example, which as has now been shown is secreted from the peritoneal mast cells in rats during the development of peritonitis [11]. Contractions of MC, in turn, may evidently affect processes of cellular migration, exudation, and resorption of fluid and colloids. In bacterial peritonitis contraction of MC is followed by destruction and death.

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ELECTRON-MICROSCOPIC STUDY OF PARCHMENT

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UDC 675.43:612.79-086.3

KEY WORDS: parchment; collagen fibrils; destruction of collagen fibrils.

If optimal conditions for the preservation of ancient manuscripts on parchment are to be ensured, it is important to know the causes of destruction of parchment during long-term storage. The present investigation was undertaken in order to study the ultrastructure of parchment and to identify the submicroscopic changes which could explain the changes in its physical properties.

EXPERIMENTAL METHOD

Nine parchments from the 11th-19th centuries, which had been well, satisfactorily, or badly kept, and a sample of modern parchment, prepared from calf skin at the "Moskozhob"edinenie" Factory for restoring old manuscripts, were studied.

Pieces of the parchments measuring less than 1 mm^2 were fixed in 1% OsO_4 solution in 0.1 M cacodylate buffer, pH 7.3, with or without the addition of ruthenium red in a concentration of 0.0005% to the fixative. Some specimens also were fixed consecutively with 3% glutaraldehyde in 0.1 M phosphate buffer and 1% OsO_4 by Caulfield's method. After dehydration in alcohols of increasing concentration the fragments were embedded in Araldite. In the course of dehydration (at the 70% alcohol stage, 16 h) they were treated with 3% uranyl acetate. Ultrathin sections were stained successively with 3% phosphotungstic acid solution and with lead

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 12, pp. 104-107, December, 1982. Original article submitted December 17, 1981.

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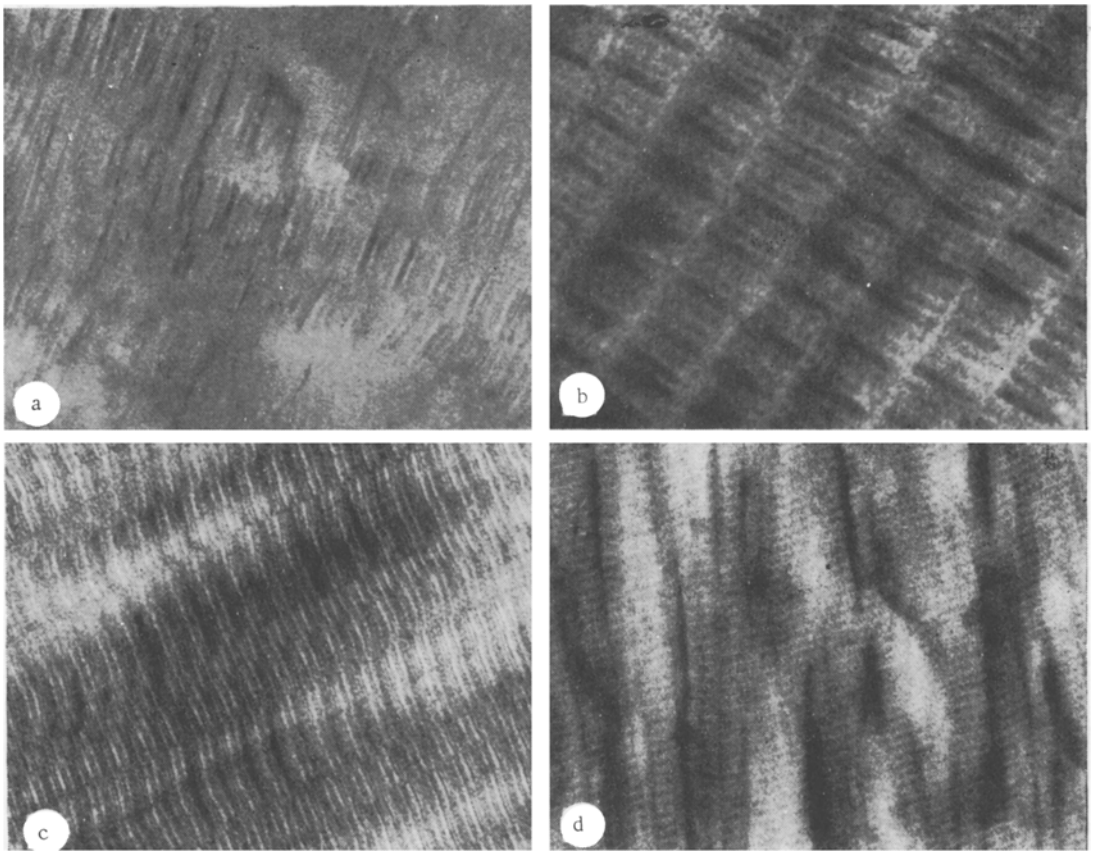

