

Protein Content of Comedones from Patients with Acne vulgaris

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Characteristic early lesions in acne vulgaris are the open and closed comedones (blackheads and whiteheads) which are well known to contain a "plug" of cornified material. Histological analysis of these lesions has indicated that their protein content, presumed in part to be keratin, may be degraded, possibly by bacterial action, though this has never been adequately demonstrated biochemically. We have analysed the keratin content in a pool of material taken from a number of open comedones (approximately 200-500 mg by weight). Using a highly sensitive silver stain technique which can detect minute quantities of protein we have also been able to analyse individual lesions. In normal keratin extracted from human stratum corneum using a Tris-urea-mercaptoethanol buffer, SDS/polyacrylamide gel electrophoresis reveals the presence of a group of polypeptides with molecular weights in the range 66 000-44 000. Comedonal material contained bands of the same molecular weight but in addition to these undegraded keratin polypeptides, showed bands corresponding to molecular weights in the region of 15 000-10 000 and 30 000-25 000 indicating that the keratins in this material are partially degraded. Similar groups of low molecular weight polypeptides were observed when keratin was digested with purified V8 protease from *Staphylococcus aureus*. It is possible that inflammation around the follicle could involve the leakage of keratin digestion products into the dermis. (Received February 11, 1985.)

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Acne is known to be a multifactorial condition (1). It predominantly affects the face, back and chest and is characterised by many different lesion types, which are divided into two main groups, the non-inflamed and the inflamed.

Available evidence favours the idea that inflamed lesions arise from non-inflamed lesions (2) which are the clinical manifestations of ductal cornification. Ductal cornification is usually associated with bacterial colonisation. Several factors have been suggested as mediators of inflammation and these include substances released from bacteria (3, 4) and from the materials forming the plug, in particular the degradation products of keratin (5).

Since little is known about the protein content of the non-inflamed lesion, we decided to examine the keratin polypeptides from comedones. We have extracted the protein from (a) a pool of 'open comedones' and (b) individual open comedones and analysed them by using very sensitive techniques. In addition we compared the protein content of open comedones with closed comedones and those of patients undergoing treatment.

It was established that both undegraded and degraded keratin polypeptides obtained from comedones reacted with anti-keratin serum and therefore potentially can act as antigens.

Table I.

<i>Patients not receiving treatment</i>	
Total number of open comedones excised	2 254
Pooled open comedonal material	2 000
Individual analysis	23 inflamed blackheads 107 whiteheads 254 blackheads
<i>Patients receiving treatment</i>	
Topical Benzoyl Peroxide	(Individual analyses 115)
Oral erythromycin (1 g/day 3 months)	

MATERIALS AND METHODS

Comedones were removed using a comedo extractor and stored at -20°C until required for use. Total protein was extracted from comedonal material at 4°C by micro-homogenization in 0.05 M-Tris/HCl buffer, pH 7.2, containing 6 M-urea and 2% mercaptoethanol.

After total protein extraction keratin was purified by dialysis against 0.1 M-citric acid/trisodium citrate buffer, pH 2.65 and isoelectrically precipitated at pH 4 (6). Keratin was analysed by electrophoresis on SDS/polyacrylamide gradient (7.5–17.5%) gels. Prekeratin from human scalp epidermis and keratin from plantar callus were run in parallel tracks for comparison and the gels were calibrated with proteins of known molecular weight. In the case of protein from pooled samples, gels were stained with Coomassie Blue. When protein samples from individual comedones had been electrophoresed only silver stain was sensitive enough to reveal the protein bands (7).

Some comedonal extracts were subjected to immunoblotting (8) following polyacrylamide gel electrophoresis, using rabbit anti-human heel callus keratin serum. The immunoblot was then compared with a Coomassie Blue-stained blot of the same sample.

