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# The effect of various polymer applications on the physical structure of nude mouse skin

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## Summary

The percutaneous absorption of water and urea through nude mouse skin in vitro was investigated. The influence of water and urea with and without different polymer carriers on nude mouse skin is reported. The solutions were brought in contact with the skin in an open cell diffusion system, the samples were removed after certain periods of time and investigated by scanning and transmission electron microscopy. The influence of polymers on the barrier properties of the stratum corneum through scaling, hydration and syneresis, which affected the percutaneous absorption of water and urea, is described.

## Introduction

Percutaneous absorption studies conducted by Ackermann and Flynn (1987) in vitro have shown increasing permeation as a function of time for water, urea and glycerol. Their results were confirmed by Van der Merwe et al. (1988) who found that factors such as temperature, time of hydration and deterioration of the skin permeation barrier played an important role in the permeability profiles obtained.

When these substances are incorporated into polymer vehicles for topical application, other factors such as the structural properties of the polymers in solution, the pH of the polymer solutions and evaporation from these polymer solutions in addition to those described by Van der Merwe et al. (1988) may influence the permeability profiles obtained.

Van der Merwe and Ackermann (1987) found structural changes to the skin and physical deterioration of the extensively hydrated stratum corneum. This phenomenon was observed after 48 h of percutaneous absorption studies with the closed cell diffusion system. Studies conducted by Ackermann et al. (1988) with the open cell diffusion system, have shown no physical deterioration of the stratum corneum after the same period of time. It could be concluded that the stratum corneum maintained its barrier function when used during in vitro studies with the open cell diffusion system.

In the light of these results, the physical influ-

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ence of the polymers on the stratum corneum of the skin was investigated by means of transmission electron microscopy (TEM). Scanning electron microscopy (SEM) was used to investigate the changes of the polymer films formed on the skin surface during these studies.

## Materials and Methods

*Materials*

The following materials were used during this study:

(1) Polymers JR 125, 400 and 30M with average molecular weights of $2.5 \times 10^5$, $4.0 \times 10^5$ and $6.0 \times 10^5$, respectively (supplied as a white powder by Union Carbide), Gafquat copolymer 734 with an average molecular weight of $1.0 \times 10^5$ (supplied as a 50% viscous alcoholic solution) and Gafquat copolymer 755N with an average molecular weight of $1.0 \times 10^6$ (supplied as a 20% viscous aqueous solution). The last two polymers were both supplied by Gafquat, South Africa.

(2) [$^{14}$C]Urea with activity 250 μCi/mg (supplied as as freeze-dried solid) and [$^3$H]water with activity 1 Ci/ml, both supplied by Amersham Laboratories, U.K.

(3) De-ionized water and sodium chloride injection BP 0.9% (w/v) (normal saline), supplied by Sabax (Pty) Ltd, South Africa.

(4) Unlabelled urea, analytical grade and unlabelled glycerol, analytical grade, supplied by G.D. Searle (Pty) Ltd, South Africa.

*Preparation of stock solutions*

All the stock solutions were prepared from the materials as received from the suppliers. These procedures were performed by using Finnpipettes and Eppendorf comfortips. The stock solutions were used as prepared. Where normal saline was used as solvent it provided isotonic solutions.

Solutions of 1% and 2% (w/v) of each of the three grades of polymer (JR 125, 400 and 30M) were freshly made up. At each concentration three solvents were used, namely: (1) 0.06% (w/v) urea in normal saline, (2) normal saline and (3) de-ionized water. The solvents were preheated to 45°C to facilitate solubility of the polymer.

The 1% and 2% (w/v) solutions of the Gafquat 734 polymer were prepared by adding 2 ml and 4 ml of the viscous alcoholic solution to 98 ml and 96 ml of each of the above mentioned solvents, respectively. The volumes were corrected after cooling. Clear solutions resulted. The pH ranged between 7.5 and 8.5 at 310 K.

The 1% and 2% (w/v) solutions of the Gafquat 755N polymer were prepared by adding 5 ml and 10 ml of the viscous aqueous solution to 95 ml and 90 ml of each of the solvents, respectively. Clear solutions resulted. The pH ranged between 5.0 and 6.0 at 310 K.

The [$^{14}$C]urea solution was made up by diluting the freeze-dried solid to 5 ml with ethanol.

The [$^3$H]water solution was made up by adding 10 ml of the original solution to 9990 ml of ethanol. 1000 ml of this solution was diluted to a 5 ml secondary solution with ethanol.

The 0.06% (w/v) urea solution was prepared by dissolving 1.212 g of the urea in 2000 ml of normal saline.

The 1.20% (w/v) urea solution was prepared by dissolving 0.242 g of the urea in 200 ml of normal saline.

