Ultrastructural Examination of Fresh Pigskin and after Frozen and Cooked

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SUMMARY: Native pigskin exhibited three main layers under the scanning electron microscope: the epidermis, the dermis and the hypodermis. The epidermis was further distinguished as the superficial cornified layer and the subjacent noncornified layer composed of variously shaped cells. The cytoplasm of these cells is packed with the keratinous fibrils running in all directions. The dermis consists mainly of bundles of the collagen fibers which run in various directions. The hypodermis is formed with fatty cells and the collagen and elastin fibers.

Heating pigskin in water induced morphological changes both to the keratinous fibrils in the noncornified layer of the epidermis and the collagen fibers in the dermis. The keratinous fibrils swelled, coagulated to pieces which enlarged and united to form a integrated spongy mass. The collagen fibers swelled, granulated and adhered to form a integrated mass.

Freezing pigskin for a long time showed little effect on the ultrastructure of pigskin.

INTRODUCTION: The pigskin covers the whole surface of the pig body. It makes up some 4% of the carcass weight (WHITTEMORE et al., 1976). Like those of other higher vertebrates, fresh pigskin consists of water, protein, fatty materials and some mineral sals. Of these, the most important part is the protein which constitutes about 33% of the total weight(SHARPHOUSE, 1971). According to LAWRIE(1985), muscle contains 19% of protein. These demonstrate the high utilizing and nutritive value of pigskin.

For hundreds of years, Chinese people have consuming pigskin as a food, with the traditional folk ones being fried pigskin, jellied pigskin and pork(with skin) braised in brown sauce etc. However, pigskin has not yet been widely accepted as a food or a kind of food processing material throughout the world due to its unpleasant taste and the difficulties encountered when processing it. It has been commonly utilized as the raw material for manufacturing leather products. This surely restricts its utilization. For the sake of making full use of pigskin to increasing the sourses of edible protein for mankind, much research work should be done on pigskin in its fundermental and processing aspects.

The present study was designed to examine the ultrastructure and the ultrastructural changes of native pigskin as affected by heating and freezing.

MATERIALS and METHODS: 1. Native pigskin was obtained from a slaughtered pig within 30min after bleeding by cutting parallel to the surface of the skin with a single-edged razor. The sample was divided into two pieces. One was kept at -18°C for 6 months and then prepared as described in 2. The other one was trimmed to strips of about 20mm long and 2mm wide. Of these strips, one was immediately prepared as described in 2, others were heated while immersed in distilled water at 40, 50, 60, 70, 80, 90 and 100°C for 30min respectively and then prepared as described in 2.

2. All samples from 1 were diced into 5mm long and 2mm wide, fixed in Karnovsky's fixative(Karnovsky, 1965) for 2hr, freeze-fractured in liquid nitrogen, postfixed and electro-conducting treated in 1% osmium tetroxide for 1.5hr, dehydrated through ethanal gradients, critical-point dried using liquid carbon dioxide, coated with gold and examined with a JEOL JSM-35CF scanning electron microscope(SEM).

RESULTS and DISCUSSION:

The ultrastructure of native pigskin:

Native pigskin exhibited three main layers under the SEM, the epidermis, the dermis and the hypodermis. The epidermis could be further distinguished as the superficial cornified layer and the subjacent noncornified layer.

The microstructure of the epidermis at low magnification is shown in fig.1. The cornified layer consists of many layers of thin, squamous cells which lack of a nucleus and appear rather dense in their cytoplasm. The outermost layers seperate in many places, leaving large spaces between them. Below the cornified layer is the noncornified layer composed of variously shaped cells. The cytoplasm of these cells is packed with fibrils which run in all directions forming a network(Fig. 2). The fibrilar network of all the cells unites to form a integrated fibrilar, epidermal network(FEN), in which the nuclei can be clearly seen but the membrane of the cells indistinguishable.

