Collagen and Glycosaminoglycans in Mummified Skin

T. AMMITZBØLL, R. MØLLER, G. MØLLER, T. KOBAYASI, H. HINO, G. ASBOE-HANSEN & J. P. HART HANSEN

Ammitzbøll, T., Møller, R., Møller, G., Kobayasi, T., Hino, H., Asboe-Hansen, G. & Hart Hansen, J. P. 1989. Collagen and Glycosaminoglycans in Mummified Skin. – Meddr Grønland, Man & Soc. 12: 93–99. Copenhagen 1990–01–26.

Two tombs among the rocks at Qilakitsoq, Greenland, contained the 500-year-old bodies of eight Eskimos mummified by a natural drying process. Samples of the human skin and sealskin were analysed for the content of collagen and glycosaminoglycans which are characteristic macromolecules of skin. The skin of the Eskimo mummies was well-preserved. In some respects, the macromolecules of the skin were identical to those of fresh human skin. Well-preserved ancient sealskin had the same biochemical composition as modern skin treated by scraping, washing, stretching and drying in Greenland. The state of preservation could be determined by cellulose acetate electrophoresis of the glycosaminoglycans, because badly preserved skin contained degradation products of glycosaminoglycans. Collagen was better preserved than other components of the skin. The composition of the glycosaminoglycans was different in the skin from the two seal species, ringed seal and harp seal. Such differences may facilitate the general identification of untanned, unhaired skin. Alum tanning removes the glycosaminoglycans from the skin and makes identification impossible.

T. Ammitzbøll, R. Møller, T. Kobayasi, H. Hino and G. Asboe-Hansen, Department of Dermatology, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. G. Møller, The National Museum of Denmark, Ny Vestergade 11, DK-1471 Copenhagen K, Denmark. J. P. Hart Hansen, Department of Pathology, Gentofte Hospital, University of Copenhagen, DK-2900 Hellerup, Denmark.

The skin of humans and animals is composed of three layers: epidermis, dermis, and subcutaneous adipose tissue (Fig. 1). Epidermis is rich in cells forming the horny layer on top of the skin. Dermis is the thick, tough layer of the skin, which is tanned to leather. Subcutis consists mainly of fat (blubber on seal). Dermis contains fibrous macromolecules forming a network filled up with ground substance and cells. The fibrous protein collagen constitutes about 70% of the dry weight of dermis. Collagen gives toughness and tensile strength to the skin. The ground substance between the collagen fibres consists mainly of water and glycosaminoglycans, a family of water-binding macromolecules. The glycosaminoglycans render the skin soft and flexible.

Analyses of collagen and glycosaminoglycans are part of the investigations performed on patients with certain skin diseases. Some of these analyses were performed

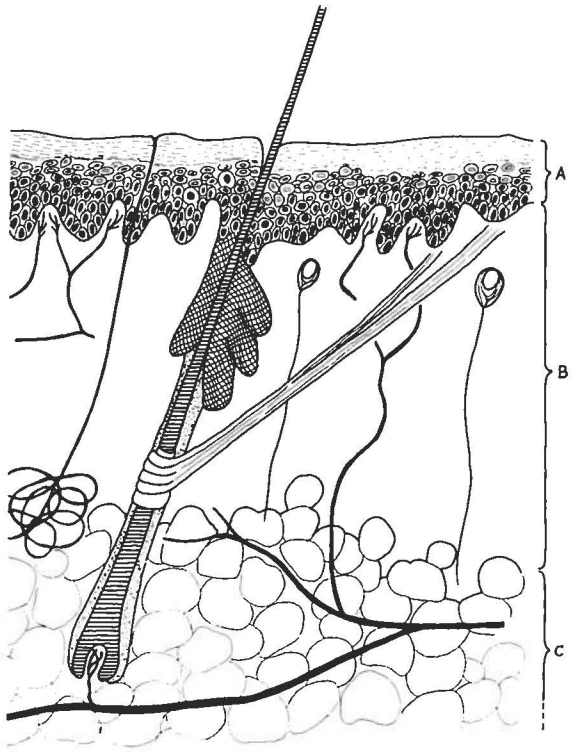

Fig. 1. The skin is composed of three layers: Epidermis (A), dermis (B) and subcutis (C). Dermis consists of cells, collagen and ground substance with glycosaminoglycans. Determinations of collagen and glycosaminoglycans were performed on samples of skin from the 500-year-old Eskimo mummies and sealskin (Gulløv, *del.*).

on samples from the 500-year-old Eskimo mummies and their skin clothes in order to compare the old material from Qilakitsoq with present-day skin.

