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DIFFERENTIAL SCANNING CALORIMETRY AS AN ANALYTICAL TOOL IN THE STUDY OF THE DUPUYTREN DISEASE

ROBERTA CURINI and GIUSEPPE D'ASCENZO
Department of Chemical Sciences, University of Camerino, Camerino (MC) (Italy)

GIACOMO ALBO
3rd Orthopedic Clinic, University "La Sapienza" Roma, Roma (Italy)

ALDO LAGANÀ
Department of Chemistry, University "La Sapienza" Roma, Roma (Italy)

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ABSTRACT

The problem of water content modification in tissues, induced by Dupuytren's disease, is treated by differential scanning calorimetry (DSC) and thermal analysis.

Two different types of water are found in the pathological tissue instead of the only one found in healthy tissue. At the same time quantitative analysis shows an increase in the total water in the pathological tissue.

Finally, the ratio of the heights of the two maxima found in the pathological tissue is proposed as a diagnostic parameter for the evaluation of the development of the pathology.

INTRODUCTION

Dupuytren's disease is a human affliction in which there is a progressive, irreversible contraction of one or more fingers [1].

The most obvious changes are the shrinkage of parts of the palmar aponeurosis and the development of typical nodules along its length.

Histological studies have shown that the most significant changes take place in the nodules, which are essentially masses of densely packed cells embedded in a collagen-rich matrix, typical of the so-called "fibrotic lesions". Structural studies [2] carried out by wide- and low-angle X-ray diffraction or by transmission electron microscopy have shown that no alterations of the molecular structure or the state of macromolecular aggregation of the collagen were detected.

On the contrary some biochemical changes were pointed out [1–4], such as:

(1) increase of collagen including the consistent appearance of type III collagen, which is virtually absent from normal adult palmar fascia;

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(2) increase of hexosamine content;
(3) presence of galactosamine in the most severely involved tissues;
(4) increase in the total number of reducible cross-links;
(5) appearance of hydroxylsinohydroxynorleucine as the major reducible cross-link.

Finally, some researchers [1] pointed out that there was a progressive increase in water content from the unaffected parts to the contractures and finally the nodules. The problem of the water content modification was of interest and needed to be investigated thoroughly by much more sophisticated analytical techniques such as thermal analysis and differential scanning calorimetry.

The aim of this paper is the study of the modifications induced by the pathology on the water in the tissues either considering the quantitative aspect, evaluating the changes of the water percent in the tissues, or from a qualitative point of view, studying the different types of water in the tissues, and verifying if the Dupuytren's pathology can induce the appearance or the disappearance of different types of water. In fact structural changes, and especially modifications of the cross-links like those observed in Dupuytren's disease, can induce modifications of the water matrix interactions, functions of the modified structural and stereochemical situations of the matrix with the consequent different possibilities of arrangement of the water molecules inside the lattice.

EXPERIMENTAL

Apparatus

TG and DTG curves were obtained using a Perkin-Elmer TGS-2 thermobalance, while the DSC curves were obtained using a Perkin-Elmer DSC-2 instrument, both equipped with a data station.

The atmosphere was very pure nitrogen (99.999%) and the gas flow was 50–100 ml min^{-1}.

Sample collection

Specimens of Dupuytren's tissues, contractures and normal surrounding tissues were obtained by surgery. The samples were then frozen at $-40°C$ until needed for analysis.

RESULTS

The samples were removed from the freezer to reach room temperature before analysis and were then carefully dissected free of any fat or extra-