The polymer and urea solutions were freshly prepared before each experiment. The polymer/labelled mixtures used during these studies were made up from the prepared stock solutions as follows:

| | |
|---|---|
| 2% polymer solutions | 500 |
| [$^3$H]water | 150 |
| [$^{14}$C]urea | 300 |
| 1.20% (w/v) urea solution | 50 |
| Total | 1000 |

The polymer solution resulted as a 1% (w/v) solution in the polymer/labelled mixtures. The 1.20% (w/v) urea solution resulted as a 0.06% (w/v) solution in the polymer/labelled mixture.

*Methods*

The samples were exposed to various solutions for different periods as described in the figure legends. The temperature of exposure was controlled at 310 K. The sample in the first diffusion cell was removed prior to each experiment so that the con-

trol sample would have the same extent of hydration. The area of skin exposed was cut from the rest with a scalpel. It was divided into two and each part prepared for TEM and SEM. The skin samples of the other three cells were removed after 2, 9 and 18 h, respectively, and treated in the same manner.

*TEM*

The skin samples were cut into small strips (2 × 1 mm) with a sharp scalpel and placed on a thin layer of cool melted 1.5% (w/v) water agar which was left to gel on a glass plate. The skin strips were then covered with a thin layer of agar and left to gel (Tiedt, 1985). The strips embedded in the agar were cut into small blocks (3 × 2 mm) and flooded with a 2% (v/v) glutaraldehyde/formaldehyde fixative for a minimum of 6 h. The agar blocks were then rinsed in a phosphate buffer (sodium acid phosphate:sodium hydroxide = 5:1, pH 7.2) for 15 min and post-fixed in 1% (w/v) osmium tetroxide for 1 h.

*SEM*

A modified version of the vapor fixation process described by Tiedt (1985) was used to prepare the skin samples. The skin samples were placed on a parafilm layer in a Petri dish. The samples were secured in the corners with pins to prevent curling. Several drops of a 2% osmium tetroxide solution, previously heated to room temperature, were placed on the parafilm layer. No direct contact between the solution and the skin samples occurred. The Petri dish cover was replaced and the dish left for 3 days to air dry. The dried samples were mounted on SEM stubs and coated with 3 nm carbon and 3 nm gold palladium. The skin samples were then studied at 10 kV with a scanning electron microscope (S.A. Phillips Transmission Electron Microscope). Micrographs were taken by means of the electron microscope camera.

**Results and Discussion**

TEM micrographs representing the influence of the different polymers on nude mouse skin (obtained from the dorsal region) at commencement of the experiments can be seen in Fig. 1. The samples were removed almost immediately after placing in contact with the solutions. Fig. 2 represents the SEM micrographs at this stage. Figs 3 and 4 are the TEM and SEM micrographs, respectively, after 2 h, while the results after 9 h are represented by Figs. 5 and 6.

The physical presence of the polymer in the layers of the stratum corneum could not be seen. The reason might be that the time of exposure of the skin samples to osmium tetroxide during the preparation of the skin samples for TEM was much shorter than that of the agar capsules containing the polymer solutions. The skin cannot be exposed for the same period of time as the agar capsules. The skin is an organic entity and too long exposure to osmium tetroxide would have resulted in total staining of the skin samples and elimination of structural definition. The polymers, on the other hand, took longer to colour before they could be detected.

The differences between the TEM micrographs of the nude mouse skin with the normal saline solution (control) and the different polymer solutions for the period up to 9 h provide sufficient evidence that the cationic polymers influenced the structure of the stratum corneum. At 18 h the preparation procedures of the skin samples for TEM presented problems in obtaining micrographs that would be acceptable for making valid conclusions. When the skin samples were removed from the diffusion cells, the stratum corneum layers parted from the epidermis. This happened in the areas where the skin was exposed to the polymer solutions. This phenomenon was observed with the polymers with high molecular weights (JR 400, JR 30M and GAF 734) and not with the control and the JR 125 and GAF 734 polymers. The reason for this might have been the combined effect of the more adhesive nature of the polymers with their high molecular weight and the deterioration of the skin. This effect resembled tape stripping of the stratum corneum discussed by Ackermann (1983). The procedures for the SEM micrographs did not present the same problem. The skin samples used were much larger and in some places the stratum corneum was still intact. The study with the SEM was a surface study

