

## Thermodynamic Characteristics of Collagen in Composition of Pathologic Tissues

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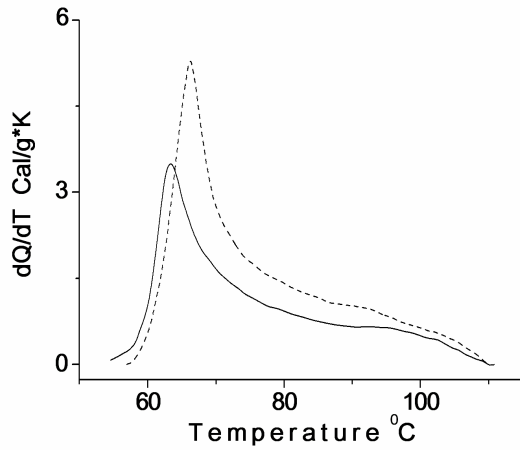
The thermodynamic denaturation parameters of collagen directly in composition of pathologic tissue of human rheumatoid heart valve (HRHV) and nail-finger tissue of persons with Marphein syndrome were determined. It was shown that the denaturation process of both tissues being in physiological solution covering wide temperature range is characterized with intensive peak of heat absorption at  $\sim 66.2^{\circ}\text{C}$  and  $86.5^{\circ}\text{C}$ , and the diffusion heat absorption covering the temperature range  $55\text{-}115^{\circ}\text{C}$  and  $70\text{-}112^{\circ}\text{C}$ , accordingly (Figs. 1, 2).

It was established that  $T_d$  of intensive peak depends on severity of the disease (Fig. 3). Removing of water from tissues by evaporation (Fig. 4) caused a shift of the transition start to higher temperatures by  $20^{\circ}\text{C}$ , the maximum of the main stage – by  $24^{\circ}\text{C}$ , and the redistribution of heats between endotherms. The total heat of denaturation ( $Q_d$ ) decreased only by  $20\pm 5\%$  and equaled to  $8.1\pm 1.0$  cal/g at 22.0 W% water of HRHV and the heat absorption increased almost twice in the temperature range  $70\text{-}115^{\circ}\text{C}$  (Fig. 5). The sharp increase of  $T_d$  depending on water content in a sample is characteristic for collagen compared to globular proteins and protein-lipid complexes [1, 2, 3]. Therefore, we affirm that endotherms at  $66.2^{\circ}$  and  $86.5^{\circ}\text{C}$ , and the weak diffusion endotherms in the case of fresh tissue in the temperature range  $55\text{-}115^{\circ}\text{C}$  and  $70\text{-}120^{\circ}\text{C}$  really correspond to denaturation of collagen fibres packed with various degrees of density. The dependences  $\Delta H_d=f(W\%)$ ,  $T_d=f(W\%)$  and  $\Delta T_d=f(W\%)$  (where W% is weight percent of water) were built on the basis of obtained data. They showed that the decrease of  $\Delta H_d$  and  $\Delta T_d$  were obtained only after removing of hydrate water from tissue and, on the contrary,  $T_d$  was permanently increased as the water content was decreased. It was shown that decrease of  $\Delta T_d$ , which is directly connected with cooperativity of systems depends on tissue types, water content and rate of drying. The mechanism of collagen stability in tissues and the observed changes in denaturation parameters of pathologic tissues are discussed.

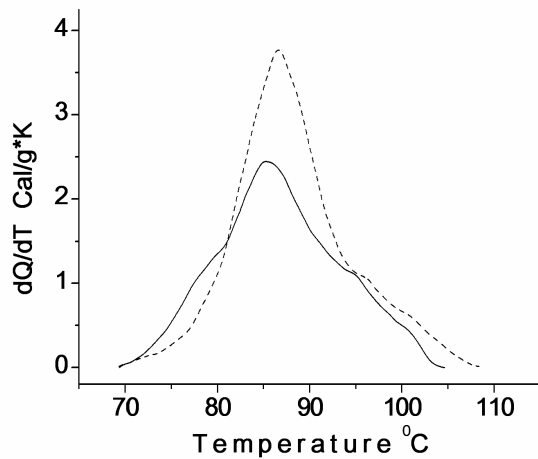
Calorimetric investigations were carried out on DSC with sensitivity  $0.1\mu\text{W}$ , volumes of measuring cells were  $0.02$  and  $0.05\text{ cm}^3$ , the calorimeter resolution of heat capacity (i.e. significant deviation from the baseline) was  $10\mu\text{J}/^{\circ}\text{C}$ . The accuracy of absolute temperature measurement was better than  $0.05^{\circ}\text{C}$ . Measurements were carried out at 1 point per 1s at scan rates from  $0.04$  to  $1^{\circ}\text{C}/\text{min}$ . The calculation of heat denaturation ( $Q_d$ ) and denaturation temperature ( $T_d$ ) and width of melting temperature ( $\Delta T_d$ ) was carried out by a program developed by us. Deconvolution of curves was conducted with Origin 6.0 (Microcal<sup>TM</sup> Software Inc.). The error in determination of  $Q_d$  was not less than 12%.  $T_d$  was determined to  $\pm 1.2^{\circ}\text{C}$  and  $\Delta T_d \sim 0.7^{\circ}\text{C}$ .