Material and methods

500-year-old mummies and sealskin

Human skin. From seven of the eight Eskimo mummies, sixteen samples of skin from groin, chest and arm were obtained. The bodies investigated were of a four-year-old boy and six women aged from eighteen to about fifty. The baby was not investigated.

Sealskin. From three ancient skins of ringed seal, fourteen samples were taken, eight of them from well-preserved areas and six from poorly preserved areas. Two samples of well-preserved skin and three samples of badly preserved skin were obtained from two ancient sealskins of harp seal. Furthermore, two samples of alum-tanned sealskins, one of ringed seal ond one of harp seal, were included. The identity of the sealskin (ringed or harp) was established by the colour and marking of the hair.

Kamiks. The 500-year-old eskimo boots, *kamiks*, were made of unhaired sealskin of unknown seal species. The material investigated included samples of two untanned *kamiks* treated with Lederweicher® and samples of 7 alum-tanned (Lutan F) *kamiks*. The treatment with Lederweicher ® or Lutan F was done at the National Museum in order to preserve the items.

Present-day human skin and sealskin

The human control group consisted of 29 age and sex matched Caucasians. Skin specimens from the groin were removed 6-36 h post mortem.

*Sealskin.*The 500-year-old sealskin was compared to fresh, untanned Greenlandic skin of three ringed seals and three harp seals. The modern sealskin were treated in Greenland by scraping, washing, stretching, and air drying. The number of samples were eight from ringed seal and seven from harp seal. The samples from the modern skin were cut from the same anatomical regions as the samples from the ancient skin. A skin of ringed seal was tanned with Lutan F at the National Museum and used in investigation.

Pre-treatment of samples

The samples of sealskin were shaved and defatted in three changes of acetone, subsequently in acetone/ether (1:1) and ether, and then dried in a vacuum dessicator. The samples were weighed before and after the extraction and drying.

Collagen analyses

Collagen is a fibrous protein built of amino acid residues. Hydroxyproline (Hyp) and hydroxylysine (Hyl) are amino acids characteristic of collagen. Proline (Pro) occurs in greater quantities in collagen than in other proteins. The concentrations of Hyp, Hyl and Pro were measured in the samples of skin and fur. The defatted, dried tissue (about 4 mg) was hydrolysed in 2 ml of 6 N HCl at 118°C for 18 h and evaporated to dryness at 60°C at 50 mbar. The residue was dissolved in 5 ml of a 0.046 M citrate – 0.415 M phosphate buffer pH 7.0 and analysed for Hyp, Hyl and Pro in an AutoAnalyzer® equipment (Blumenkrantz & Asboe-Hansen 1977; Blumenkrantz 1980). The results were expressed as nmol amino acid per mg defatted, dried tissue.

Analyses of glycosaminoglycans

Glycosaminoglycans are a family of macromolecules made of sugar moieties, uronic acid and hexosamine. Typical glycosaminoglycans in skin of humans and animals are chondroitin sulphate (C) hyaluronic acid (HA) and dermatan sulphate (DS) (Fig. 2). The glycosaminoglycans constitute less than 1% of the dry weight of skin. The method of investigation is described in detail elsewhere (Møller *et al.* 1985). The defatted, dried skin (about 10 mg) was cut into small pieces and digested with pronase. The glycosaminoglycans were isolated by precipitation with ethanol and complex formation with cetyltrimethylammoniumbromide. The uronic acid content of the isolated glycosaminoglycans was determined and expressed as nmol uronic acid per mg defatted, dried tissue. Part of the glycosaminoglycans was used for cellulose acetate electrophoresis. The electrophoresis strip was stained with Alcian Blue, which gave the individual bands of glycosaminoglycans a blue colour. The intensity of the blue colour was measured by densitometric scanning of the strips. Each peak on the densitometric curve represents a glycosaminoglycan, C, HA, etc.