RESULTS

Comedones were analysed as indicated in Table I. The analysis by SDS/polyacrylamide gel electrophoresis of keratin extracted from several hundred blackheads (total weight 100–400 mg) following partial purification by isoelectric precipitation is shown in Fig. 1. Peptides can be seen which are presumed to be the products of keratin degradation on the basis of comparison with prekeratin extracted from normal scalp epidermis and with callus keratin (Fig. 2). Digestion of authentic keratin with *Staph. aureus* V8 protease (Fig. 2) produces two groups of peptides with molecular weights in the range 30 000–25 000 and 15 000–10 000.

Sensitive protein detection methods which involve the use of silver stain have enabled us to examine the protein content of individual comedones starting with less than 1 mg of material (Fig. 3). It has also enabled the comparison to be made of protein from open comedones with that from closed comedones (Fig. 4). From the samples examined so far we have found that both lesions contain partially degraded keratin.

The immunological properties of comedonal degradation products were investigated by using the technique of immunoblotting with an anti-callus keratin serum. The gel in Fig. 5a shows the electrophoretic analysis of protein from a pool of open comedones. Fig. 5b is a Coomassie Blue stained protein blot which demonstrates that peptides have successfully transferred to the nitrocellulose membrane, and Fig. 5c shows the corresponding immunoblot.

Anti-keratin antibody bound to some but not all of the comedonal peptides. In general there was more antibody bound to higher molecular weight peptides than to those of lower molecular weight.

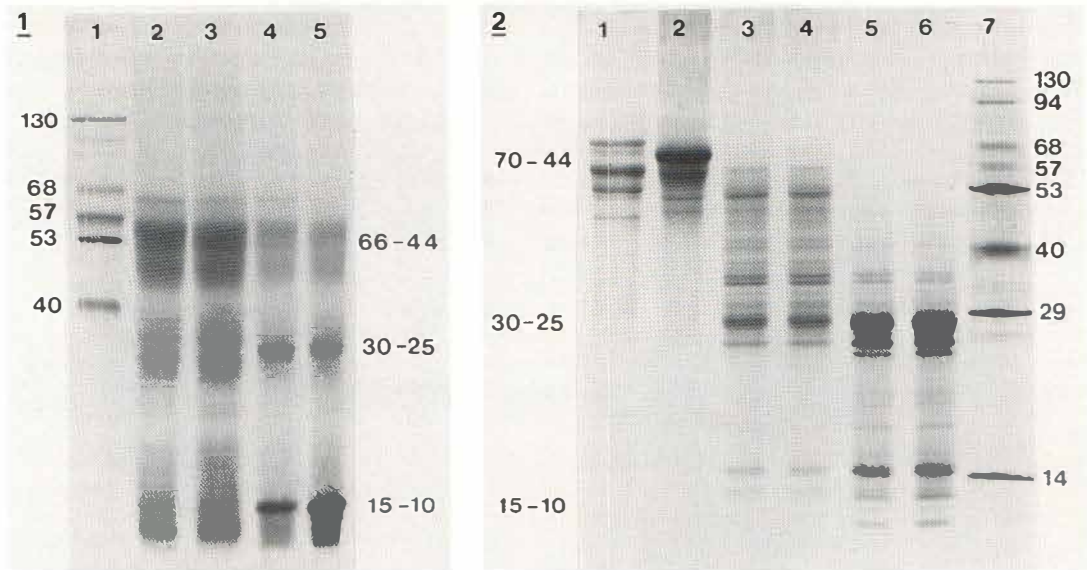


Fig. 1. Electrophoresis of keratin protein from pooled comedonal material. SDS/polyacrylamide gel electrophoresis of protein extracted from pooled open comedonal material is shown in tracks 2-5. Tracks 2 and 3 contain identical material, as do tracks 4 and 5. As can be seen there is some difference in the degree of degradation even in pooled material extracts. Essentially, however, two groups of peptides are formed with molecular weights in the range 30 000-25 000 and 15 000-10 000. Standard proteins of known molecular weight are shown in track 1. Molecular weights of proteins are shown ($\times 10^{-3}$).