### References:

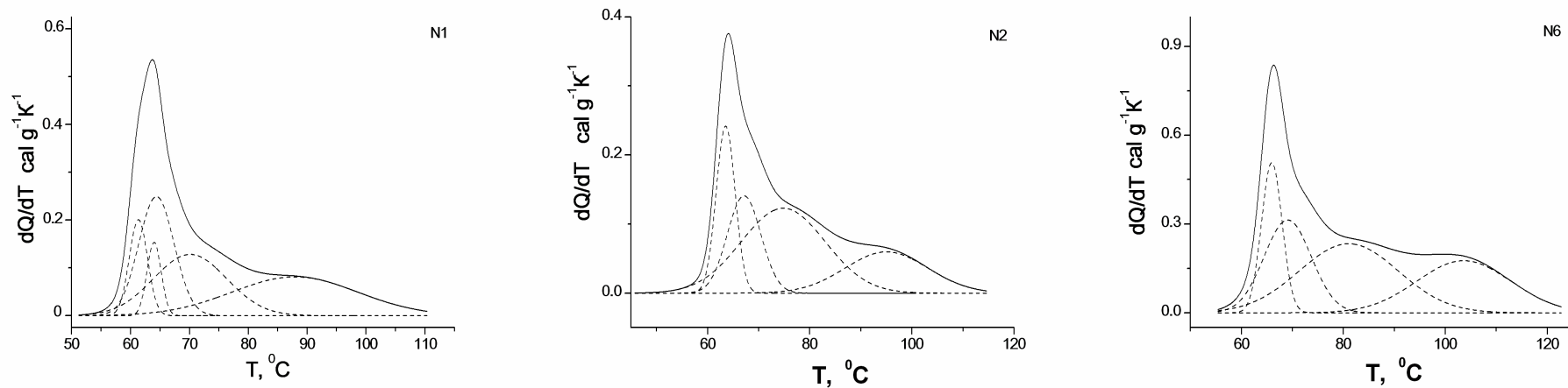
1. J. Monaselidze, N. Bakradze. Doklady Acad, Nauk, USSR, 189-192, 5, 1205. (1968).
2. Miles, Gelashvili. Biophys. J., vol.76, 3243-3252. (1999).
3. P. Privalov, Adv. Prot. Chem., 33, 167-240, (1979).



**Fig. 1.** Calorimetric curves of heat absorption of pathologic tissue of HRHV at different severity of the disease. Dotted line – measurement cell contained 9.45 mg of fresh tissue, dry biomass was 2.07 mg and 30.5 mg of physiological solution. Solid line – measurement cell contained 8.3 mg of fresh tissue, dry biomass was 1.66 mg and 30.0 mg of physiological solution.

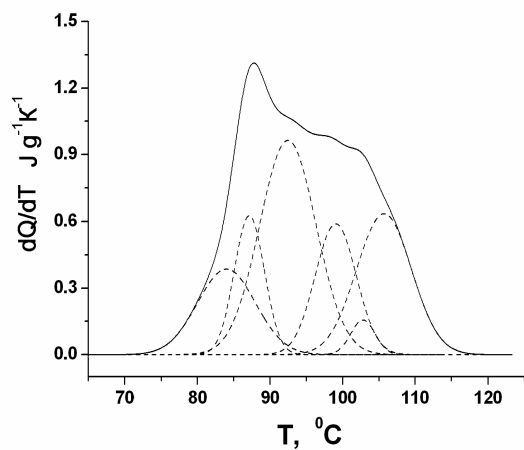


**Fig. 2.** Calorimetric curves of heat absorption of tissue of human nail-finger. Dotted line – (norm); measurement cell contained 5.0 mg of fresh tissue, dry biomass was 3.8 mg and 10.0 mg of physiological solution. Solid line – (pathology); measurement cell contained 4.3 mg of fresh tissue, dry biomass was 3.7 mg and 10.2 mg of physiological solution.



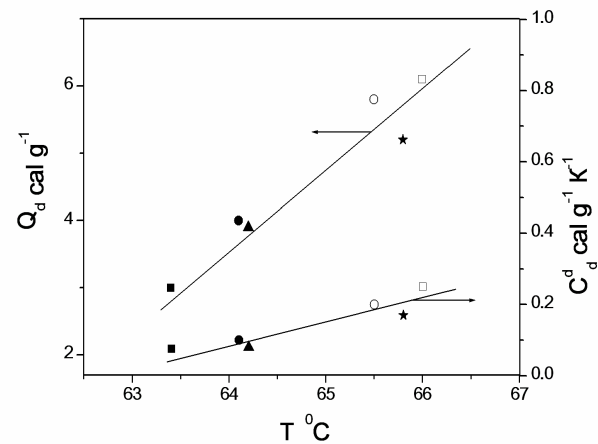
**Fig. 3.** Calorimetric curves of heat absorption of pathologic tissue of HRHV at different severity of the disease.

**Fig. 4.**



**Fig. 4.** Calorimetric curve of heat absorption of preliminarily dried pathologic tissue of HRHV at 25 °C; C=78.2 %.

**Fig. 5**



**Fig.5.** Dependence of tissue (HRHV) heat denaturation ( $Q_d$ ) and denaturation increment ( $\Delta C_d$ ) on temperature at various severity of the disease.