Electron microscopy

Small pieces of skin from Eskimo mummies were rehydrated in 0.5 M cacodylate buffer pH 7.4 and then fixed in a solution of glutaraldehyde (2%) in cacodylate buffer, osmicated, dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Jeol electron microscope 100 CX at 80 kV (Hino *et al.* 1982).

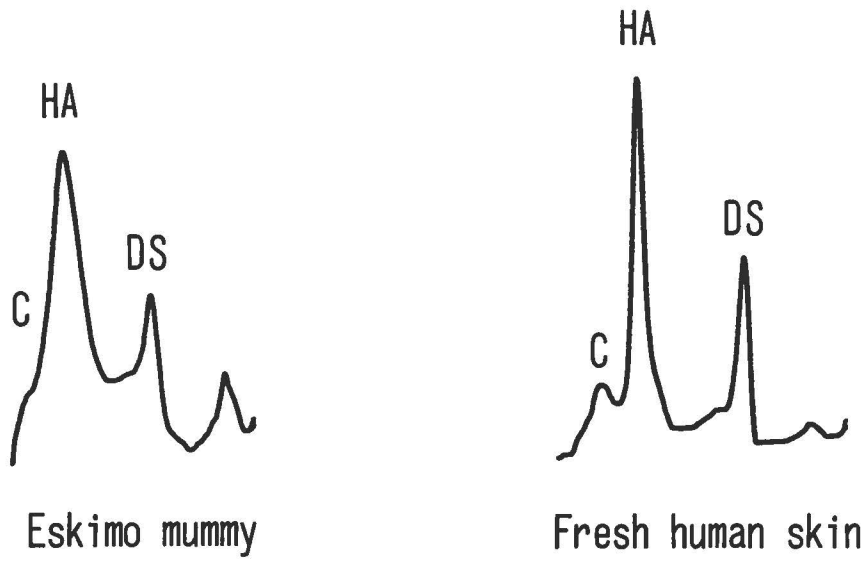

Fig. 2. Glycosaminoglycans isolated from well-preserved skin of Eskimo mummy showed an electrophoretic pattern almost identical to that found in fresh human skin. The curves are densitometric scans of glycosaminoglycans separated on cellulose acetate electrophoresis strips. C: Chondroitin sulphate, HA: hyaluronic acid, DS: Dermatan sulphate.

Statistical evaluation

The Wilcoxon Rank Sum Test for unpaired data was used for the statistical analyses. Biological significance was recognized at $p<0.05$.

Results

Eskimo mummies

The skin of the 500-year-old bodies was stiff and hard as wood and was almost without extractable lipids and water. The acetone/ether extractable fraction was 5% as compared to 75% in fresh human skin.

The concentration of glycosaminoglycans-derived uronic acid in the mummified skin was 4.5 nmol/mg defatted, dried tissue, range 1–10 nmol/mg. This was 56% of that in fresh human skin (8.0 nmol/mg defatted, dried skin, range 5.7–10 nmol/mg). The glycosaminoglycans still present in the 500-year-old human skin were separated by cellulose acetate electrophoresis. The electrophoresis strips were densitometrically scanned (Fig. 2). Each peak of the densitometric scan represents a type of glycosaminoglycan: C = Chondroitin sulphate, HA = Hyaluronic acid, DS = Dermatan sulphate. The relative proportion of the different types of glycosaminoglycans was similar in the mummified skin and in fresh human skin. The broad peaks in the densitogram of glycosaminoglycans from the mummies are due to some degradation of the glycosaminoglycans. The results showed that more than fifty per cent of the glycosaminoglycans were still present in the mummified human skin and could be extracted and identified as such.

Table 1. Collagen content of skin. The concentrations of hydroxyproline, hydroxylysine and proline (nmol/mg defatted, dried skin) are given as mean (range) for skin of Eskimo mummies, 500-year-old sealskin, and control material of recent date.