Fig. 2. Electrophoresis of purified epidermal prekeratin and stratum corneum keratin after digestion with *Staph. aureus* V8 protease. Normal epidermal scalp prekeratin after SDS/polyacrylamide gel electrophoresis is shown in track 1 with polypeptides in the molecular weight range 70 000-44 000. Keratin from heel callus is shown in track 2 with polypeptides in the molecular weight range 66 000-44 000. Digestion of keratin with V8 protease for 30 min at 25°C produced peptides (tracks 3-6) and peptides of lower molecular weight were more evident in tracks 5 and 6 than in tracks 3 and 4, due to an increased amount of protease. (Protease to keratin ratio (w/w) tracks 3 and 4 1:200; tracks 5 and 6, 1:20.) The peptides produced fell into two groups. Those of 30 000-25 000 molecular weight and those of 15 000-10 000 molecular weight. Standard proteins of known molecular weight are shown in track 7. Molecular weights of proteins are shown ($\times 10^{-3}$).

DISCUSSION

The mechanism of comedo formation in acne is unknown although some authors (9) have attributed it to an increased formation of horny cells in the sebaceous follicle and an increased coherence of these cells. Keratins are but one of several major proteins found in the comedone and the nature of the keratins in such lesions has never been examined rigorously. In the present work utilisation of silver stain procedures has been invaluable in analysing protein obtained from individual comedonal lesions. The silver stain used here was one of several tested on a previous occasion (7).

Comedonal material, not surprisingly, contains keratin polypeptides on the basis of (a) their solubility in 0.1 M citric acid/sodium citrate buffer, (b) their molecular weights (i.e. characteristic bands in the 66 000-44 000 region) and (c) their ability to react with anti-keratin serum. The polypeptides observed were those typical of stratum corneum keratin, but in addition, comedonal material contained degraded keratin polypeptides with molecular weights in the region 30 000-25 000 and 15 000-10 000. Some of these degradation products also reacted with the antiserum.