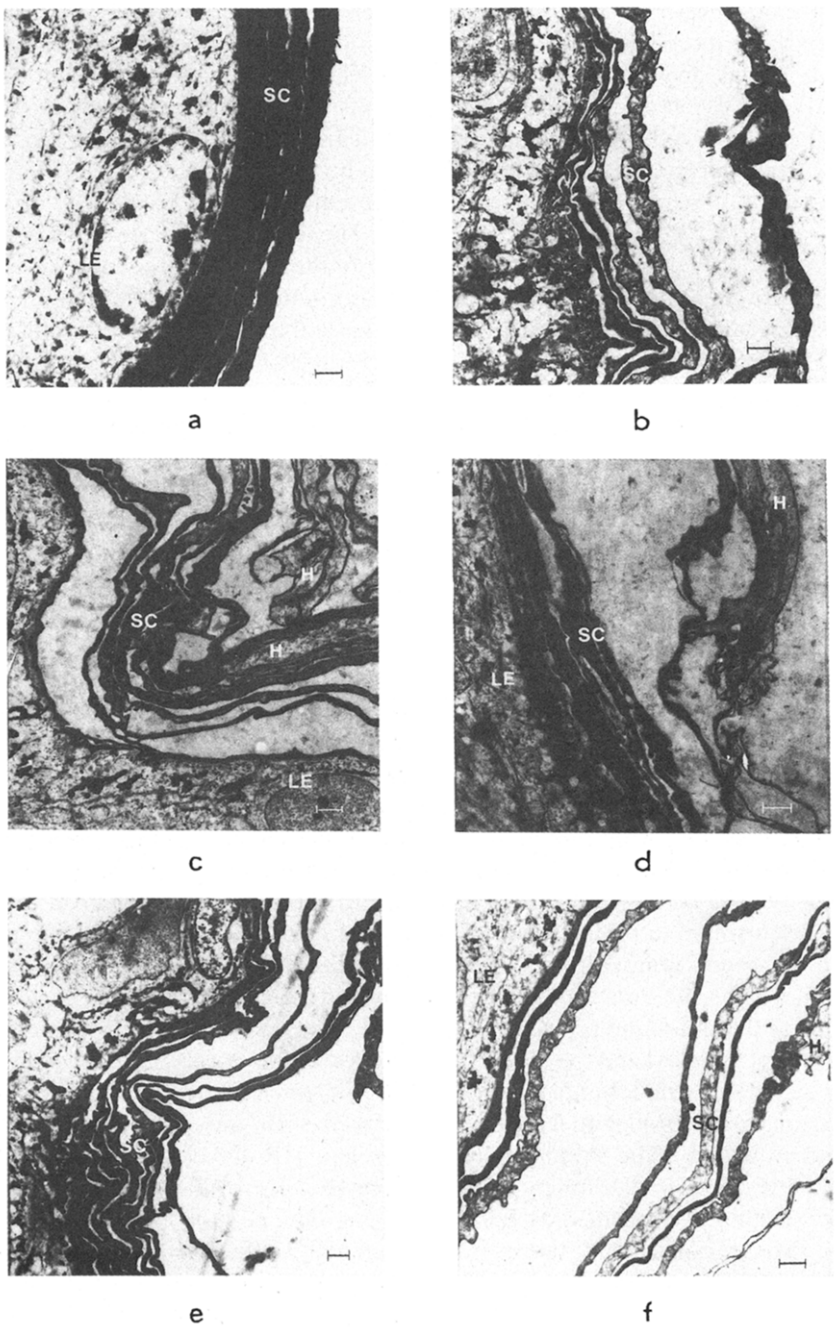


Fig. 1. TEM micrographs of the skin at the commencement of the experiments (× 5200, bar = 1 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (H, hydration; SC, stratum corneum; LE, living epidermis).

of the stratum corneum and even when dissociated from the living epidermis, the stratum corneum could be used. The control samples, at the commencement of the experiment, showed the densely packed layers of the stratum corneum. At 2 h these layers were relatively closely packed and

