by reduction of NaB^3H_4 as described by Monder[7]. Specific radioactivity of (21-^{3}H)-prednisolone was 36 μCi/mole. The enzyme was prepared from the cytosol fraction of hamster liver by centrifugation, ammonium sulfate fractionation, sephadex G-100 gel filtration and CM-sephadex C-50 column chromatography as described by Lee et al.[6].

Reaction mixtures were prepared to contain 0.03 μmole, total radioactivity of 1 μCi, of (21-^{3}H)-prednisolone and 1 mg of enzyme in 0.025 M tris-HCl buffer, pH 8.0, in a total 2 ml of reaction volume. Reactions were carried out at 37 °C for 6 h and stopped by freezing the mixture at −70 °C. The condensate was obtained by lyophilization of frozen incubation mixture. The remaining residue was dissolved in 2 ml of 1 N HCl solution and extracted with 5 ml of ethyl acetate twice. The distribution of radioactivity in various fractions obtained from incubation mixture is shown in table 1. The radioactivity of the condensate and ethyl acetate extract, containing both substrate and metabolite, were 9.8 and 67.8% of substrate added, respectively. The recovery of overall radioactivity averaged 79.2%.

Table 1. Distribution of radioactivity in fractionated incubation mixture

Fractions*	Radioactivity DPH × 10^{-3}	%
Condensate	240.2	9.8
Ethyl acetate extract 1	1655.7	67.8
Ethyl acetate extract 2	7.3	0.3
Aqueous phase	31.7	1.3
Total	1934.9	79.2

*The condensate was obtained by lyophilization of the reaction mixture containing radioactivity of 1 μCi and the resulting residue dissolved in 2 ml of 1 N HCl was extracted with ethyl acetate twice.

Table 2. Substrate specificity of the enzyme

Substrates	Detritiation* I DPM	%	II DPM	%
Prednisolone	1195	2.04	4327	3.60
6 -fluoroprednisolone	668	1.62	2032	2.46
6 -methylprednisolone	1493	2.24	3386	2.46
Cortisol	1403	1.04	2682	1.18
11-deoxycorticosterone	8528	17.77	17575	18.31

*Incubation mixture was prepared to contain 0.74 mg of protein and 0.5×10^{-3} μmole (I) and 1.0×10^{-3} μmole (II) of (21-^{3}H)-corticosteroids with varying specific activities.

The initial detritiation velocity was proportional to the amount of enzyme added. The rate fell off during prolonged incubation. The enzyme solution did not show any detritiation activity after being boiled for 3 min.

The accumulated ethyl acetate extracts were dried under reduced pressure and the resulting residue dissolved in methanol was applied onto silica gel plates made of silica gel G.F. 254. After the plates were developed with chloroform:methanol (98:2), 2 bands were observed under UV-light. One, at R_f 0.4, corresponded to the substrate itself; the other remained at the origin. 2 radioactive peaks coinciding with the UV opaque components were detected on the plate with a radiochromatogram scanner. The TLC behavior of the metabolite in a non-polar solvent system suggested that it was more polar than the substrate. The portion containing non-mobile metabolite was scraped off the plate and eluted with methanol, and further purified with TLC, using a polar acidic solvent system (upper phase of toluence:acetic acid:water = 50:50:10). The main UV absorbing band with R_f value of 0.35 on the plates was extracted with methanol. The isolated acidic metabolite was esterified with diazomethane and subjected to crystallization with acetone-ether system.

High and low resolution mass spectra of the acidic metabolite methyl ester obtained using direct inlet system on the AEI MS 30 were identical to those of authentic methyl 11 β, 17 α, 20 ζ-trihydroxy-3-oxo-1, 4-pregnadien-21-oate ester. The fragmentation pattern derived from these spectra revealed a relatively intense molecular ion at M^+ 392.0194, $C_{22}H_{30}O_6$, and were characteristic of a steroid nucleus of Δ^4-3-ketone with a 17 glycolate methyl ester[8].

The relative detritiation rates of a number of (21-^{3}H) corticosteroids achieved in 2 h incubation are listed in table 2. The initial detritiation rate of glucocorticoids tested was significantly lower than 11-deoxycorticosterone, indicating that oxidation of tritium at C-21 of corticosteroids was affected by the nature of the ring substituents.

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The effect of antifibrinolytic agents on wound healing in vitro

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Summary. The effect of antifibrinolytic agents (antiplasminogen activators) on wound healing was studied in vitro. All these substances caused the proliferating epithelium to change direction and migrate to stratum corneum, resulting in a everted epiboly formation.