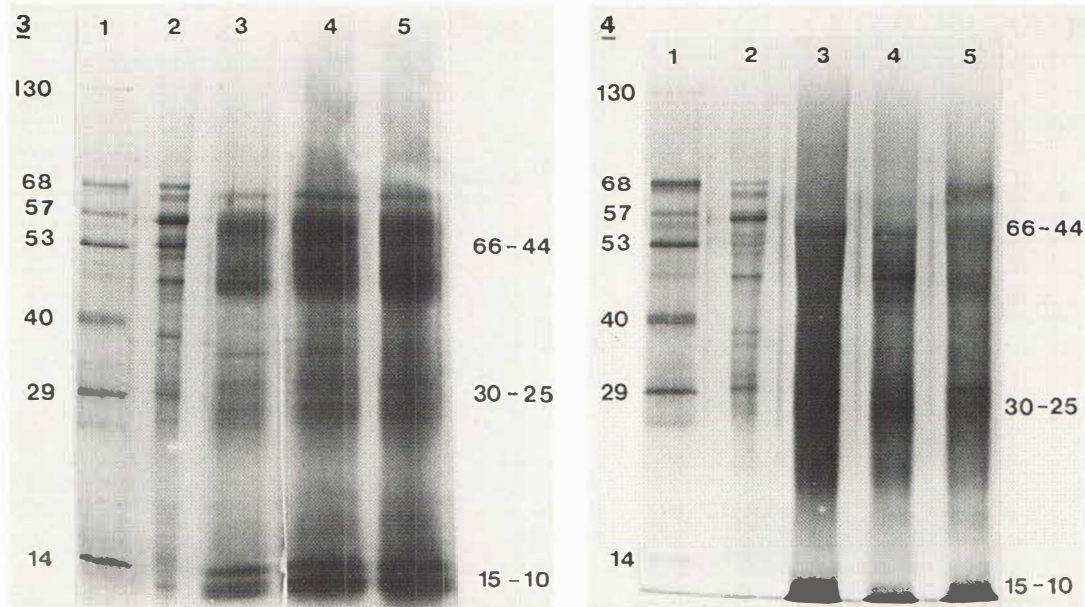


Fig. 3. Analysis of keratin from individual open comedones. Keratin was extracted from individual open comedones and subjected to SDS/polyacrylamide gel electrophoresis. After electrophoresis the gel was stained with silver stain. Keratin material from open comedones can be seen in tracks 3–5. As can be seen two groups of peptides are again apparent as well as polypeptides which have molecular weights identical to those of keratin from stratum corneum. Epidermal prekeratin is shown in track 2 and standard proteins of known molecular weight are shown in track 1. Molecular weights of proteins are shown ($\times 10^{-3}$).

Fig. 4. Analysis of keratin from individual closed comedones. Keratin was extracted from individual closed comedones and subjected to SDS/polyacrylamide gel electrophoresis. After electrophoresis the gel was stained with silver stain. Keratin material from closed comedones can be seen in tracks 3–5. As can be seen there appears to be more degradation than is usually apparent in protein from open comedones. Epidermal prekeratin is shown in track 2 and standard proteins of known molecular weight are shown in track 1. Molecular weights of proteins are shown ($\times 10^{-3}$).

Open comedones, inflamed open comedones, closed comedones and comedones from patients on treatment all contained degraded keratin material. The lack of change in lesions from patients on treatment could be due to the fact that the comedones may have been in existence before treatment was started.

Similar groups of peptides to those found in comedonal material, i.e. in the molecular weight ranges 30 000–25 000 and 15 000–10 000 were observed when purified stratum corneum keratin was digested with purified commercially available *Staph. aureus* V8 protease or with other proteolytic enzymes (subtilisin, chymotrypsin).

The organism *Propionibacterium acnes* (*P. acnes*) is frequently found in the sebaceous follicle (10), and *P. acnes* is also known to produce extracellular proteases at least in culture (11). The fact that *P. acnes* is commonly found on the skin (12) is only circumstantial evidence for its being responsible for the proteolytic cleavage of keratin by production of protease. Experiments designed to demonstrate that *P. acnes* proteolytic enzyme(s) are capable of digesting keratin are now in progress in our laboratory.

Inflammation produced around the follicle in acne lesions could be due to both soluble and insoluble keratin digestion products passing into the dermis. Preliminary immunofluorescent work with keratin antiserum, which, as shown here, interacts with some of the