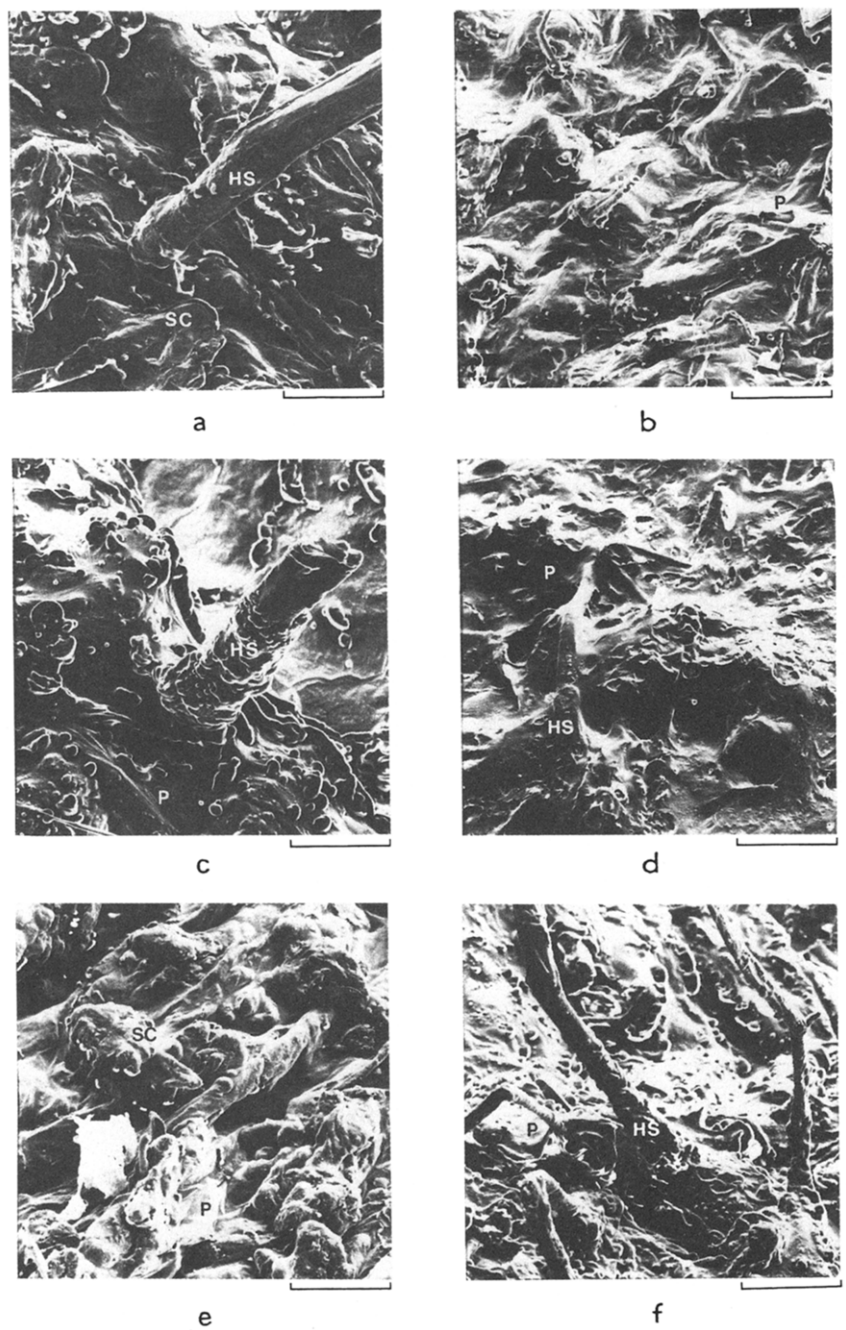


Fig. 2. SEM micrographs of the surface of the skin at the commencement of the experiment (× 220, bar = 100 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (HS, hair shaft; SC, stratum corneum; P, polymer).

showed no difference from the control at 9 h. This supports the statement made by Ackermann et al. (1988), that the stratum corneum layers stay intact during studies with the open cell diffusion system. In contrast to the effect observed for the control solution, the polymers caused disruption of the