Fig. 1. Collagen fibrils of parchment: a) cross-striation of fibrils of modern parchment: 96,900 ×; b) fibrils of modern parchment after fixation with the addition of ruthenium red. Rutheniophilic dark bands can be distinguished. Magnification 95,000 ×; c) well preserved parchment from the 13th century. Periods with 10-12 subperiods are clearly visible. Magnification 86,000 ×; d) rutheniophilic amorphous material between collagen fibrils (arrows). Magnification 32,000 ×.

citrate by Reynolds' method, after which they were studied in the JEM-100S electron microscope under a magnification of 3000 to 50,000.

EXPERIMENTAL RESULTS

The specimen of modern parchment was a sheet with rather smooth surfaces, consisting of tightly packed collagen fibrils. Narrow spaces detectable here and there between the fibrils were filled with amorphous material of average electron density. The fibrils were straight and formed bundles running in different directions. In cross section they were circular.

Longitudinally cut fibrils under high power showed distinct cross-striation with characteristic periods and subperiods (Fig. 1a). The levels of the periods of neighboring fibrils often did not coincide. The thickness of the fibrils was 80-90 nm and the length of the periods 54-56 nm. In some places the outlines of the cross sections, the periods and, in particular, the subperiods could not be seen clearly. They were seen most clearly in preparations treated with ruthenium red (Fig. 1b).

Well-preserved specimens of parchment from previous centuries were similar in ultrastructure to the samples of modern parchment (Fig. 1c). However, in some places the surface of the sheets was very uneven because of separation of the collagen bundles (fringing). In the thickness of the sheets slits or irregularly shaped cavities were present between the bundles. Often they communicated with the surface. Various microorganisms were present on the surface of some specimens.

Separation of the bundles of collagen fibers was more marked in samples whose preservation was only satisfactory (Fig. 2a). In ultrathin sections some areas consisted apparently of isolated "islands" of collagen fibrils. More often, cracks were seen, running from the