The numerous fibrils observed in the noncornified layer of the epidermis were believed to be the tonofibrils. They are in fact bundles of 60-80Å filaments. All epidermal cells have a cytoplasm packed with the filaments which are condensed to a highly ordered structure in the cornified layer(MONTAGNA, 1974; MATOLTSY, 1975; BLOOM et al., 1986). These epidermal cytofilaments are composed of cytokeratins of lower and higher molecular weight (MEYER, 1986) which give an α-diffraction pattern(GIROUND et al., 1951).

Lying between the epidermis and the hypodermis, the dermis forms the thickest layer in the pigskin. It consists of numerous collagen fibers and some elastin and reticular fibers. As these fibers are generally in a interwaving form, it is difficult to distinguish between them under the SEM. However, as the collagen fibers are numerous in the dermis, they can be easily identified. They appear to be 20-100nm in diameter and various length(Fig. 10). Huge numbers of them aggregate to wavelike bundles which run in all directions forming a spongy network. It was found that the upper bundles appeared comparatively thin and short while those deeper ones were thick and long(Fig. 1).

Beneath the dermis is the hypodermis. It is formed with fatty cells and the collagen and elastin fibers. The hypodermis is a deeper continuation of the dermis.

The ultrastructural changes of pigskin as affected by heating:

1. the epidermis

Little difference was observed between the morphological feature of the native cornified layer and that of the heated ones, but the structural change of the keratinous fibrils in the noncornified layer(KFINL) and of the FEN was found evident.

40°C: Some of the KFINL appeared slightly swollen and gave global appearance in some regions of the FEN. The FEN became comparatively loose. (Fig. 3)

50°C: Most of the KFINL were swollen and some of them were found having merged and coagulated. (Fig. 4)

60°C: Coagulation of the swollen KFINL was widespread. Pieces of the coagulated KFINL which were connected by thick fibers were obvious. As a result, the FEN was not recognizable. (Fig. 5)

70°C: The pieces of the coagulated KFINL became bigger, which gave rise to formation of the spongy masses. Holes could be observed on the surface of these masses. (Fig. 6)

80°C: The structural feature of the KFINL was found similar to that at 70°C. (Fig. 7)

90°C: The spongy masses were bigger than those at 70°C. (Fig. 8)

100°C: The spongy masses became very large. Inaddition, they were in uniting condition forming a integrated spongy mass. (Fig. 9)

The results above demonstrated that when native pigskin was heated in water gradually to higher temperature

the KFINL underwent a progressive structural change. The procedure of this change could be summarized as: some of the KFINL swelled → most of the KFINL swelled and some of the swollen KFINL merged and coagulated → most of the swollen KFINL coagulated forming pieces → the pieces of the coagulated KFINL enlarged forming spongy masses the spongy masses gradually united → a final integrated spongy mass was formed. As noticed, 60-70°C appeared to be the critical range during which the KFINL changed severely.

The cornified layer showed higher thermal stability than the noncornified layer. It was believed that the molecular structure of the main structural protein of the epidermis - α-keratins was responsible for this.

RUDALL(1946) demonstrated the increasing thermal stability of strips of tissue cut from successively higher layers of epidermis. He believed that with the growth and moving upward of the epidermal cells, more disulfide bonds were formed. These were supported by the higher thermal contracting temperature of the higher layers of epidermis.

According to MONTAGNA(1974), when histochemical preparations for -SH groups are viewed under the phase-contrast microscope, the reaction appears to be located in the tonofibrils. Furthermore, GIROUND and LEBLOND (1951) suggested that the -SH groups of fibrous protein change into the disulfide bonds during the formation of the cornified cells and thus give the stratum corneum both the strength and chemical inertness.

BADEN et al.(1976, 1978) reported that among the changes that occured in the fibrous proteins of the stratum corneum was formation of intra- and/or inter-chain disulfide bonds.

BLOOM et al.(1986) indicated that the epidermal cells contain large amount of keratins of different molecular weight, while the keratins in the cornified layer are extensively cross-linked by disulfide bonds.

Hence, it is suggested here that the KFINL have a lower thermal stability because their component - α-keratins are poor in disulfide bonds, while the cornified layer of the epidermis has a higher thermal stability because the α-keratins in it are rich in disulfide bonds.