Number	Species	Number of samples	Hydroxyproline	Hydroxylysine	Proline
7	Eskimo mummy	16	540 (320–700)	31 (21–37)	820 (410–1140)
29	Modern human	29	600 (510–700)	30 (24–37)	960 (840–1100)
3	Ringed seal, 500-year-old				
	well-preserved	8	510 (460–540)	30 (26–34)	770 (720–840)
	poorly preserved	6	640 (560–680)	37 (31–39)	860 (750–910)
3	Modern skin of ringed seal	8	490 (440–590)	29 (25–38)	760 (680–860)
2	Harp seal, 500-year-old				
	well-preserved	2	680 (560–800)	46 (39–53)	1010 (890–1120)
	poorly preserved	3	710 (700–720)	45 (40–49)	990 (950–1010)
3	Modern skin of harp seal	7	640 (590–710)	38 (34–46)	910 (810–1050)

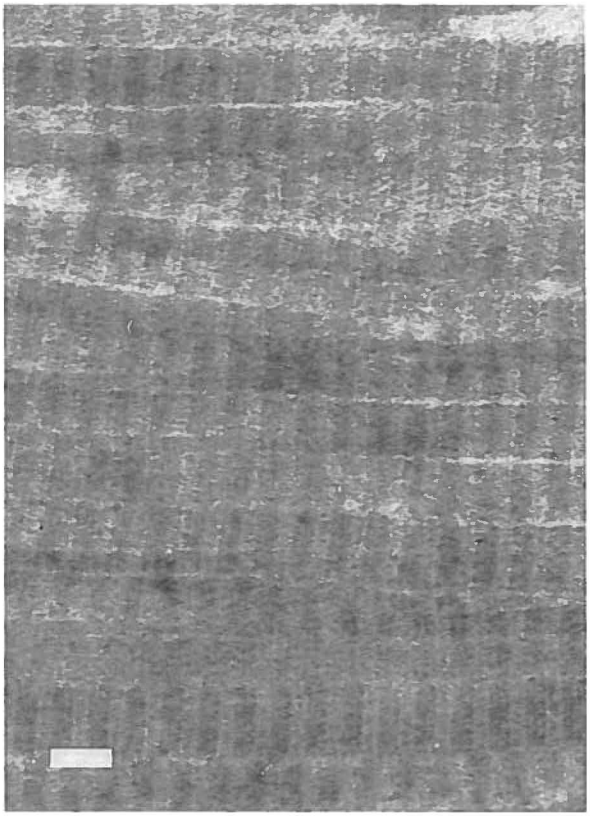

Fig. 3. The ultrastructure of collagen in the dermis from the upper arm of the 500-year-old mummified remains of a 50-year-old Eskimo woman found at Qilakitsoq. The picture is identical to that of fresh human skin. Electron micrograph, X 20,000. Bar equals 100 nm.

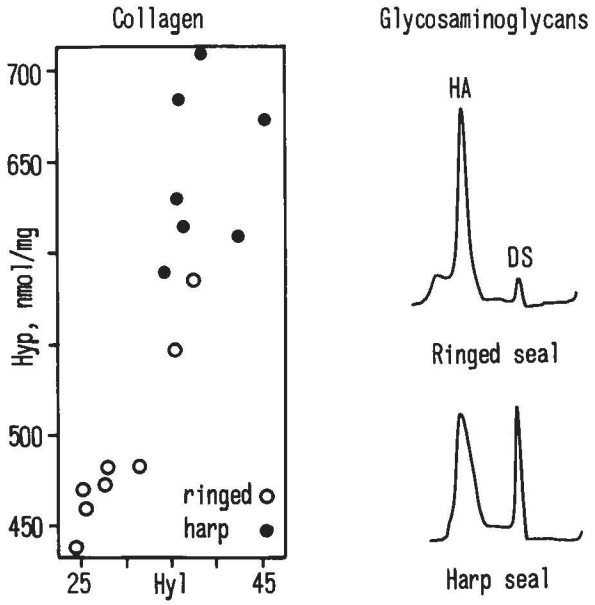

Fig. 4. Analysis of fresh skin showed that the two Greenlandic seal species, ringed seal and harp seal, had significant differences in the dermal content of collagen and glycosaminoglycans. The collagen content was expressed as the concentrations (nmol/mg defatted, dried skin) of the amino acids hydroxylysine (Hyl) and hydroxyproline (Hyp). The glycosaminoglycans were isolated from the skin and separated by cellulose acetate electrophoresis. The curves are densitometric scans of the electrophoresis strips, HA: Hyaluronic acid, DS: Dermatan sulphate.