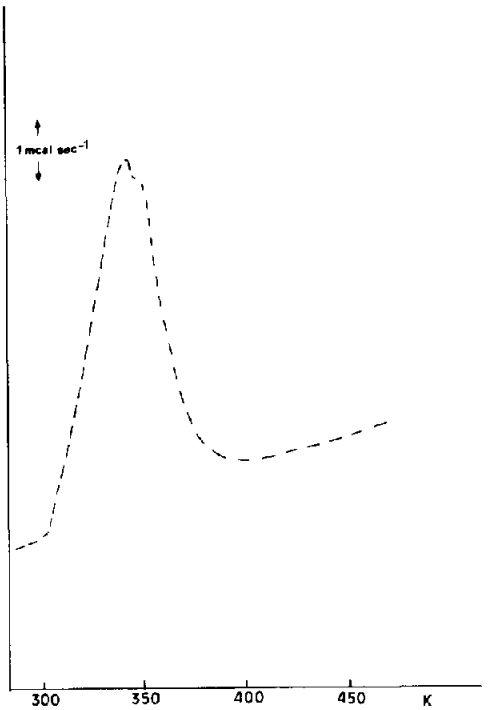

Fig. 1. Patient No. 1, pathological stage V. DSC curve of the pathological tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}; 1st max/2nd max = 1.06.

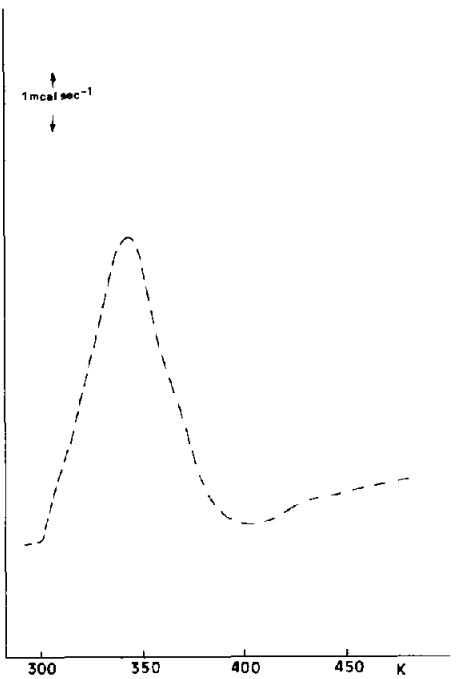

Fig. 2. Patient No. 1. DSC curve of the healthy tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}.

neous tissue. The samples obtained were then divided into three parts, which were analysed separately to check the reliability of the data. The samples were then weighed and analysed by DSC in a dry nitrogen atmosphere, to avoid oxidation phenomena, using a heating rate of 10°C min^{-1}, always analysing in sequence a pathological tissue and the corresponding healthy tissue.

Figure 1 shows the DSC curve of a pathological tissue. The process concerning the water loss occurs through two overlapped endothermic peaks, showing the presence of two different kinds of water in the tissue, whose maxima are at 344 and 350 K, respectively.

Figure 2 gives the DSC curve of the corresponding healthy tissue. The water loss is represented by only one peak with a maximum at 344 K indicative of the presence of only one kind of water similar to that corresponding to the first maximum in the pathological tissue.

Moreover, comparing the areas subtended by the two curves corresponding to the same sample mass (Figs. 1 and 2), it is possible to note that the first is larger than the second, i.e., the water content in the pathological tissue is higher than that in the healthy tissue.

Figures 3 and 4 show the DSC curves corresponding to the pathological and the healthy tissue, respectively, of a second pátient. Samples of a

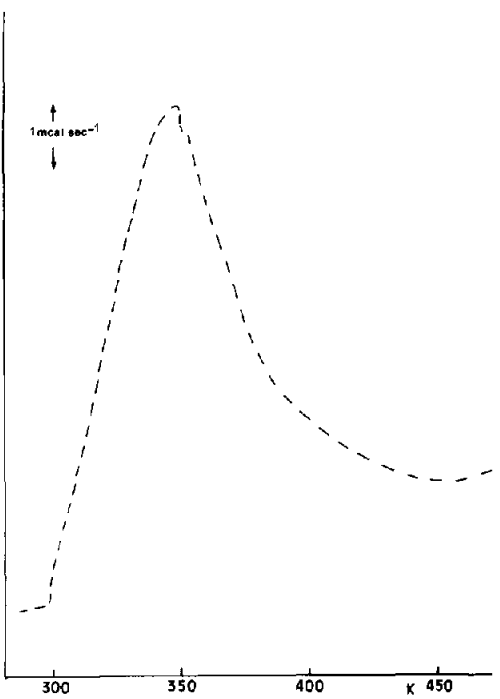

Fig. 3. Patient No. 2, pathological stage V. DSC curve of the pathological tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}; 1st max/2nd max = 1.06.