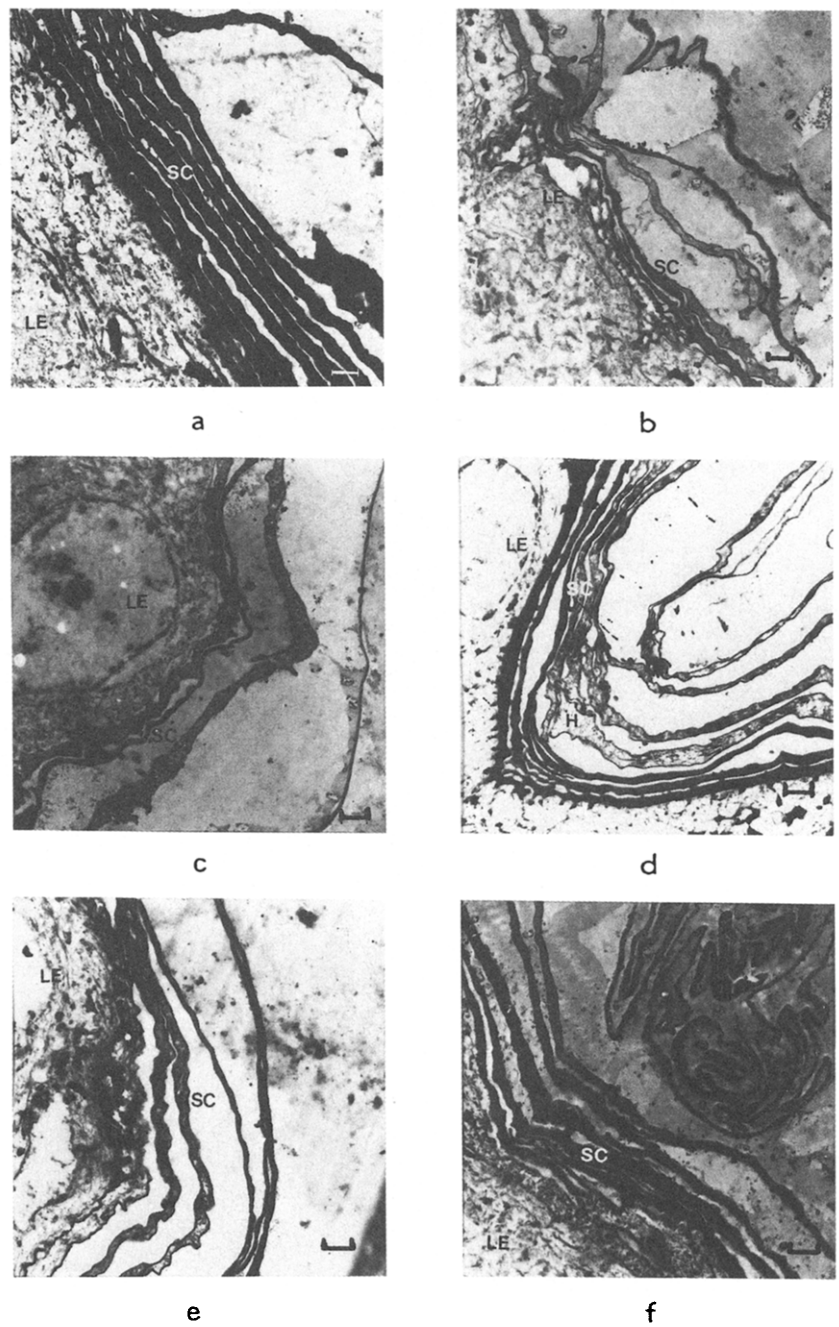


Fig. 3. TEM micrographs of the skin after 2 h (× 5200, bar = 1 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (H, hydration; SC, stratum corneum; LE, living epidermis).

stratum corneum soon after application (Fig. 1). This effect was observed with all the polymers and occurred at the commencement of the experiment (approx. 10 min after application, Fig. 1), up to 9 h (Fig. 5). The disruption of the stratum corneum layers by the JR 125 and GAF 734 polymer sol-

