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ACANTHOLYSIS INDUCED BY PROTEOLYTIC ENZYMES
I. PORCINE PANCREATIC ELASTASE

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Abstract. In an attempt to induce acantholysis in cultivated normal human epidermis, porcine pancreatic elastase was used. After 30 minutes, the intercellular contact layers of desmosomes appeared discontinuous. Following the disappearance of the intercellular filamentous structures, desmosomes ruptured into two, and in 3 hours, typical acantholysis was observed. After 15 minutes, prior to the desmosomal changes, dermo-epidermal separation started at the subepidermal space. After 2 hours of elastase influence, anchoring filaments and the basal lamina were degraded. Five hours from the start of culture, the anchoring fibrils remained undigested by elastase. The mechanism of elastase influence on epidermis is assumed to depend rather on its proteolytic activity than on its elastolytic activity.

Key words: Acantholysis; Porcine pancreatic elastase; Ultrastructure; Skin culture; Desmosomes; Dermo-epidermal separation

In a previous paper (4), the authors studied desmosome formation and concluded that it starts when the epidermal keratinocytes come in contact with adjacent cells, and intercellular contact layers appear as the last of all stages in desmosome formation. This paper, by contrast, describes the destruction of desmosomes, viz. acantholysis. Elastase or pancreatopeptidase E produced from porcine pancreas was used. This breaks down elastin as well as other proteins (2, 10, 12). A few authors have reported that elastase separates the dermo-epidermal junction and produces acantholysis in the epidermis (1, 7–9, 11). Using the electron microscope, we studied acantholysis and dermo-epidermal separation in normal human skin in relation to duration of culture.

MATERIAL AND METHODS

Normal human skin of a middle-aged woman was obtained at mammoplastic surgery. The surface was cleaned with 70% alcohol. For cultivation, small pieces of split thickness skin were prepared with a 2 mm punch. They were placed on stainless steel organ culture grids (Falcon) in plastic organ culture dishes with absorbent rings (Falcon).

Porcine pancreatic elastase, a lyophilized, chromatographically prepared product of Worthington, New Jersey, USA, containing 8.7 units/mg, 99% protein (6363 ESFF 50A453) was dissolved in Hanks' balanced salt solution at a concentration of 0.4 mg/ml. When the cultivation was started, it was added to the culture medium. The final concentration of elastase in the culture medium was 0.08 mg/ml, 0.696 units/ml.

Culture medium was prepared from Eagle's minimum essential medium with Earle's salts 100 ml, heat-inactivated fetal calf serum 10 ml, L-glutamine (200 mM) 2 ml penicillin–streptomycin (Gibco, Scotland) 10000 I.U. each and gentamycin 5 mg. The pH was adjusted to 7.4 with Hepes buffer (1 mol, pH 7.3, Gibco).

The skin material was incubated with culture medium containing elastase in an incubator (Ehret®) at 37°C with 5% CO_2 in air.

For electron microscopy, the material was fixed every 15 min, 21 times in all, for up to 5 hours from start with 3.0% glutaraldehyde in a 0.15 M cacodylate buffer at pH 7.4, containing 7.5% sucrose for 3 hours. After postfixation with 0.5% of osmium tetroxide in the same buffer for 1 hour, the samples were dehydrated in a series of alcohol of increasing concentration, and embedded in Epon 812. Ultrathin sections were cut with a Reichert Ultracut ultramicrotome, stained with uranyl acetate and lead citrate, and studied with an electron microscope type JEOL 100CX.

The control specimens were prepared by a similar method without elastase in the culture medium.

RESULTS

In order to find the right concentration of elastase for the experiment, a few preliminary trials were performed. When 0.5 mg/ml of elastase was used, separation between the epidermis and the dermis started after 10 min. On the other hand, 0.01 mg/ml could not produce acantholysis, even after 36 hours.

Control specimens, i.e. skin cultured in a medium not containing elastase, showed a completely normal appearance from the beginning to the end of the