2. the dermis

Our observation was mainly on the morphological change of the CF.

40°C: Most of the CF exhibited little morphological change, but some of them showed slightly swollen pattern and were transversely connected by filaments in some regions. A few CF were revealed having slightly granulated. (Fig. 11)

50°C: All of the CF appeared swollen. The transverse filaments were clearly visible. Inaddition, the adherence of some CF together was observed. Many CF were found having granulated. (Fig. 12)

60°C: The adherence of the CF was severe. A lot of the swollen CF were found sticking to each other forming very thick fibers. Much more CF could be seen having granulated. (Fig. 13)

70°C: The adherence became much more severe, which induced formation of the pieces. All of the CF appeared having granulated severely giving beadlike appearance. (Fig. 14)

80°C: The pieces of the adhered CF were bigger. Many spaces existed among them. The fibrous appearance of the CF was not discernible. (Fig. 15)

90°C: The morphological feature of the CF was similar to that at 80°C. (Fig. 16)

100°C: The pieces of the adhered CF were much bigger. They appeared further adhering to each other which gave rise to formation of a integrated mass with only small spaces existing in it. (Fig. 17)

From the results above, it could be seen that when native pigskin was heated in water gradually to higher temperatures, the CF in the dermis also underwent a progressive morphological change. The change appeared to begin around 40°C, to be severe during 60-70°C, and to be further enhanced by higher temperatures.

According to RATON(1988), the collagen fibers are composed of numerous collagen micromolecules of three helical polypeptide chains. As the collagen molecules become denatured by heat, they collapse and go into a random coil conformation(gelitin). If the cross-links are present, the molecules are held together by these native cross-links. This can produce a isometric shrinkage of the collagen molecules and fibers.

The morphological change of the CF at 40°C as shown in Fig. 11 was believed to be the result of the hydrothermal isometric shrinkage of the CF. 60-70°C of heating caused most of the CF to denature and induced all the CF to adhere to form pieces. Higher temperatures of heating resulted in adherence of these pieces to form a integrated mass.

It should be further noted here that the morphological change of the CF as affected by the gradual heating was a progressive procedure, from slightness to severeness and from parts to the whole.

The ultrastructural changes of pigskin as affected by freezing:

The ultrastructural feature of the pigskin frozen for half a year was found similar to that of the native ones. However, we observed that many of the CF in the dermis were transversely connected by very thin filaments (Fig. 18). Transverse filaments were also observed between the CF in the native dermis, but their number was rather small. The reason for this remains to be explained through further studying.

CONCLUSIONS: 1. Native pigskin consists of three main layers, the epidermis, the dermis and the hypodermis. The epidermis is composed of the superficial cornified layer and the subjacent noncornified layer. The noncornified layer of the epidermis is formed with variously shaped cells whose cytoplasm is packed with keratinous fibrils which form a network. The dermis consists mainly of the collagen fibers which aggregate to bundles of different size. These bundles run in all directions. The hypodermis is composed of fatty cells and the collagen and elastin fibers.

2. When native pigskin is heated in water gradually from 40°C to 100°C, both the keratinous fibrils in the noncornified layer of the epidermis and the collagen fibers in the dermis undergo progressive morphological changes. The keratinous fibrils first swell, then coagulate and become spongy, finally form a integrated spongy mass. The collagen fibers first swell, then granulate and adhere, finally form a integrated mass.