The collagen content in the 500-year-old human skin was reduced by only 10% as compared to fresh human skin. This is shown by determination of hydroxyproline, hydroxylysine and proline (Table 1). Investigation of mummified skin by electron microscopy (Fig. 3) showed well-preserved collagen fibrils with the characteristic axial periodicity of 67 nm as seen in fresh human skin.

Identification of sealskin from different species

The biochemical analysis of fresh skin and 500-year-old skin showed that the two related Greenlandic seal species, ringed seal and harp seal, had significant differences in the dermal composition of collagen and glycosaminoglycans (Fig. 4). The densitometric scanning of the glycosaminoglycans after cellulose acetate electrophoresis showed two peaks of equal size (hyaluronic acid and dermatan sulphate) in skin of harp seal, whereas skin of ringed seal was dominated by a high hyaluronic acid peak. The ratio of dermatan sulphate to hyaluronic acid was 0.36 (range 0.25–0.55) in harp seal and 0.08 (range 0.01–0.12) in ringed seal. The difference was significant. Skin of harp seal had a higher concentration of collagen (hydroxyproline and hydroxylysine) than was found in skin of ringed seal (Table 1, Fig. 4). Such differences were significant in both modern skin and 500-year-old well-preserved skin from Qilakitsoq.

State of preservation of old skin

Most of the skin clothes and sealskins from Qilakitsoq were well-preserved with light flesh side and intact fur. However, some parts of the skin were dark, hairless and poorly preserved. Unfortunately, the dark, poorly preserved areas of the skin clothes were partly lost during the tanning procedure (Fig. 5). The biochemical investigations showed that the poorly preserved areas were characterized by degraded glycosaminoglycans and the well-preserved skin contained well-preserved glycosaminoglycans.

Fig. 5. The 500-year-old *kamik* illustrates that the poorly preserved areas of the skin clothes were lost during alum tanning at the National Museum.

Fig. 6 illustrates the identical pattern of glycosaminoglycans in modern skin of ringed seal (Fig. 6A) and in 500-year-old skin of ringed seal (Fig. 6B). The results also indicated that the modern and ancient Greenlandic skins have been treated in the same way by scraping, washing and drying. The poorly preserved skin from Qilakitsoq contained degradation products of glycosaminoglycans, which gave an irregular pattern on cellulose acetate electrophoresis (Figs. 6C and 7B).

The concentrations of collagen-derived amino acids were identical in well-preserved ancient sealskin and in modern skin (Table 1). The poorly preserved skin of ringed seal showed a significantly increased concentration of hydroxyproline, hydroxylysine and proline as compared to modern skin. A significantly increased ratio of hydroxyproline to proline was observed in poorly preserved sealskin indicating that collagen (hydroxyproline) was better preserved than other components including proteins (proline) and glycosaminoglycans.

Kamiks

The Greenlandic *kamiks* (boots) were made of unhaired, water-proof sealskin. It is difficult to identify the seal species from which the unhaired skin was taken. Well-preserved samples from two untanned *kamiks* from Qilakitsoq were analysed and the glycosaminoglycans in both revealed two peaks of equal size, hyaluronic acid and dermatan sulphate (Fig. 7A), which showed that the 500-year-old *kamiks* were made of harp seal. Another sample from one of the *kamiks* contained degraded glycosaminoglycans (Fig. 7B) showing that this part of the *kamik* was poorly preserved.

Tanned skin

Most of the *kamiks* were tanned with alum (Lutan F) at the National Museum before the biochemical analyses were performed. The investigation included six tanned *kamiks* (Fig. 7C), two samples of tanned ringed sealskin and one sample of tanned skin of harp seal. All the tanned skin had lost the glycosaminoglycans during the alum tanning. Therefore, it was not possible to identify the seal species from which a tanned skin originates by means of a glycosaminoglycan analysis.