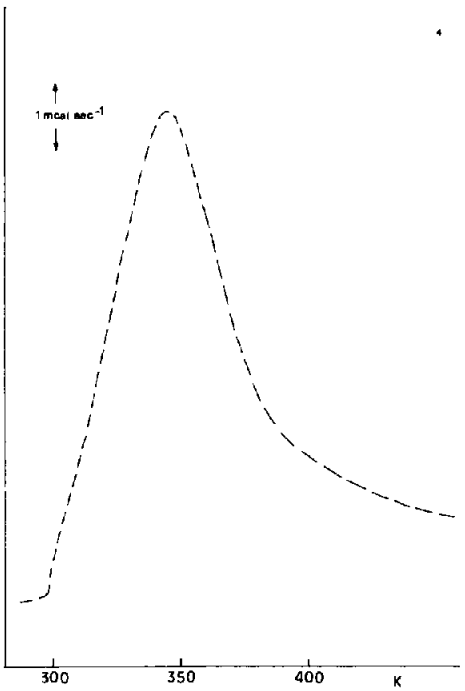

Fig. 4. Patient No. 2. DSC curve of the healthy tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}.

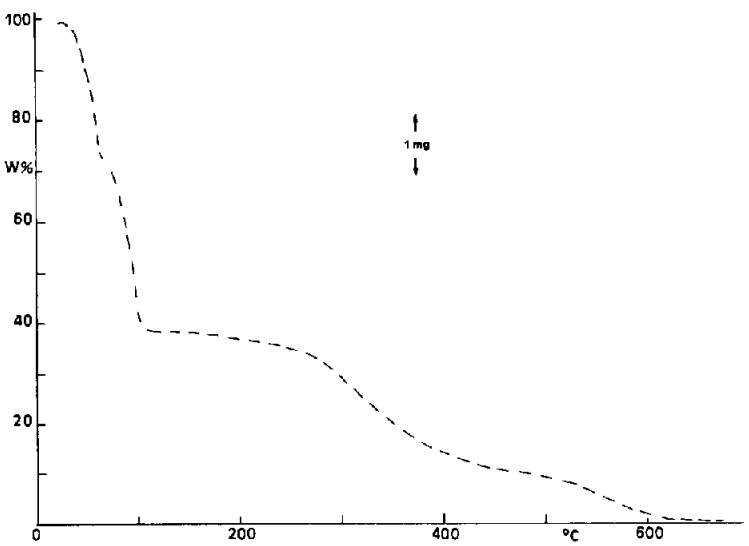

Fig. 5. Patient No. 1. TG curve of the pathological tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}.

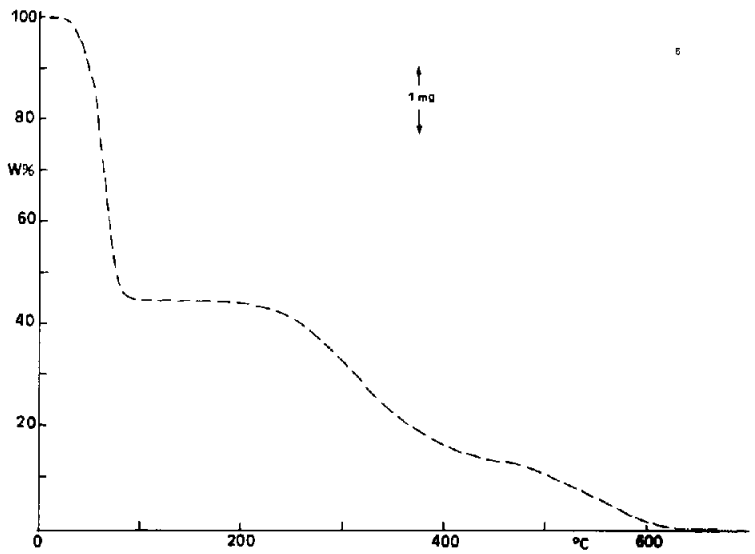

Fig. 6. Patient No. 1. TG curve of the healthy tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}.

number of different patients were thus analysed, and the same behaviour was always obtained.