3. Native pigskin undergoes little ultrastructural change during the long period of freezing.

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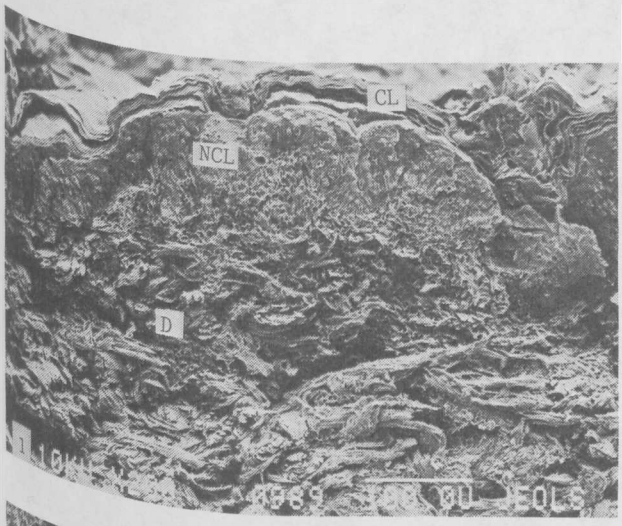
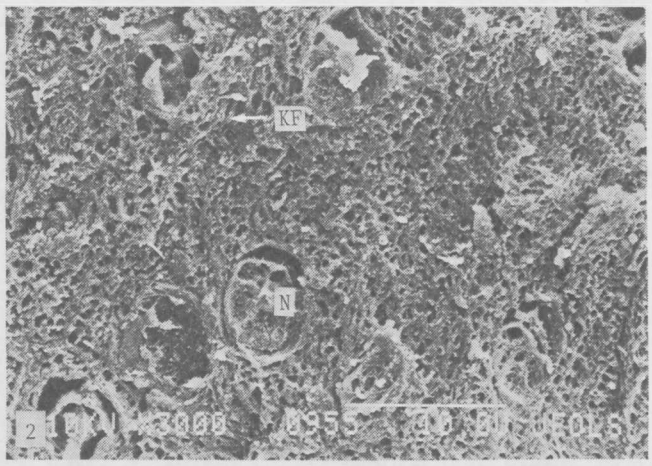
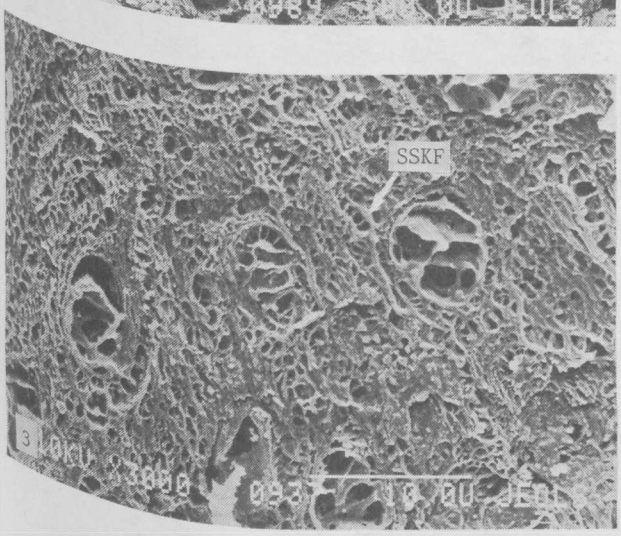
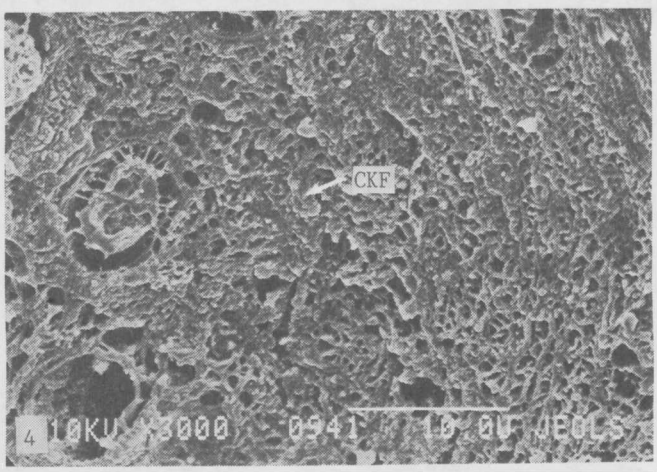
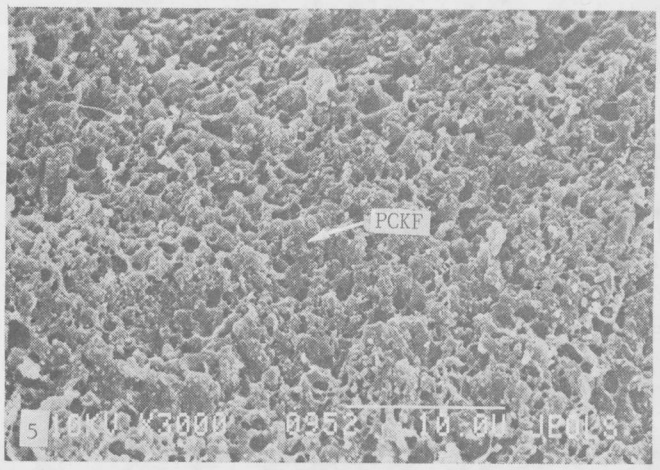
The descriptive caption for the figures: Fig. 1: Native pigskin. X342; CL: the cornified layer; D: the dermis; NCL: the noncornified layer. Fig. 2: The noncornified layer of the native epidermis. X5089; KF: the keratinous fibril; N: nucleus. Fig. 3 — Fig. 9: The morphological change of the KFINL as affected by heating pigskin to different temperatures in water. X5089. Fig. 3: 40°C, SSKF: the slightly swollen keratinous fibril. Fig. 4: 50°C, CKF: the coagulated keratinous fibrils. Fig. 5: 60°C, PCKF: the piece of the coagulated keratinous fibrils. Fig. 6: 70°C, SP: the spongy mass. Fig. 7: 80°C. Fig. 8: 90°C. Fig. 9: 100°C. Fig. 10: Bundles of the CF in the native dermis. X6609. CF: the collagen fiber. Fig. 11 — Fig. 17: the morphological change of the CF of the dermis as affected by heating pigskin to different temperatures in water. X6609. Fig. 11: 40°C, upper left: the transverse filaments between the CF (arrow). Fig. 12: 50°C. Fig. 13: 60°C. Fig. 14: 70°C, upper left: beadlike appearance of the CF. Fig. 15: 80°C, S: the space. Fig. 16: 90°C. Fig. 17: 100°C. Fig. 18°C: The CF in the frozen dermis. X10708. Upper left: the transverse filaments between the CF (arrow).







