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Short Communications

Factors affecting the permeability of water through nude mouse skin in vitro. II. Antibiotics in diffusion media

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Summary

The effect of the addition of streptomycin sulphate (100 μg/ml) and a combination of chloramphenicol sodium succinate (50 μg/ml) and amphotericin B (50 μg/ml) to diffusion media on the increasing permeation phenomenon of water was studied in a closed diffusion cell system. No significant difference ($P < 0.05$) could be found between the control experiment and the experiment with streptomycin sulphate. A significant decrease ($P < 0.05$) of the increasing permeation phenomenon occurred after 24 h in the chloramphenicol sodium succinate/amphotericin B experiment. The microbial breakdown of the permeation barrier contributed only to a small extent to the increasing permeation phenomenon of ^{3}H-water. It is important to prevent the proliferation of micro-organisms especially when experiments of 12 h or longer duration are conducted. A broad spectrum antibiotic, combined with an antifungal agent should be used on both sides of the skin in closed diffusion cell systems.

Deterioration of the stratum corneum of nude mouse skin in a closed diffusion cell system as a function of time was reported by Van der Merwe and Ackermann (1987). A phenomenon of increasing permeability was also reported which may be due to several factors of which temperature, time of hydration, urea concentration and stirring of the donor- and receiver cells were discussed by Ackermann et al. (1985) and Van der Merwe et al. (1988). Microbial breakdown of the membrane structures may also be a cause of deterioration, especially in the in vitro situation where the skin is immersed in an aqueous solution throughout the experiment. The aqueous conditions provide an excellent growth medium for microbial proliferation. Extensive hydration causes physical deterioration of the permeation barrier (Van der Merwe et al., 1988) which may result in micro-organisms entering the skin more easily.

Addition of an antibiotic to the diffusion medium should prevent or minimize microbial growth. In a preliminary study it was found, after isolation of the organisms present after various

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TABLE 1

Permeability coefficients

Time (h)	*n*	Permeability coefficients × 10 (cm/h)		
		Normal saline	Normal saline + 100 μg/ml streptomycin sulphate	Normal saline + 50 μg/ml chloramphenicol sodium succinate + 50 μg/ml amphotericin B
6	4	0.11 ± 0.03	0.13 ± 0.03	0.8 ±0.02
12	3	1.6 ±0.4	1.9 ±0.3	1.1 ±0.4
24	2	17 ±1	14 ±1	7.7 ±3.7
48	1	31 ±0	29 ±0	10 ±0 *

Permeability coefficients of a 9.9×10^{-4} M ^{3}H-water solution at 37 °C with only normal saline as diffusion medium and with 100 μg/ml streptomycin sulphate and a combination of 50 μg/ml chloramphenicol sodium succinate and 50 μg/ml amphotericin B added to the diffusion medium as a function of time. Values are means ± S.D.

* Value obtained by extrapolation.

time intervals, that streptomycin sulphate in a concentration of 100 μg/ml prevented growth of all types of micro-organisms isolated. Blank et al. (1967) also used a weak solution of streptomycin in combination with penicillin in the receiver phases during experiments conducted over a period of days. In a preliminary study addition of streptomycin sulphate in a concentration of 100 μg/ml to the diffusion medium on both sides of the skin caused a decrease in the increasing permeation phenomenon of [^{14}C]methanol. Therefore, streptomycin sulphate (100 μg/ml) was used in the following study on both sides of the skin in order to assess the effect of the antibiotic on the increasing permeation phenomenon of ^{3}H-water (9.9×10^{-4} M). In order to expand the spectrum of antimicrobial activity to include both bacteria and fungi, chloramphenicol sodium succinate (50 μg/ml), a broad-spectrum antibiotic in combination with the antifungal amphotericin B (50 μg/ml), was used in another experiment. Normal saline was used as a control. The same general procedure as described by Van der Merwe and Ackermann (1987) was followed, except that the contents of both the donor and receiver cells were stirred in all 4 diffusion cell systems. All the experiments were performed at 37 °C. At each sampling the contents of the receiver cells were replenished with fresh normal saline containing antibiotic in order to maintain the concentration of streptomycin sulphate at 100 μg/ml and that of chloramphenicol sodium succinate and amphotericin B at 50 μg/ml throughout the experiment. The results of these experiments are presented in Table 1.

No significant difference ($P < 0.05$) could be found between the control experiment and the experiment with streptomycin sulphate with regard to the increasing permeation phenomenon. However, a significant decrease ($P < 0.05$) of the increasing permeation phenomenon occurred after 24 h in the chloramphenicol sodium succinate/amphotericin B experiment. It became evident that the microbial breakdown of the permeation barrier contributed only to a small extent to the increasing permeation phenomenon of ^{3}H-water.

It is important to prevent the proliferation of micro-organisms especially when experiments of 12 h or longer duration are conducted with a closed diffusion cell system. It is recommended that a broad spectrum antibiotic, combined with an antifungal agent should be used on both sides of the skin in such experiments.

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