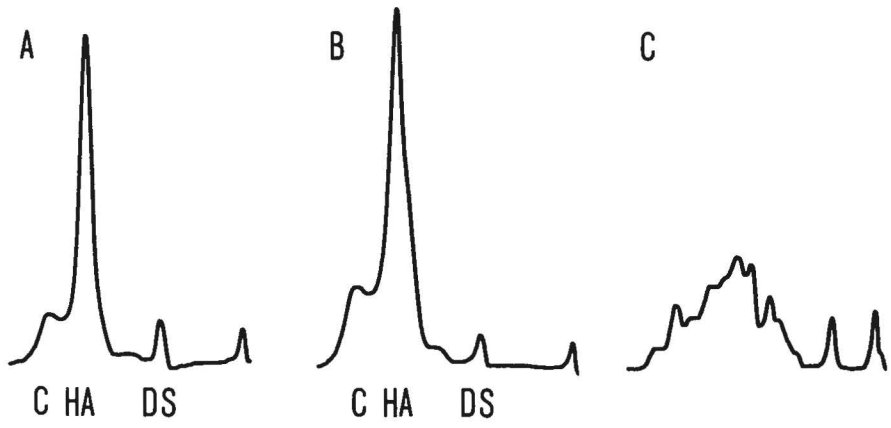

Fig. 6. Densitometric scanning of glycosamionoglycans separated by cellulose acetate electrophoresis. C: Chondroitin sulphate, HA: Hyaluronic acid, DS: Dermatan sulphate. Modern skin of ringed seal (A) had a composition identical to well-preserved 500-year-old skin of ringed seal (B). Poorly preserved skin of ringed seal from Qilakitsoq contained degraded glycosaminoglycans (C).

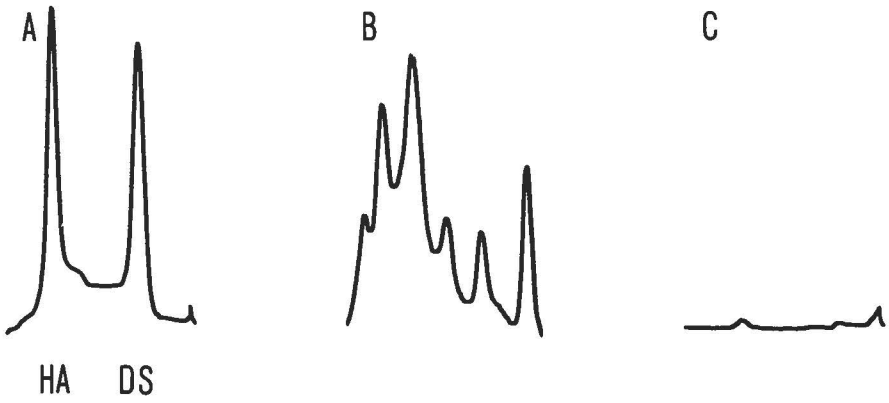

Fig. 7. Glycosaminoglycans were isolated from 500-year-old *kamiks*. Two untanned *kamiks* (A) showed a glycosaminoglycan pattern identical to that of a fresh harp seal (Fig. 4). Another piece of one of the untanned *kamiks* of harp seal contained degraded glycosaminoglycans (B) as an indication of poor preservation. *Kamiks* tanned with Lutan F contained no glycosaminoglycans, as illustrated by cellulose acetate electrophoresis (C), which yielded no peaks of glycosaminoglycans.

Discussion

The dead Eskimos were entombed in two dry clefts and the bodies were dried up before the skin putrefied. The bodies therefore appeared as mummies with a very hard, stiff and shrunken skin. The mummified skin was soluble in hydrochloric acid and could be sufficiently digested by the enzyme pronase. It was thus possible to apply biochemical analyses of dermal macromolecules to the material. Electron microscopy studies revealed intact collagen fibres and, together with the biochemical methods, confirmed the high degree of preservation of the 500-year-old human skin.