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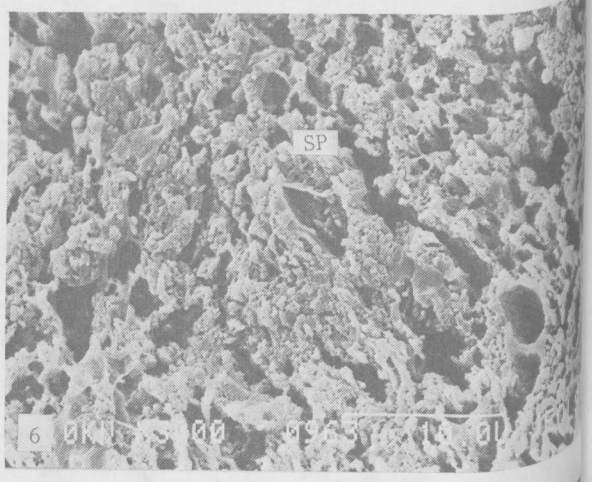
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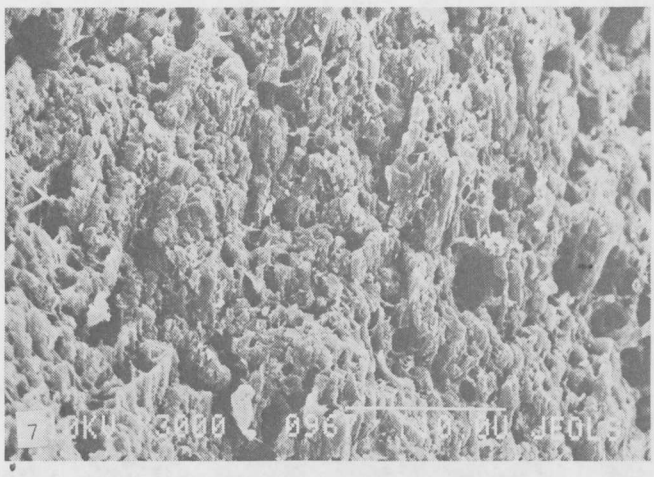
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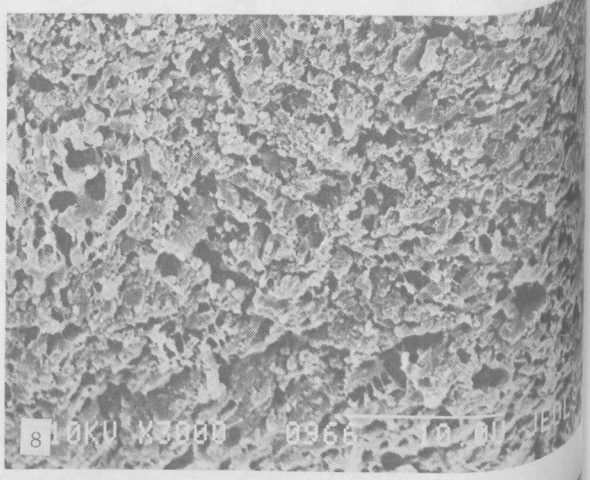
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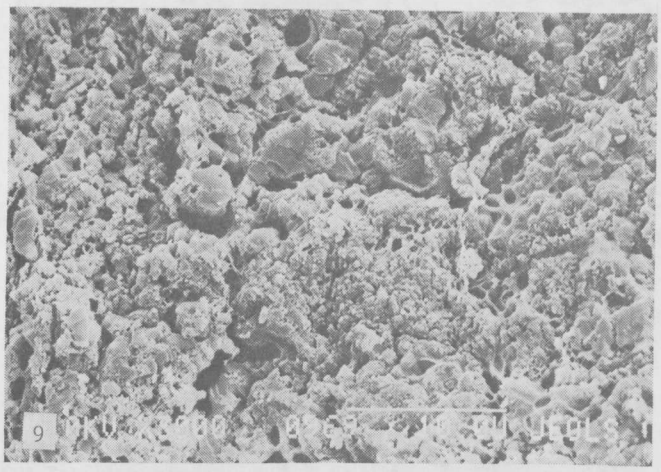
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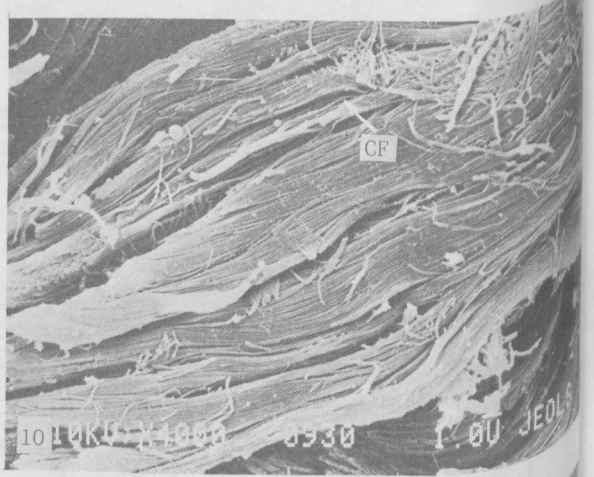