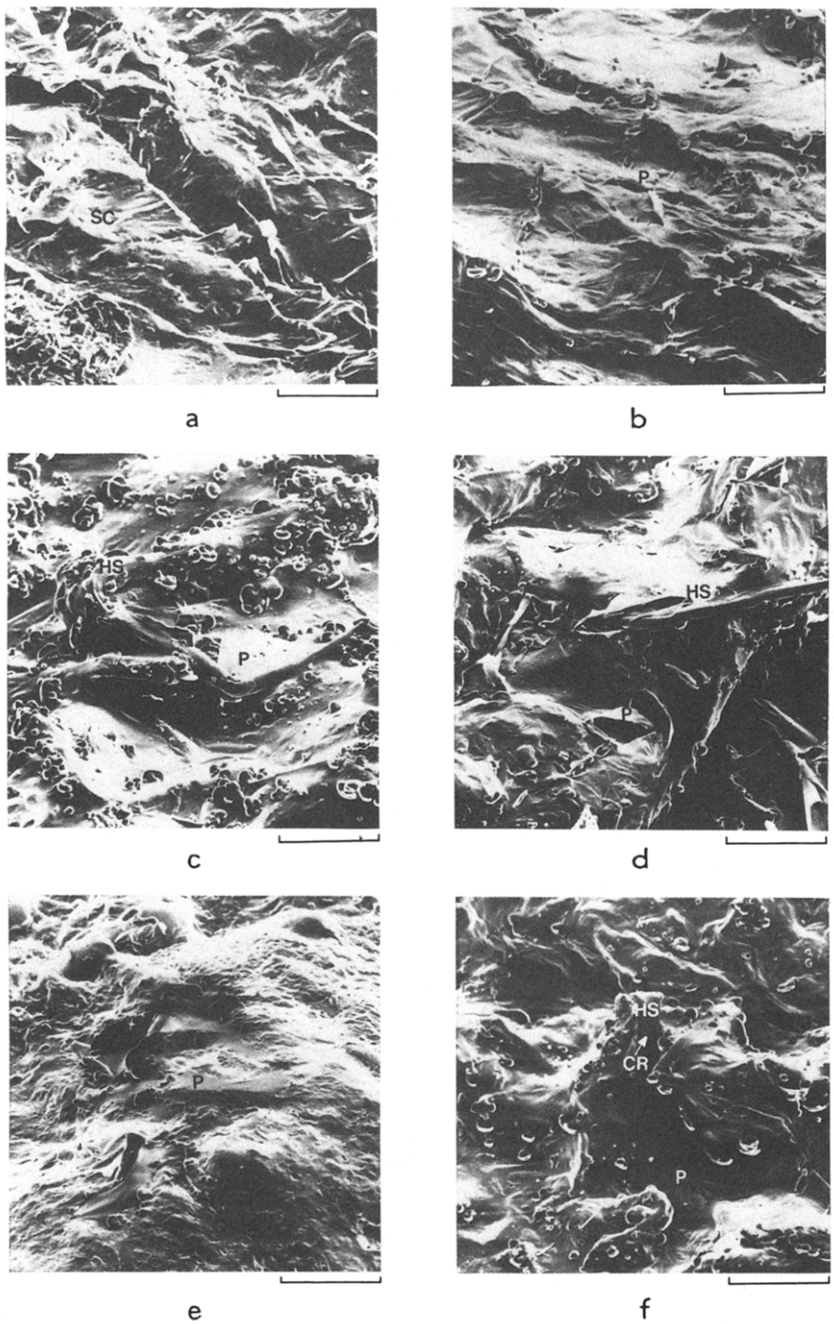


Fig. 4. SEM micrographs of the surface of the skin after 2 h (× 220, bar = 100 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (HS, hair shaft; SC, stratum corneum; CR, cracks; P, polymer).

utions were also present after 18 h. Similarities between the micrographs of these polymer solutions after 18 h and the micrographs taken of the polymers at 9 h were observed. It may therefore be concluded that disruption of the stratum corneum by the polymer JR 400, JR 30M and GAF 755N