The mechanism by which epithelial cells are directed, migrate and establish contact with the connective tissue in wound healing is incompletely known. Fibrin has been proposed to have a guiding role in this process[1] and suggested as an adhesive agent to be used in reconstructive surgery[2]. The interaction between proliferating epithelial cells and fibrin is unknown, but a fibrinolytic activity by migrating epithelial cells has been proposed[3] and epithelial cells from the buccal mucosa of man have been shown to release plasminogen activator, especially during earlier stages of cell maturation[4].

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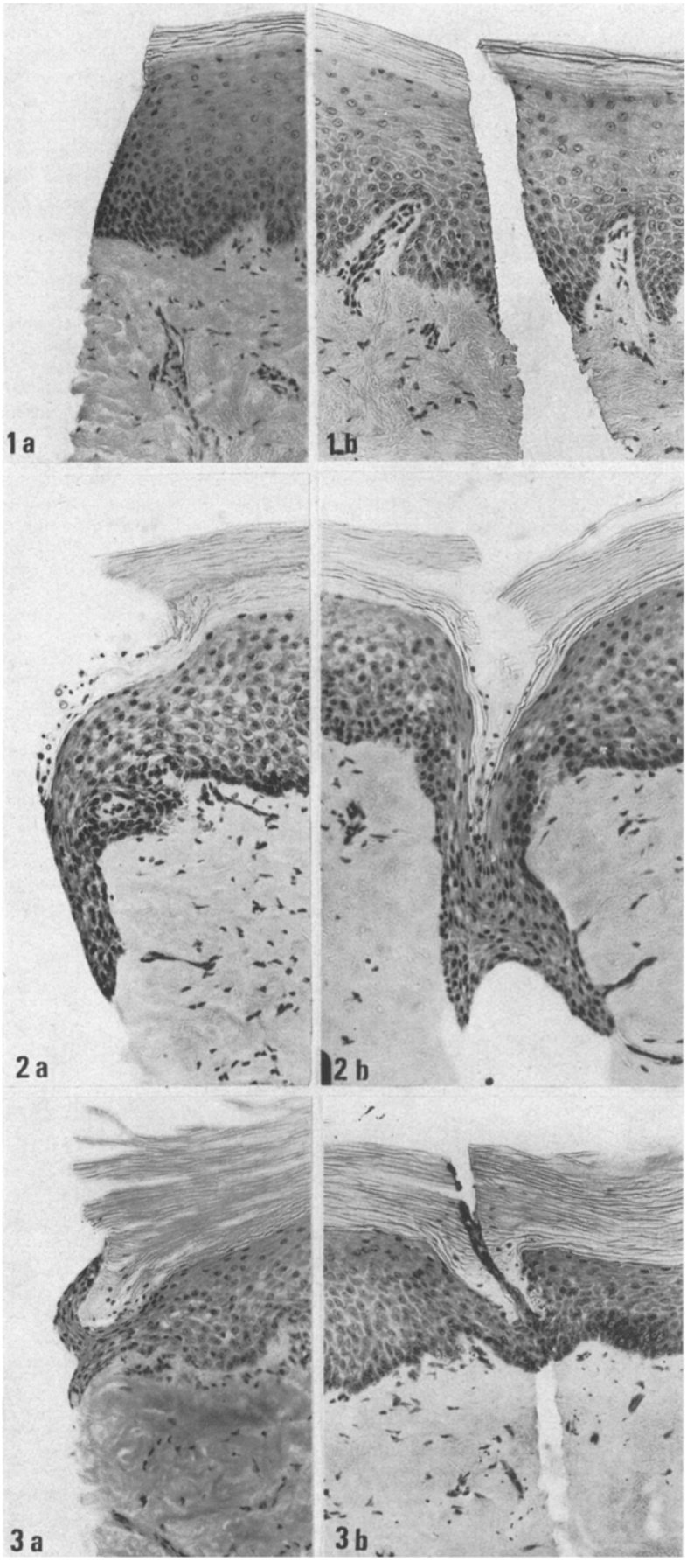

Fig. 1. Appearance of cut surface (a) and incisional wound (b) at the time of explantation. × 170.
Fig. 2. Appearance of cut surface (a) and incisional wound (b) after 48 h in MEM medium. × 170.
Fig. 3. Appearance of cut surface (a) and incisional wound (b) after 48 h in MEM medium supplemented with 0.075 M δ-amino valeric acid (a) and 0.0075 M tranexamic acid respectively. × 170.