It was fascinating to discover that the well-preserved sealskins from Qilakitsoq had the same composition of glycosaminoglycans and collagen as modern Greenlandic skins. These results showed that the ringed seal had not undergone changes of the connective tissue due to environmental or genetic influence during the past 500 years. Since the glycosaminoglycans are sensitive to washing and tanning procedures, the results also indicated that the Eskimos of 500 years ago treated the sealskin in almost the same way as today, i.e. scraping, washing, stretching, and drying. Treatment of fur with fixatives (formaldehyde) or tanning removes the water soluble glycosaminoglycans. Unfavourable storage conditions also decompose the glycosaminoglycans as seen in poorly reserved skin from Qilakitsoq.

The skins of different animal species have characteristic differences in thickness, mechanical properties and the character of the fur. It is novel information that ringed seal and harp seal had differences in the chemical composition of the skin. In particular, the relative proportion of the individual glycosaminoglycans differs in the two seal species. As a result of this discovery, two of the 500-year-old *kamiks* were identified as made of harp seal. This method may perhaps be developed to allow for the general identification of unhaired skin which has not been treated with agents destroying or dissolving the glycosaminoglycans (tanning, fixation etc.).

Previous investigations of archaeological material have been based on collagen, which is the major constituent of connective tissue (skin, bone, etc.). Two Egyptian mummies at the National Museum in Copenhangen contained hydroxyproline (collagen) in the skin, but only 75% of that in fresh human skin (Ammitzbøll *et al.*, unpublished; Hino *et al.* 1982). From fossil bone, hydroxyproline and proline were isolated and used for radiocarbon dating and for stable carbon isotope ratio determinations (Stafford *et al.* 1982). Immunofluorescence studies demonstrated the preservation of Type I and Type III collagen in skin of mummies from Peru (Wick *et al.* 1980). The preservation of the parchments of the Dead Sea Scrolls was studied using X-ray diffraction and racemization analyses on collagen (Weiner *et al.* 1980). These analyses were also applied to material from Qilakitsoq and showed that the collagen fibres in the 500-year-old sealskin and human skin were extremely well-preserved (Traub *et al.*, unpublished). Furthermore, the glycosaminoglycan, chondroitin sulphate, as well as uronic acid and hydroxyproline have been identified in fossil antlers (3,000–130,000 years old) by Scott & Hughes (1981).

Acknowledgements

The autors are greatly indebted to Mr. Ole Christensen, Mrs. Bente Sell and Mrs. Eva Hoffmann for skilful technical assistance, and to Mrs. Inger Johansson for typing the manuscript.

References

Blumenkrantz, N. & Asboe-Hansen, G. 1977. Methods for analysis of connective-tissue macromolecules by determination of certain constituents. – In: Glick, D. (ed.), Methods of Biochemical Analysis. Vol. 24: 39–91. Wiley, New York.

Blumenkrantz, N. 1980. Automated triple assay for proline hydroxyproline and hydroxylysine on one single sample. – Clin. Biochem. 13: 177–183.

Hino, H., Ammitzbøll, T., Møller, R. & Asboe-Hansen, G. 1982. Ultrastructure of skin and hair of an Egyptian mummy. – J. Cutan. Pathol. 9: 25–32.

Møller, R., Serup, J. & Ammitzbøll, T. 1985. Glycosaminoglycans in localized scleroderma (morphoea). – Connect. Tissue Res. 13: 227–236.

Scott, J. E. & Hughes, E. W. 1981. Chondroitin sulphate from fossilized antlers. – Nature 291: 580–581.

Stafford Jr., T. W., Duhamel, R. C., Haynes Jr., C. V. & Brendel, K. 1982. Isolation of proline and hydroxyproline from fossil bone. – Life Sci. 31: 931–938.

Weiner, S., Kustanovich, Z., Gil-Av, E. & Traub, W. 1980. Dead Sea Scroll parchments: unfolding of the collagen molecules and racemization of aspartic acid. – Nature 287: 820–823.

Wick, G., Haller, M., Timpl, R., Cleve, H. & Ziegelmayer, G. 1980. Mummies from Peru. Demonstration of antigenic determinants of collagen in the skin. – Int. Archs. Allergy Appl. Immun. 62: 76–80.