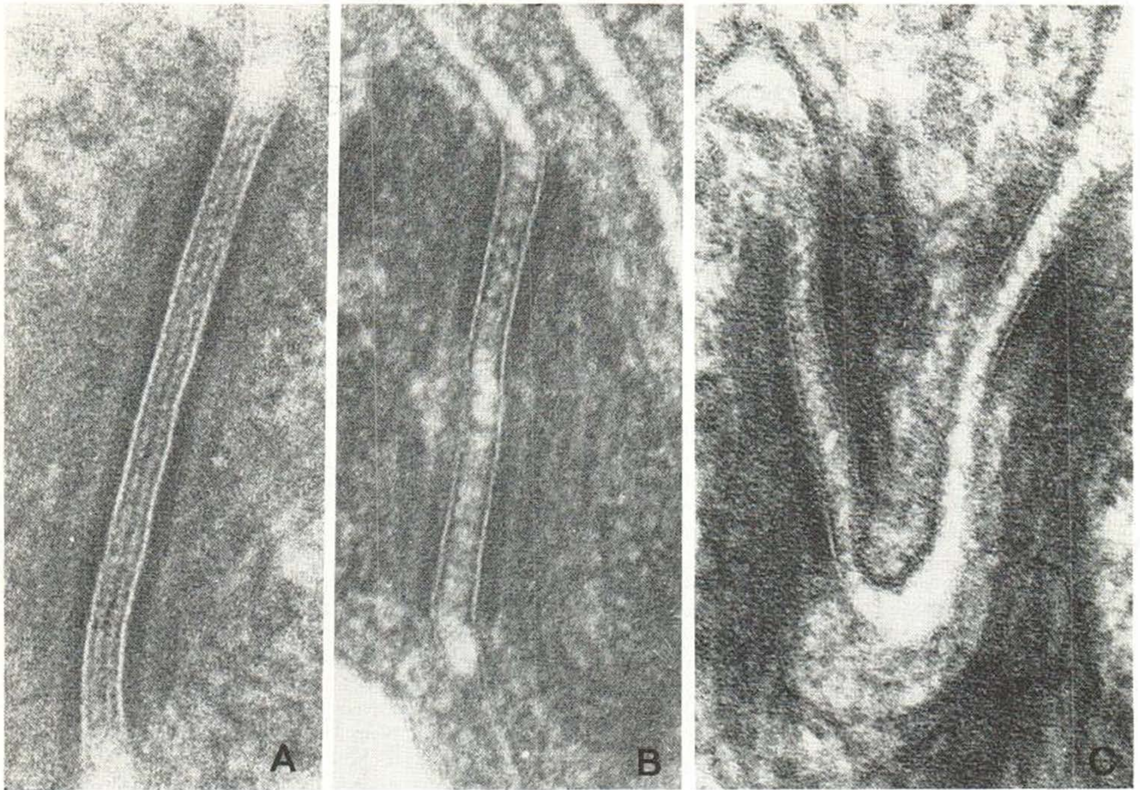


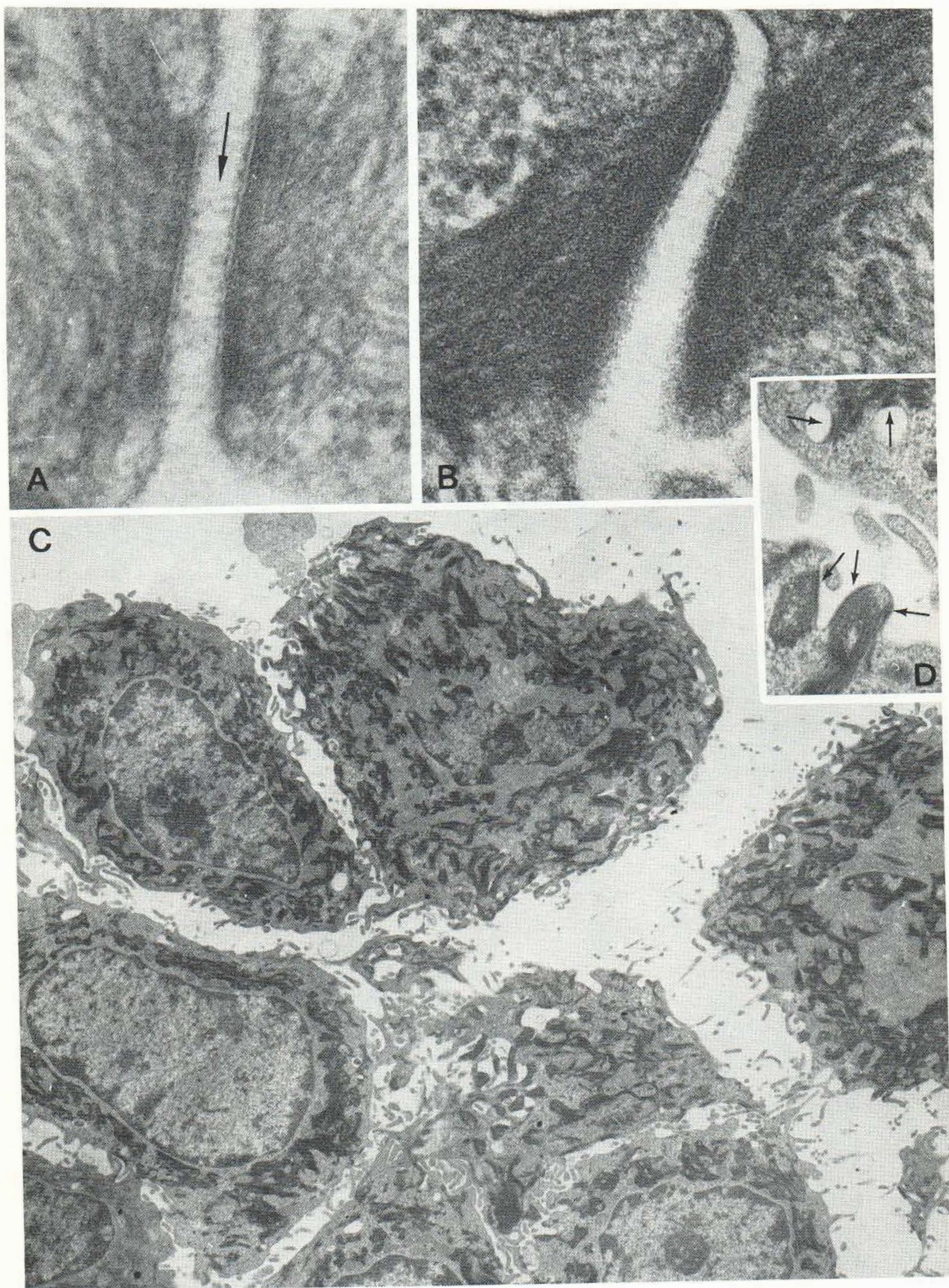
Fig. 1. (A) Normal desmosome of control specimen after 5 hours. ×150 000. (B) After 30 min from start of culture with elastase, the intercellular contact layer displays a 'moth-eaten' appearance. ×150 000. (C) After 1.5 hours, the intercellular contact layer has disappeared and the intercellular substance, viz. fine filamentous structures, has diminished. ×150 000.

