicate structural changes in the stratum corneum barrier or an extraction of some components of the stratum corneum by DMSO, which is known to be a good solvent [15].

Earlier discussion of the ratios of Kp at 90% DMSO to those at 70% DMSO indicated that the increase in permeability for propanol is at least double that for hexanol (47-fold and 23-fold, respectively). Thus, the diffusional route of propanol must have become relatively easier than for hexanol. Because hexanol is relatively lipid-soluble and propanol relatively water-soluble, this would indicate that there has been some change in the lipid environment in the stratum corneum.

Cowie and Toporowski [22] have shown that DMSO forms a hydrogen bonded complex with water. This has been established as being in the molar ratio 1:2 (equivalent to about 67% v/v DMSO), with a bond strength approximately 1.3 times as strong as water-water bonding [23]. The data in Figs 1 and 2 indicate that the penetration-enhancing property begins to overcome the solvent effect of DMSO only at concentrations of DMSO in which the water-DMSO bonding capacity was completely satisfied, i.e., above 67%.

In such systems, there will be DMSO molecules with unsatisfied hydrogen bonding capacity, and these will be potentially more reactive. A reasonable explanation of such observations is that the DMSO molecules form hydrogen-bonded complexes with stratum corneum lipids, resulting in structural or configurational changes that give rise to the increased permeability. These changes would be concentration-dependent, but would probably only be seen at concentrations when "free" DMSO molecules were available (that is above about 70% DMSO). From the present data it is clearly not possible to preclude other possible mechanisms, such as lipid extraction for example, which may also occur.

There is also a concentration difference between the start of increasing values of Kp and the effect becoming irreversible (Figs 1 and 2). Over the range 70 to 80% DMSO, the action of DMSO was to increase stratum corneum permeability, but the effect may be reversible, while above 80% DMSO it is irreversible. This would indicate that more than one mechanism of action of DMSO, one reversible and one irreversible, may be involved in producing penetration enhancement. However, from the present data this cannot be stated with certainty, nor can detailed mechanisms be proposed. Further work is being undertaken to investigate this observation.

From the foregoing discussion it is concluded that below 70% DMSO reduces the penetration rate of the alkanols by a solvent effect. Above 70% DMSO, the permeability of stratum corneum is increased with DMSO concentration, an effect which may be reversible up to 80% DMSO. Hydrogen bonding of DMSO with the stratum corneum barrier appears to be involved in producing the increased permeability observed.

REFERENCES

1. Stoughton RB, Fritsch WI: Influence of dimethyl sulfoxide on human percutaneous absorption. Arch Dermatol 90:512–517, 1964
2. Maibach HI, Feldmann RJ: The effect of DMSO on percutaneous penetration of hydrocortisone and testosterone in man. Ann NY Acad Sci 141:423–427, 1967
3. McDermot HL, Finkbeiner AJ, Wills WJ, Heggie RM: The enhancement of penetration of an organophosphorus acetylcholinesterase through guinea pig skin by dimethyl sulfoxide. Can J Physiol Pharmacol 45:299–303, 1967
4. Scheuplein RJ, Blank HI: Permeability of the skin. Physiol Rev 51:702–747, 1971
5. Scheuplein RJ, Ross L: Effects of surfactants and solvents on the permeability of epidermis. J Soc Cosmet Chem 21:853–873, 1970
6. Idson B: Biophysical factors in skin penetration. J. Soc Cosmet Chem 22:615–634, 1971
7. Idson B: Percutaneous absorption. J Pharm Sci 64:901–924, 1975
8. Barry BW: Drugs and Pharmaceutical Sciences: Dermatological Formulations: Percutaneous Absorption. New York, Marcel Dekker, 1983
9. Selkirk AB, Douglas M: The influence of formulation on the percutaneous absorption of proflavine hemisulphate. Pharm Acta Helv 51:160–163, 1976
10. Sweeney TM, Downes AM, Matoltsy AG: The effect of dimethyl sulfoxide on the epidermal water barrier. J Invest Dermatol 46:300–302, 1966
11. Allenby AC, Fletcher J, Schock C, Tees TFS: The effect of heat, pH and organic solvents on the electrical impedence and permeability of excised human skin. Br J Dermatol 81(suppl 4):31–39, 1969
12. Sekura DL, Scala J: The percutaneous absorption of alkyl methyl sulfoxides. Adv Biol Skin 12:257–269, 1972
13. Elfbaum SG, Laden K: The effect of dimethyl sulfoxide on percutaneous absorption: A mechanistic study, Part I. J Soc Cosmet Chem 19:119–127, 1968
14. Akhter SA, Barry BW: Flurbiprofen penetration and enhancement through cadaver skin. Value of 'in vivo mimic' approach with low enhancer concentrations. J Pharm Pharmacol 35:30P, 1983
15. Kurihara-Bergstrom K, Flynn GL, Higuchi WI: Physicochemical study of percutaneous absorption enhancement by dimethylsulfoxide: kinetic and thermodynamic determinants of dimethylsulfoxide mediated mass transfer of alkanols. J Pharm Sci 75:479–486, 1986
16. Kligman AM: Topical pharmacology and toxicology of dimethyl sulfoxide—Part 1. JAMA 193:140–148, 1965
17. Astley JP, Levine M: Effect of dimethyl sulfoxide on permeability of human skin in vitro. J Pharm Sci 65:210–215, 1976
18. Chandrasekaran SK, Campbell PS, Michaels AS: Effect of dimethyl sulfoxide on drug permeation through human skin. AIChE Journal 23:810–816, 1977
19. Dugard PH, Embery G: The influence of dimethyl sulfoxide on the percutaneous migration of potassium butyl [^{35}S] sulphate, potassium methyl [^{35}S]sulphate and sodium [^{35}S] sulphate. Br J Dermatol 81(suppl 4):69–74, 1969
20. Szmant HH: Chemistry of DMSO, Dimethyl Sulfoxide. Vol 1. Basic Concepts of DMSO. Edited by Jacob SW, Rosenbaum EE, Wood DC. New York, Marcel Dekker, 1971, 1–97.
21. Elfbaum SE, Laden K: The effect of dimethyl sulfoxide on percutaneous absorption: A mechanistic study, Part II. J. Soc Cosmet Chem 19:163–172, 1968
22. Cowie JMG, Toporowski PM: Association in the binary liquid system dimethyl sulfoxide–water. Can J Chem 39:2240–2243, 1961
23. MacGregor WS: The chemical and physical properties of DMSO. Ann NY Acad Sci 243:20–23, 1975