CALORIMETRIC INVESTIGATION OF COLLAGEN HYDRATION

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The physiochemical properties of cells and tissues largely depend on their content of water, which is in the free and bound state [8, 9]. Water bound with the polar and ionic groups of collagen, unlike free water, is unable to undergo phase transition when the temperature falls to $-120°C$, and it plays a special role in conformational changes in biopolymers [4, 5, 10, 11].

The writers showed previously [6] that disorganization of the carbohydrate-protein complex, leading to changes in hydration and other physicochemical properties of collagen, takes place in connective tissue in the postmortem period.

The object of this investigation was to study the dynamics of relations between free and bound water in Achilles' tendons at different times after death.

EXPERIMENTAL METHOD

Achilles' tendons from people aged 45-55 years dying from trauma were the test objects. Material was obtained at autopsy under 24 h after death. The material for testing was kept in closed vessels at 22°C. Thymol was used as antiseptic.

The water content in the samples was determined 24, 48, 96, and 120 h after death. To obtain tendons with a definite moisture content the samples were kept over saturated solutions of standard salts. The total water content was determined by Fischer's method and by drying to constant weight at 115°C [2]. Collagen hydration was studied on a differential scanning calorimeter of Perkin-Elmer DSC-2 (Sweden) type. Samples of tendons weighing 5-7 mg were placed in aluminum cuvettes, sealed to prevent drying during measurement, and frozen to $-30°C$. The temperature was then raised at the rate of 10°C/min. The reproducibility of the water melting endotherms was $\pm 2°C$. The quantity of free water, not protein-bound, was calculated from the area of the melting endotherm.

The content of water bound with polar and ionic groups of protein was calculated from the difference between the total water content and the content of free water.

EXPERIMENTAL RESULTS

Examination of Fig. 1, 1, 2, shows that in samples of tendons with a water content of 0.18-0.35 g H_2O/g tissue, all the water was in an unfreezable state.

Degeneration of the phase transition of water into a smooth cooperative transition of the order–disorder type is known to indicate that the water is in a protein-bound state [7]. In that case the tissues can evidently be frozen without the risk of damage to protein structures on account of crystallization of the water in the interstructural spaces. During thawing of Achilles' tendons containing 0.71 g H_2O/g tissue an endothermic ice melting curve is described (Fig. 1, 3).

The phase transition was shown to take place not at a definite temperature, as in the case of ordinary water, but within a temperature range of about 15°C. The maximum of the melting endotherm was shifted by about 2°C below zero.

The content of unfreezable water, bound with protein polar and ionic groups, calculated as the difference between the total water content and the content of free water obtained from the area of the melting endotherm of the frozen water, in Achilles' tendons, measured 24 h after death was 0.44 g H_2O/g tissue. This value for protein-bound water was the same as that obtained by other workers [4].

With an increase in the postmortem period the melting endotherms of water were shifted toward lower temperatures (Fig. 1, 4-6). The fall in the temperature of ice may be due to the fact that free water dissolves electrolytes, which are known to accumulate in the tissues during autolysis [1].

Curves showing changes in the content of total, free, and bound water in Achilles' tendons at different times after death are given in Fig. 2. They show (Fig. 2, 1) that the total water content of Achilles' tendons kept in closed vessels did

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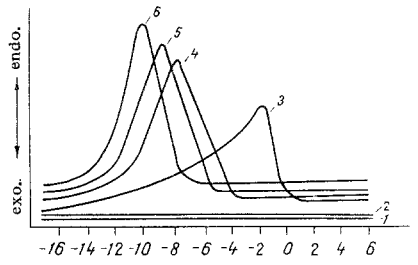

Fig. 1

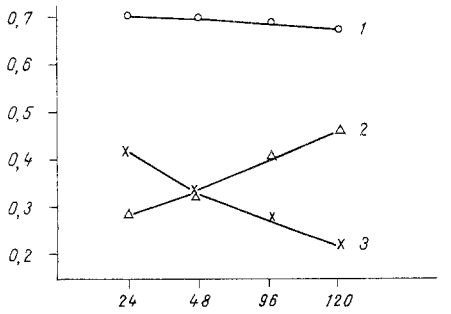

Fig. 2

Fig. 1. Melting curves of freezable water in human Achilles' tendons with different water content. Abscissa, temperature (in °C); ordinate, 1-6) 0.18, 0.35, 0.71, 0.71, 0.68, and 0.64 g H_2O/g tissue respectively. 1-3) 24 h after death, 4-6) 48, 96, and 120 h after death respectively.

Fig. 2. Total water content, and free and bound water in human Achilles' tendons as functions of duration of postmortem storage. Abscissa, time of storage after death (in h); ordinate, water content (in g H_2O/g tissue). 1) Total water content; 2) free water; 3) bound water.

not change very much, and after 120 h it was reduced by only 6-7%. A considerable redistribution of the bound and free water was found under these circumstances. The quantity of free water, capable of undergoing phase conversion, was shown to increase (Fig. 2, 2), whereas the content of bound, unfreezable water fell by 33-50% (Fig. 2, 3).

It can be concluded from these observations that the decrease in the content of bound water in the presence of an excess of free water in the system was not connected with any effect of drying. The destruction of carbohydrate-protein complexes of connective tissue, observed by the writers previously in the post-mortem period, can evidently lead to transition of bound water into the free state [6] . It can be tentatively suggested that a decrease in the content of bound water is one of the factors capable of weakening the structural stability of collagen molecules relative to the action of proteolytic enzymes in the later stages of tissue storage.

The results described above can be used during conservation of organs and tissues and also for forensic medical investigations [3].

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