procedure, i.e. after 5 hours. No changes occurred in desmosomes (Fig. 1 A) or in the dermo-epidermal junction.

Thirty minutes from the start of cultivation in a medium containing elastase, the intercellular contact layers of the desmosomes displayed a 'moth-eaten' appearance, the midlines being partly discontinuous (Fig. 1 B). The filamentous structures between both outer leaflets of cell membranes also started to be digested. After *1.5 hours*, the intercellular contact layers and the filamentous structures diminished (Fig. 1 C), and most of these filaments were actually digested by elastase after *2 hours* (Fig. 2 A). After *3 hours*, the keratinocytes had separated from their neighbouring cells and showed typical acantholysis (Fig. 2 C). The main parts of the desmosomes were separated into two (Fig. 2 B). These ruptured desmosomes with distinct attachment plaques and tonofilament masses were seen on the surfaces of the acantholytic cells. Some of them were internalized into the cytoplasm (Fig. 2 D).

Separation of the epidermis from the dermis started after *15 min*. The subepidermal space widened somewhat (Fig. 3 A), and some of the anchoring filaments became elongated. After *30 min*, though the main part of the dermo-epidermal junction still was intact, some hemidesmosomes were detached at the subepidermal space between the junction plate and the basal lamina (Fig. 3 B). The anchoring filaments were torn off, showing a