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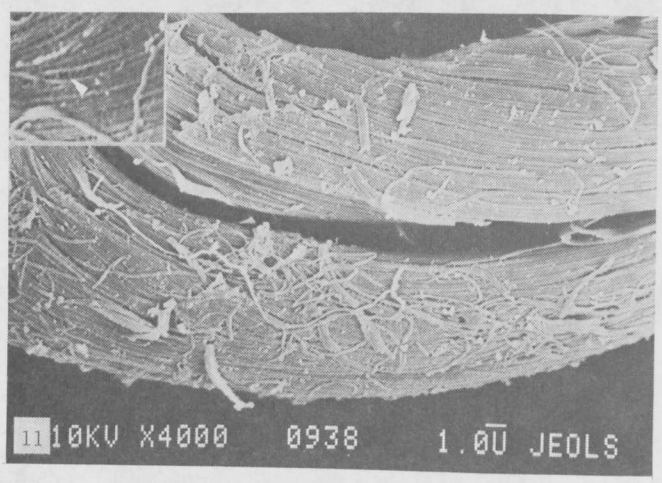
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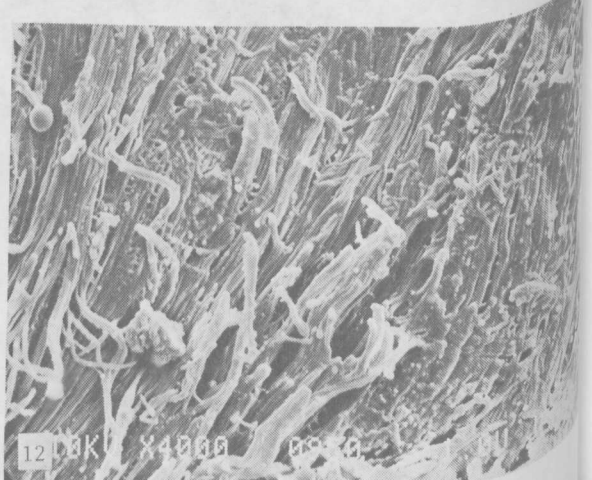
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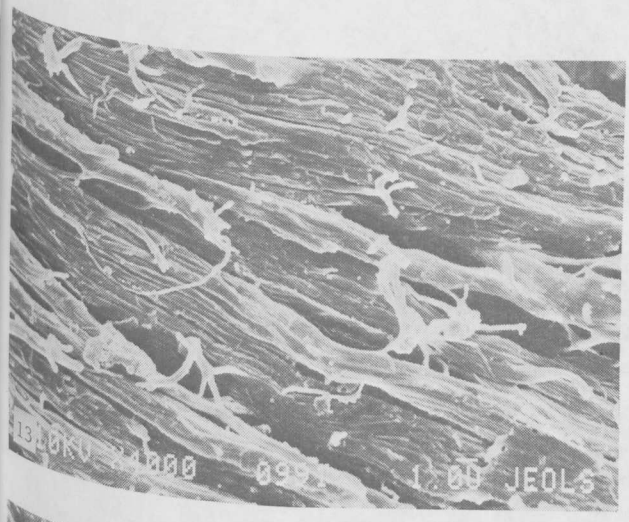
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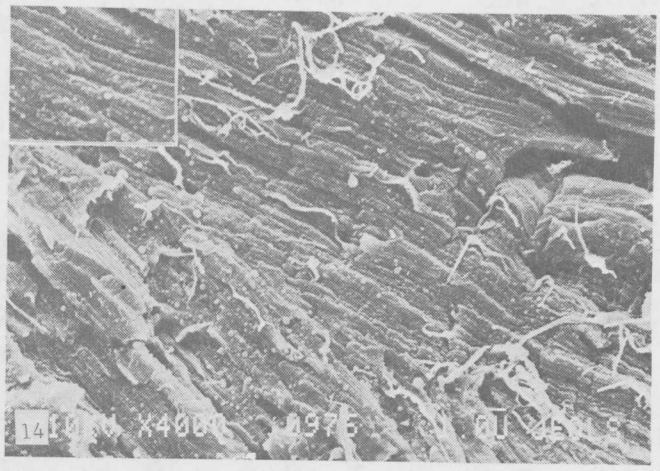