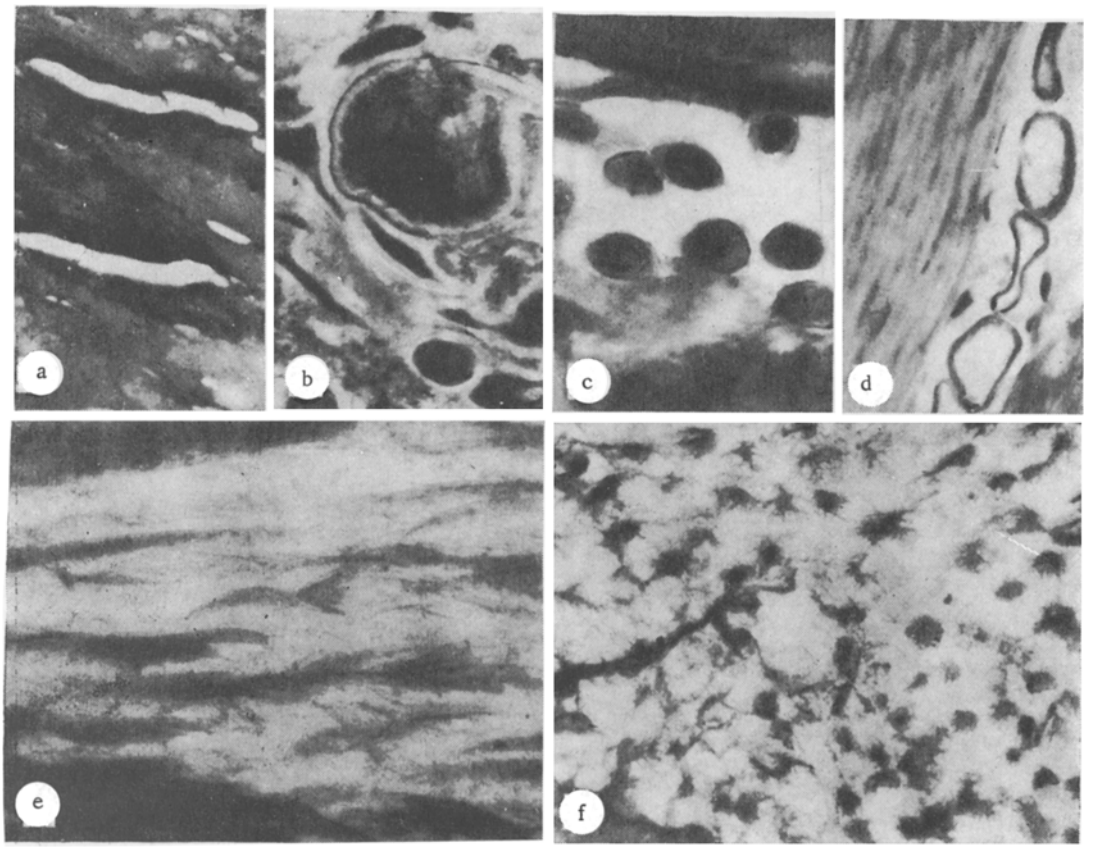

Fig. 2. Destruction of parchment: a) cracks between bundles of collagen fibrils in a piece of parchment preserved only satisfactorily. Magnification 15,600 ×; b) part of a colony of micromycetes. Magnification 15,500 ×; c) bacteria in a crack between bundles of fibrils. Magnification 32,000 ×; d) chain of bacteria on eroded surface of parchment. Some bacterial cells have neither cytoplasm nor nuclear material. Magnification 17,000 ×; e) longitudinally cut fragmented collagen fibrils. Magnification 52,000 ×; f) star-shaped transverse sections through disintegrating fibrils. Magnification 38,000 ×.

surface into the thickness of the sheet. The surface of the parchment was mostly fringed, and only in a few places was it smooth. On the surface and in the cracks bacteria were present. In one specimen (a German manuscript from the 14th century) a colony of micromycetes was found (Fig. 2b), in which deformed cells with a cell wall, cytoplasmic membrane, nucleus, and nuclear membrane could be distinguished. The intercellular spaces in the colony were filled with amorphous material of average density.

Poorly preserved parchment, in ultrathin sections, appeared to consist of fragments of collagen bundles with a very uneven surface. Here and there it resembled a continuous sheet with an eroded surface, with numerous small and large cracks and cavities, containing chains and irregular accumulations of round and irregularly shaped microorganisms (Fig. 2c). Only the cell wall and cytoplasmic membrane remained of many of them, and empty spaces replaced the cytoplasm and nuclear material (Fig. 2d).

Besides colonies of microorganisms, accumulations of amorphous material and collagen fibrils in different stages of fragmentation also could be seen. Fragmentation of fibrils took place through a stage of microfibrillary splitting, as is well demonstrated in longitudinal sections of disintegrating fibrils (Fig. 2e). Their transverse sections were blurred, star-shaped, or irregular in shape (Fig. 2f). Large areas of such parchment consisted of fragments of collagen fibrils intermingled with microorganisms and floccules of amorphous material.

On the whole, the better the degree of preservation of the parchment as estimated organoleptically, the larger the areas in which the fibrils had distinct periods and subperiods.

Rutheniophilic material was present in all specimens. It was intimately connected with the collagen fibrils and formed what looked like bands on their surface, repeating with the characteristic period for fibrils. The amorphous interfibrillary component of the parchment also was rutheniophilic (Fig. 1d). The depth of penetration of ruthenium red into the thickness of the specimens was on the whole inversely proportional to the packing density of the collagen bundles and of the fibrils in the bundles.

The methods of preparation of the specimens for electron-microscopic study of connective tissue proved to be perfectly suitable for parchment also [1-7]. Among the specimens studied there were Byzantine (11th-12th centuries), German (11th-12th and 13th-14th centuries), Old Russian (14th-15th centuries), and Western European (16th-17th centuries) manuscripts and a Torah of the 18th-19th centuries. All had the identical ultrastructure, i.e., they consisted of more or less tightly packed bundles of collagen fibers. No elastic fibers, connective-tissue cells or their remains, or remains of epidermis, blood vessels, nerves, hair follicles, or sweat or sebaceous glands could be identified in the parchments.

The characteristic periods and subperiods of the collagen fibrils were detected in all specimens except parchment of an 11th century Byzantine manuscript which was badly damaged, and this points to the great stability of the supramolecular collagen structures with time. This stability is evidently due to the interfibrillary cementing component. Judging from the electron density and presence of rutheniophilic material, it can be postulated that the amorphous cementing material must consist of proteins, proteoglycans and, possibly, glycoproteins.

In parchments several centuries old two types of changes were observed: type I) cracks between collagen bundles. They perhaps appeared because of excessive drying of the parchment; type II) destruction of collagen fibrils as a result of lytic activity of microorganisms — bacteria and micromycetes (in only one specimen). Severe deformation of the bacterial cells and the absence of cytoplasm and nucleoid in some of them indicate that these cells were most probably nonviable. Judging from the appearance of the disintegrating fibrils, they had first been subjected to microfibillary unraveling. The picture of disintegration of fibrils were never observed in specimens not containing microorganisms.

Most frequently the two types of changes coexisted. In some specimens, however, only cracks were found. Crack formation evidently precedes and facilitates the penetration of bacteria into the thickness of the parchment.

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