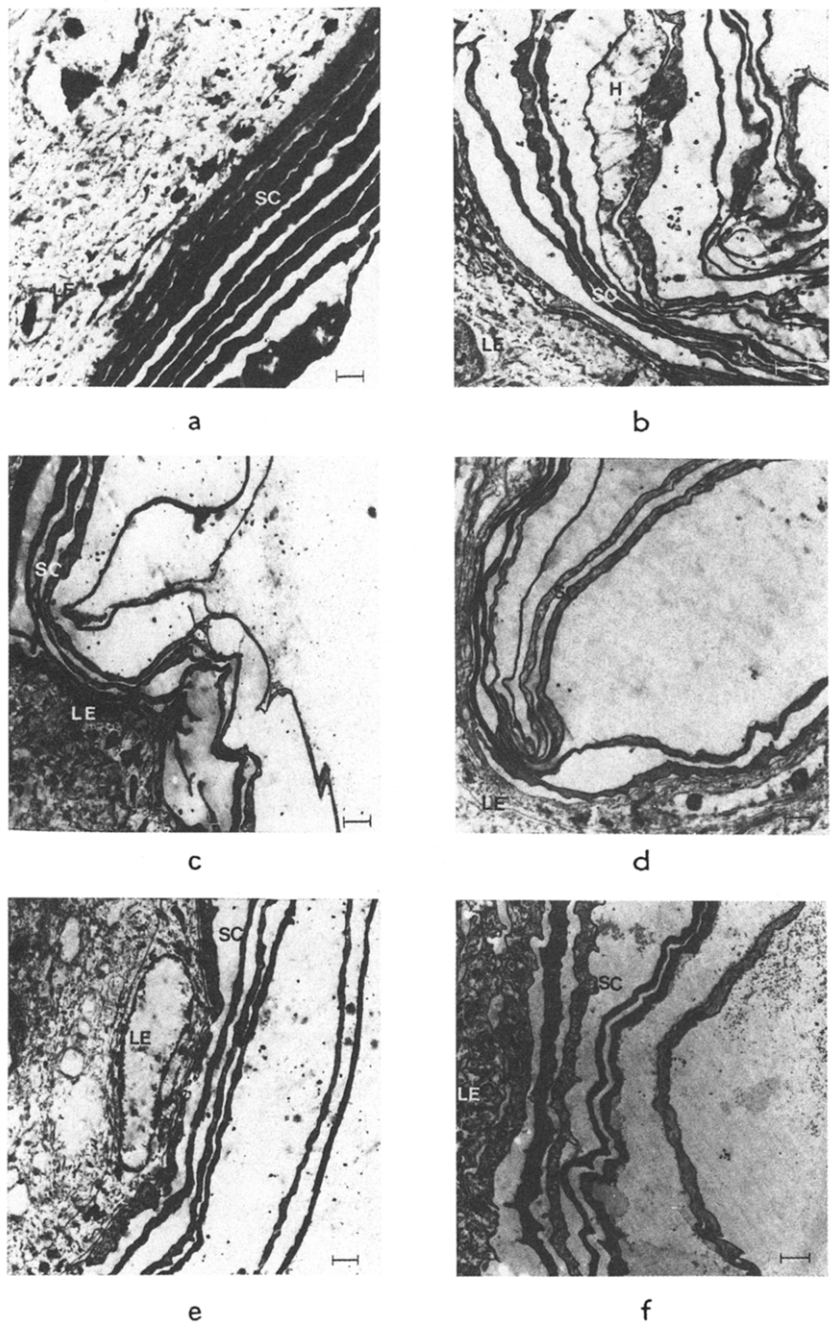


Fig. 5. TEM micrographs of the skin after 9 h (× 5200, bar = 1 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (H, hydration; SC, stratum corneum; LE, living epidermis).

solutions would be probable after 18 h. The micrographs show that the polymers also affect the smoothness of the skin surfaces adversely. The micrographs of the control solution show a smooth appearance of the skin surfaces, while the polymer solutions clearly accentuate the contours of the skin surface.

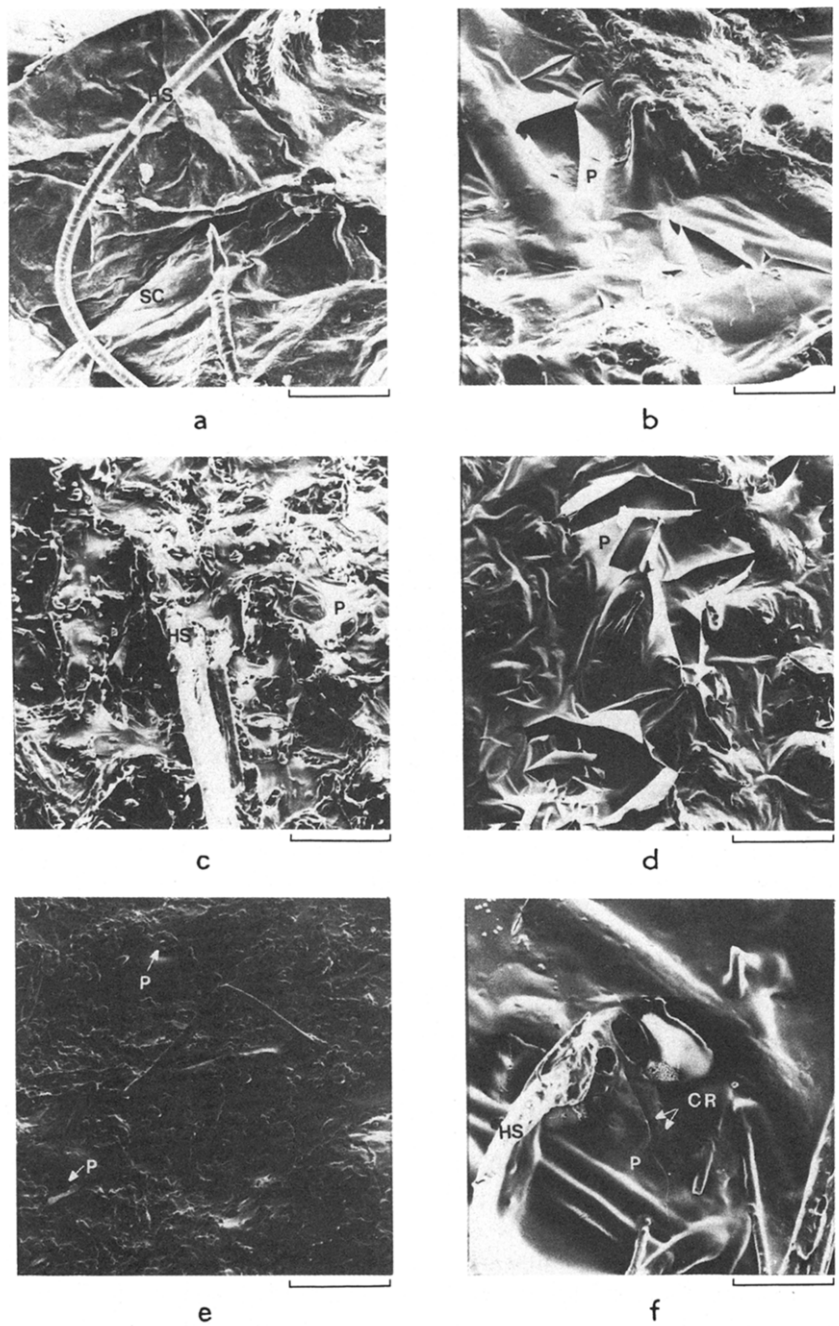


Fig. 6. SEM micrographs of the surface of the skin after 9 h (× 220, bar = 100 μm): (a) normal saline, (b) JR 125, (c) JR 400, (d) JR 30M, (e) GAF 734, (f) GAF 755N (HS, hair shaft; SC, stratum corneum; CR, cracks; P, polymer).