Effect of antifibrinolytic and control substances on epithelial proliferation

Tested substances	Concentration (M)	Everted ep. proliferation
Anti-plasminogen activators		
Epsilon-amino caproic acid	0.75	pos.
	0.075	pos.
	0.0075	pos.
	0.00075	neg.
Tranexamic acid	0.075	pos.
	0.0075	pos.
	0.00075	pos.
cis-4-(Aminomethyl)cyclo-	0.075	pos.
hexan carboxylic acid	0.0075	pos.
Paraaminobenzamidine HCl	0.048	necrosis
	0.0096	necrosis
δ-Aminovaleric acid	0.75	pos.
	0.75	pos.
	0.0075	pos.
	0.00075	pos.
Nitrophenyl-p-guanidinoben-	0.075	pos.
zoate HCl	0.0075	pos.
Control substances		
L-Leucine	0.075	neg.
D-Leucine	0.075	neg.
L-Norleucine	0.075	neg.
D-Norleucine	0.075	neg.
Glycine	0.075	neg.

In view of these considerations, the implication of fibrinolytic mechanisms in epithelial cell proliferation was considered. Consequently, the effect of antifibrinolytic agents on the behaviour of epithelium in wound healing was studied. Standardized pieces from palatal mucosa of adult exsanguinated cats were maintained up to 6 days in an organ culture system in the presence of 50% O_2, 45% N_2 and 5% CO_2 and Eagle's MEM supplemented with L-glutamine and antibiotics. In addition an incisional wound was created in each explant by cutting at right angles to the explant through the epithelium down into the submucosa (figure 1a, b). Antifibrinolytic agents and control substances respectively were added to the culture medium before explantation (table). The explants were harvested, fixed and stained after 0, 12, 24, 36, 48, 72, 96, 120 and 144 h and studied in light microscope.

In the absence of antifibrinolytic agents, the following observations were made. After 12 h the epithelium had migrated from stratum germinativum along the connective tissue on the surfaces of the explant, a so-called epiboly formation. The epiboly showed the greatest proliferative activity during the first 48–72 h (figure 2a). In the incisional wound, an epithelial downgrowth was observed in all explants (figure 2b). The epithelial buds fused and formed an epithelial bridge in most of the explants after 12 h.

In the presence of antifibrinolytic agents, the epiboly did not proliferate on the connective tissue but migrated in the opposite direction. This everted epiboly proliferated attached to the stratum granulosum, reached the stratum corneum and even spread on the upper surface. Not until 48 h after explantation did a second epiboly appear migrating over the connective tissue surface. Between the everted and the downgrowing epiboly, a sharp grove was formed (figure 3a).

In the incisional wound, the antifibrinolytic agents did not inhibit the epithelial fusion or interfere with the bridge formation. On the upper surface of the stratum corneum, buds of proliferating epithelium had appeared, while the epithelial downgrowth was minute if any (figure 3b). This changed pattern of epithelial proliferation was a regular result after addition of every substance which has been shown to have antifibrinolytic (antiplasminogen activator) activity (table). Such an epithelial climbing has not been reported earlier in wound healing[5], and it has been maintained that epithelial cells do not spread on sheets of other epithelia[6,7].

The antifibrinolytic agents used in the present study obviously did not interfere with epithelial cell proliferation or migratory capacity as indicated by the undisturbed kinetics of cell fusion and bridge formation in the incisional wound. The cause of the changed direction of epithelial migration is obscure but might result from the effect of antifibrinolytic agents on the properties of the cut surfaces. It is reasonable to assume the presence of a thin fibrin layer covering all surfaces of the explants, but during the maintenance in vitro this fibrin may be eliminated by the activator released from the explants, as activator of plasminogen has been shown to be produced by mucosal epithelial cells[8,9].

In the presence of antifibrinolytic agents, however, the elimination of fibrin was probably blocked and the persisting fibrin might make possible the migration of proliferating epithelial cells on stratum granulosum and stratum corneum. However, interference with other enzyme mechanisms cannot be excluded. Epsilon-amino caproic acid has been shown to inhibit carboxy peptidase B-activity[10]. It has also been reported that this substance may interfere with surface antigens[11]. However, Spooner et al.[12] could not confirm the effect of epsilon-amino caproic acid on red cell agglutination by anti D-serum. Such a concept is also hard to reconcile with the fact that the epithelium primarily did migrate upwards and later downwards. The clinical implication of these observations may be of some interest as the use of antifibrinolytic substances might offer means to direct epithelial growth.

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