Fig. 2. (A) Culture with elastase after 2 hours. Filamentous structure in intercellular space (arrow) vanishing. ×150 000. (B) After 3 hours, most desmosomes are separated into two. Attachment plaques and tonofilaments are still distinct. ×150 000. (C) Lower magnification of epidermis 3 hours after culture start. Typical acantholysis. ×6 000. (D) Numerous ruptured desmosomes at peripheral surface of acantholytic cells. Several are internalized into cytoplasm (arrows). ×30 000.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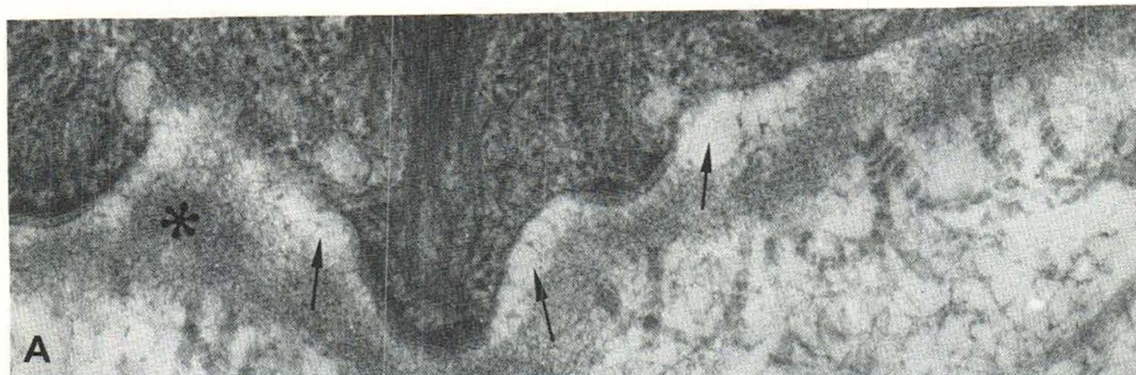
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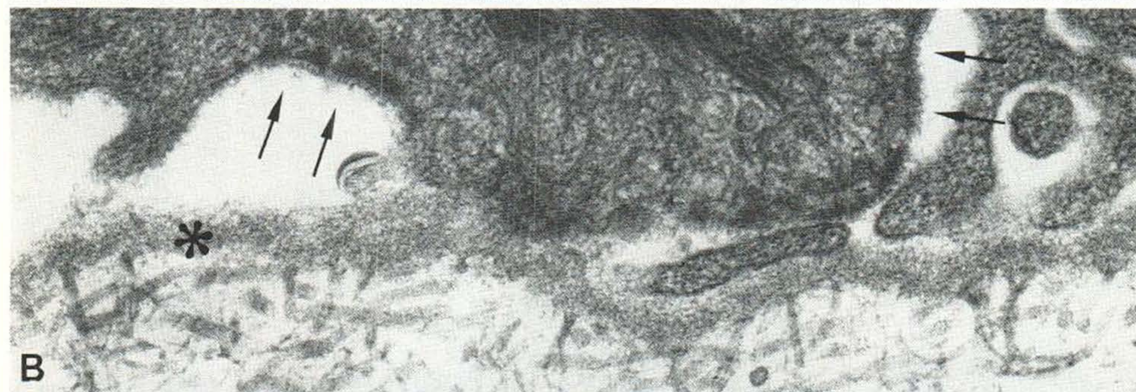
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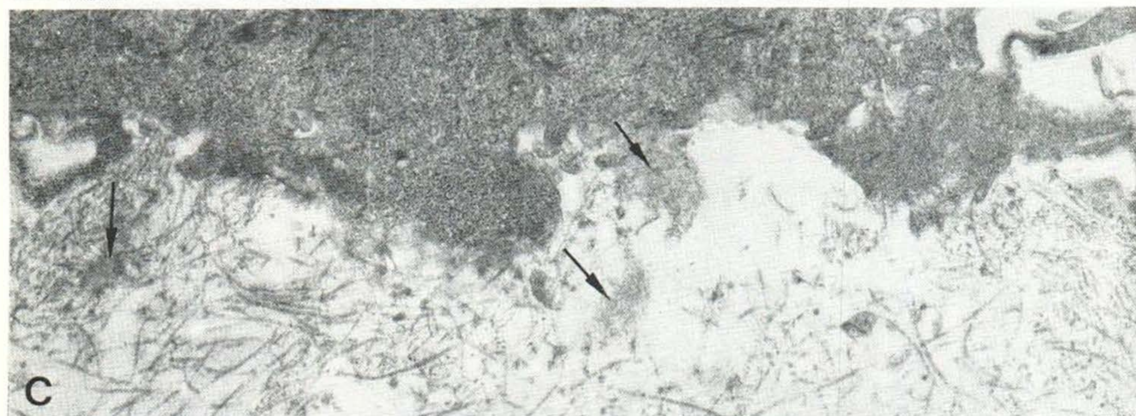
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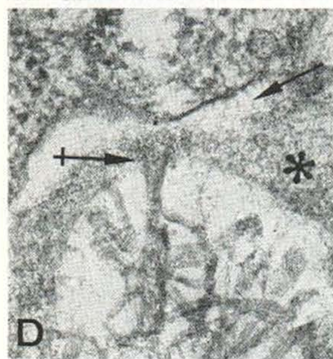
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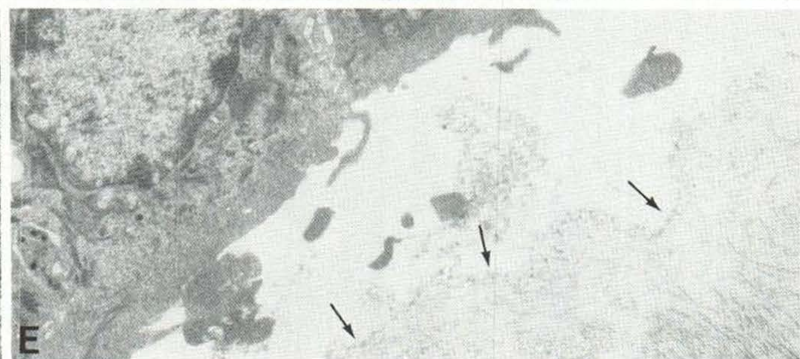
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brush-like appearance. After *1 hour*, the basal lamina was fragmented and disappeared, with the exception of a few remnants (Fig. 3C). Hemidesmosomes were no longer found at the basal cell surface. In some places where basal lamina was still observed, the subepidermal space was widened. The basal lamina and the anchoring fibrils, however, remained tightly connected (Fig. 3D). After *2 hours*, the epidermis was completely separated from the dermis. Very faint basal lamina pieces were left, while anchoring fibrils remained undigested by elastase (Fig. 3E). Elastase had digested the anchoring filaments and degraded the basal lamina. Even after *5 hours* the anchoring fibrils remained unaffected.