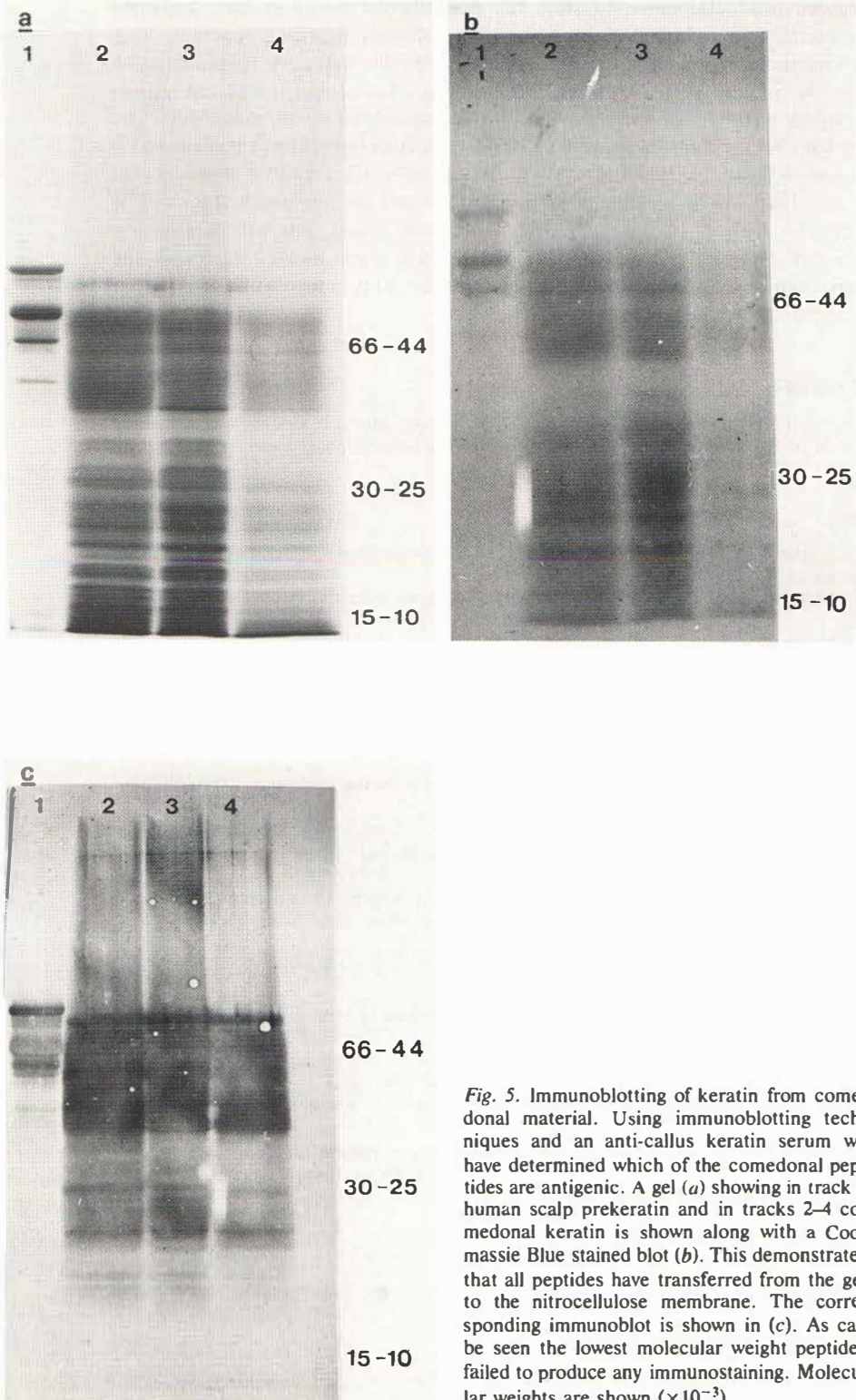


Fig. 5. Immunoblotting of keratin from comedonal material. Using immunoblotting techniques and an anti-callus keratin serum we have determined which of the comedonal peptides are antigenic. A gel (*a*) showing in track 1 human scalp prekeratin and in tracks 2-4 comedonal keratin is shown along with a Coomassie Blue stained blot (*b*). This demonstrates that all peptides have transferred from the gel to the nitrocellulose membrane. The corresponding immunoblot is shown in (*c*). As can be seen the lowest molecular weight peptides failed to produce any immunostaining. Molecular weights are shown ($\times 10^{-3}$).

keratin breakdown products, failed to stain the perifollicular areas in early inflamed lesions. Well established inflamed lesions, however, showed a strong reaction. This indicates that keratin breakdown products are unlikely to be the initiators of inflammation but are involved in its later stages. Although keratin digestion products may not initiate inflammation, many members of the population possess anti-keratin antibodies (13). The most likely explanation for this observation is that keratin is an intracellular protein and is not normally exposed to the immune system. When cells are ruptured however, for example in a cut, there will be leakage of keratin protein and the immune system will be brought into contact with it. It is possible that in an acne lesion cells will be similarly ruptured by bacterial proteolytic activity and keratin will come into contact with the immune system, both as soluble and insoluble polypeptides. This in turn will cause further inflammation at the site of the lesion.

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