The only difference was that the peak maxima localisation was ranged in a temperature arc of 10 K, holding inside each couple of samples of pathological–healthy tissue, however, a close correspondence between the first maximum of the pathological and the single maximum of the healthy tissue, while the second maximum of the pathological tissue always lies at a temperature higher by 5–6 K.

At the same time Figs. 5 and 6 were recorded by thermogravimetric analysis, which show that the water content in the pathological tissue is 61–62%, while that in the healthy tissue is 53–54%.

DISCUSSION

The differences between pathological and healthy tissue are:

(1) the presence in the pathological tissue of a new type of more strongly bound water;

(2) the increase in the overall water content.

However, it is interesting to note that the total water increases in the pathological tissue, but the water of the first type, corresponding to that of the healthy tissue, decreases.

The phenomenon could be discussed in terms of:

(1) increase in the number of cross-links with a consequent strengthening of the lattice and higher possibilities for the water molecules to be retained inside the tissue;

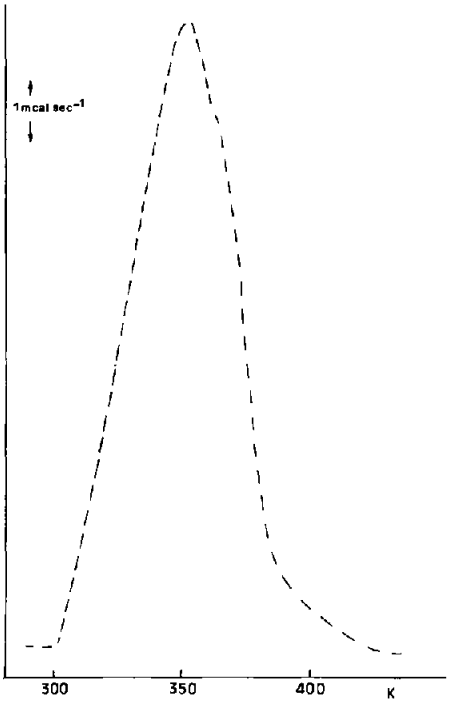

Fig. 7. Patient No. 3, pathological stage IV. DSC curve of the pathological tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}; 1st max/2nd max = 1.16.

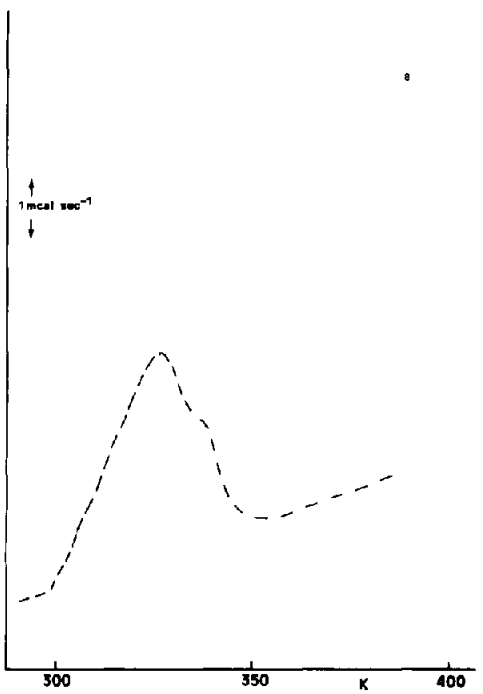

Fig. 8. Patient No. 4, pathological stage II. DSC curve of the pathological tissue. Atmosphere, dry nitrogen; heating rate, 10°C min^{-1}; 1st max/2nd max = 1.73.

(2) increase in the number of hydroxylic groups on the aminoacidic systems with a consequent increase and stabilization of the water. Finally the increase in the water of the second type and the decrease in that of first type are functions of the progressive development of the lesion, so that from the diagnostic point of view the evaluation of the development of the pathology could be made using the ratio of the heights of the two maxima of the pathological tissue (1st max/2nd max) as a diagnostic parameter.

Analyses carried out over more than 20 samples at different pathological development have shown a good correlation between the development of the lesion and the decrease in the ratio of the heights, 1st max/2nd max.

Figures 7 and 8 show two examples. Studies are in progress to analyse a sufficient number of samples useful for a statistical treatment of the data.

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