The disruption of the stratum corneum layers and the effect on the smoothness of the skin by the polymer solutions were probably caused by the high affinity between the polymers and the stratum corneum layers. The polymers were shown to have high substantivity to the keratin surfaces of

the stratum corneum due to their cationic nature (Goddard and Leung, 1982). These interaction forces may be stronger than those between the adjacent stratum corneum cell layers. The polymer solutions probably lowered the interfacial tension which have led to the folding of the stratum corneum layers. However, the stratum corneum adhered strongly to the surface of the living epidermis and as the polymer interacted with the stratum corneum the epidermis followed. This may be the reason why the stratum corneum did not appear as smooth with the polymer solutions as without it in the control experiments.

A hydration effect of the polymer solutions on the stratum corneum layers was observed (e.g. Figs. 1f and 3b). This phenomenon was not evident in all the micrographs presented. It must be remembered however, that each of the micrographs represented a small fraction of the total stratum corneum layer exposed. It was, therefore, impossible to present the hydration effect in all the micrographs. With the evidence from these micrographs and the studies conducted by Goddard and Leung (1982), showing a hydration effect of cationic polymers on the skin, it can be concluded that the polymers could hydrate the stratum corneum to a certain extent.

The SEM micrographs provided little information that could support the effects seen with the TEM micrographs. The polymer films formed on the stratum corneum can be clearly seen in the micrographs of the skin segments with the different polymer solutions. Some of the films were thicker (JR 30M) than others (GAF 734). Although all the polymer films showed the same strong adhesion to the skin surfaces not all of them started scaling at the same time. From the SEM micrographs at the commencement of the experiments (Fig. 2b–f) it is clear that the scaling is absent. The phenomenon is not an experimental artifact. The polymer solutions filled all the contours of the skin and smoother surfaces resulted. The difference between the skin with and without the polymer solutions after 9 h (Fig. 6) can be clearly seen. Scaling of polymers JR 125 and JR 30M started after 2 h (Fig. 4b,d). Cracks in the polymer film of GAF 755N were observed after the same time interval. At 9 h (Fig. 6) the scaling was more prominent. At 18 h the scaling of JR 125, JR 30M and GAF 755N was extensive while the JR 400 and GAF 734 polymer films started to crack. The formation of multilayers by the polymers that scaled is evident. The scaling of the polymers might be due to the dehydration of the polymer solutions. It was found that the polymers that scaled first (JR 125, JR 30M and GAF 755N) presented lower percutaneous absorption of water and urea than that of JR 400 and GAF 734.

In another publication by Siebert et al. (1990) it was shown that water had a much higher permeation in combination with JR 400 than in the control sample or in combination with any of the other polymers. In Figs. 4c and 6c small droplets are observed on the surface of the JR 400 polymer. It is assumed that this polymer displays a syneresis effect, which may result in enhanced permeability. A possible explanation for this effect is that the polymer chains, that are normally folded or in a helix when the polymer is in the solid state, become more extended when in solution. When the polymer dries, the chains return to the smaller folded or helix state, resulting in pressing the water out of the polymer matrix, both into the skin and to the upper surface.

It can be concluded that the polymers influenced the barrier properties of the stratum corneum and, therefore, affected the percutaneous absorption of water, urea and glycerol. No difference was observed between the effect of the control and the polymer solutions on the physical structure of the skin. The epidermis and dermis were still intact after 18 h but had shown microbial attack. Valid conclusions could be made for the TEM study up to 9 h. Thereafter, difficulty was encountered with the preparation of the skin samples. The SEM micrographs presented little information that would support the results obtained from TEM. However, the extent of scaling of the polymers has shown some correlation to the percutaneous absorption of water, urea and glycerol from these polymer solutions (Siebert et al., 1990). The polymers that started to crack and scale after 2 h (JR 125, JR 30M and GAF 755N) showed a lower overall percutaneous absorption than the polymers (JR 400 and GAF 734) that started to crack and scale after 9 and 18 h, respec-

tively. The higher permeability in the presence of JR 400 can also be explained by a syneresis effect observed on the SEM micrographs.

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