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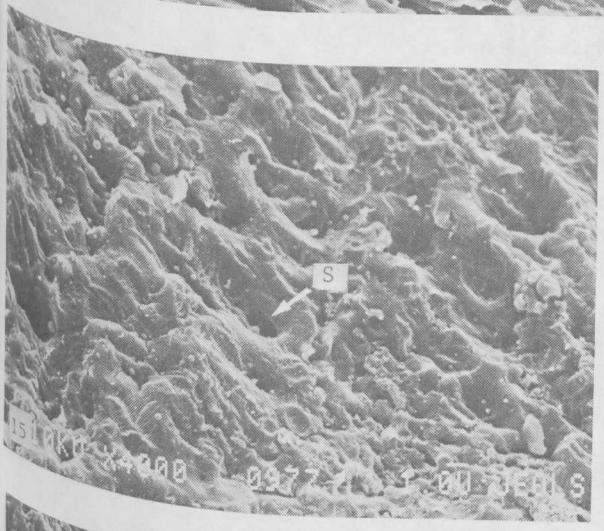
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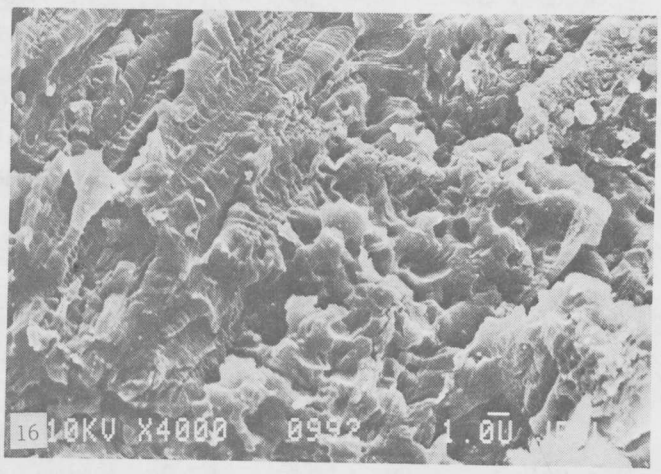
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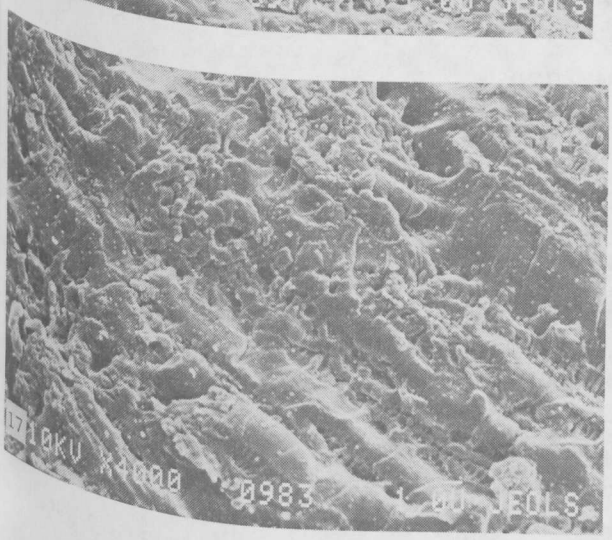
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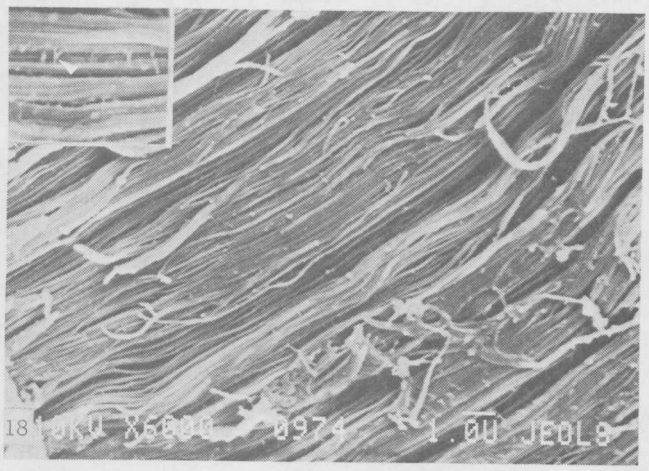
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