Elastase started to attack the dermo-epidermal junction earlier than the desmosomes. When the epidermis was separated from the dermis, separate keratinocytes still formed an epidermal sheet.

DISCUSSION

It has been known for some time that elastase digests elastin of the matrix of elastic fibres, anchoring filaments and basal lamina, whereas anchoring fibrils and elastic fibrils resist this action (3, 8, 9, 12). This was confirmed in the present study. Similar phenomena may be induced by papain, and are seen in the skin disease epidermolysis bullosa hereditaria lethalis in which the basal lamina remains attached to the dermis (5). In contrast, collagenase induces dermo-epidermal separation similar to the condition in epidermolysis bullosa dystrophica in which the basal lamina is attached to the epidermis (6). Elastase induces dermo-epidermal separation and acantholysis in the epidermis, while collagenase separates the epidermis from the dermis and leaves it as a sheet without inducing epidermal acantholysis. Collagenase can digest anchoring filaments, but not intercellular filamentous structures of desmosomes in the epidermis (1, 3, 6, 8).

During desmosome formation, the intercellular contact layers appear at the end of the process (4). In the present study, detachment of desmosomes by elastase started from the intercellular contact layers. Thereafter, the filamentous structures of the desmosomes vanished. Similar morphological changes may be caused by papain (5) and trypsin (13). Papain is believed to degrade the protein–polysaccharide–connective tissue matrix, leading to symmetrical splitting of desmosomes without prior retraction of tonofilaments (5). The intercellular substance of desmosomes may be digested by trypsin, showing that the main extracellular components consist of protein (13). Wolff & Schreiner (14, 15), using enzymatic digestion, believe the intercellular substances contain mucopolysaccharides, because they are not removed by protease. Narayanan & Anwar mentioned that elastase is thought to attack peptide bonds adjacent to neutral amino acids of substrate (10). It has also been reported that elastase has proteolytic as well as elastolytic capacities (3). We assume that acantholysis and dermo-epidermal separation may depend on a proteolytic activity of elastase rather than on an elastolytic activity.

Elastase is reported to be composed of a few fractions even after crystallization twice (10, 11). In the present study, we used highly purified elastase, chromatographically prepared. We found that 0.08 mg/ml, 0.696 units/ml of elastase was sufficient to induce acantholysis in the normal human epidermis after 3 hours, while Klein (7) claimed that 0.12 mg/ml of elastase was optimal for the separation of epidermis from dermis of back skin of mice in 20–25 min.

Fig. 3. (A) 15-min culture with elastase. Separation between epidermis and basal lamina (*) starting. Subepidermal space widened (arrows). Anchoring filaments elongated. ×60000. (B) After 30 min the main part of the dermo-epidermal junction is still in contact. Several hemidesmosomes are detached from the basal lamina (*), between the latter and the junction plate. The anchoring filaments are torn off, giving a brush-like appearance (arrows). ×60000. (C) After 1 hour, the basal lamina is broken into pieces (arrows), and disappearing. ×15000. (D) Where the dermo-epidermal junction is still intact, separation (arrow) between the subepidermal space and the basal lamina (*) is observed. Anchoring fibrils remain tightly attached (arrow with cross) to the basal lamina. ×60000. (E) After 2 hours, the epidermis is completely separated from the dermis. Very faint basal lamina material is left. Most anchoring fibrils remain distinct and undigested (arrows). ×6000.

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