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THE APPLICATION OF THERMOANALYTICAL METHODS IN THE INVESTIGATION OF BIOLOGICAL SUBSTANCES*

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The equilibria and thermodynamics of a number of naturally occuring isolated compounds (e.g. proteins, amino acids, carbohydrates) have recently been studied in several laboraties in different temperature ranges, and thermoanalytical methods have been used to study structural changes of biological materials, among them human tissues.

In our investigations we succeeded in appling the derivatograph for the assay of glycosaminoglycans and for the characterization of the stability of crosslinked proteins in intact human and animal tissues. By means of this method age-related and pathological changes and repair reactions were studied in various connective and vascular tissues.

Other temperature-dependent techniques (DSC, polarizing microscopy) were used successfully in another series of experiments. Alterations in the characteristic order-disorder transition temperatures of human serum lipoproteins could be demonstrated in pathological conditions; the altered physical structure of lipoproteins might give an additional explanation to the assumed mechanism of different metabolic disorders.

In the past few decades the application of thermoanalytical methods has been extended to the investigation of several types of naturally occurring compounds of biological origin. Table allows a view into the great variety of analyzed samples, the characterized or measured properties and the thermal methods used in the studies.

In our laboratories, in addition to morphological, chemical and biochemical methods, thermal analysis has successfully been introduced into the research work. We succeeded in using the derivatograph for the investigation of mammalian connective tissues and DSC for studying serum lipoproteins.

Age-related and pathological changes in connective tissues

Connective tissue is continuous throughout the body and in all its manifestations appears as a mixture of semifluid gel, the ground substance, in which are bathed fibrous and cellular elements varying in size, shape and relative numbers from site to site. The ground substance is composed mainly of proteoglycans. Proteoglycans are macromolecules consisting of glycosaminoglycan (GAG) chains at-

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Table 1
Thermal investigation of biological samples

Origin of the sample	Characterized or measured property	Applied method
	1. Proteins	
Egg, milk, wool, serum, connective tissues of different organs	hydration, helix-coil transition, folding, assembly, stabilization, denaturation, catalysis, influence of physical or chemical modifications	DTA, DSC
	kinetic parameters of decomposition	TG, DTG
	analysis of degradation products	TG + evolved gas analysis, isothermal mass change
	structural stability	derivatograph and IR spectrometry
fibrillar proteins of connective tissues	hydrothermal shrinkage	DTA, DSC
	fibrillogenesis	DTA and spectrometry
	surface adsorbance	DSC
	2. Carbohydrates, polysaccharides	
tobacco leaf, bacterial and invertebrate polysaccharides, cottonwood	degradation products, cleavage mechanism	isothermal mass change, analysis of pyrolysis products, derivatograph
potato	gelatinization of starch	DTA
mammalian tissues	qual. and quant. analysis	derivatograph and IR spectrometry
	3. *Nucleic acids*	
	melting, denaturation	DSC
	4. Lipids	
myelin of brain	*lipid transitions*	DSC
membranes of microorganisms and mammalian cells	phospholipid and cholesterol transitions	DSC and X-ray scattering
animal fat	behaviour during processing	derivatograph
adrenocortex, blood, vascular tissue	phase transitions	DSC and X-ray scattering and polarizing microscopy
	5. Structural water	
membranes	hydration-dehydration	DTA
connective tissues of different organs	quant. analysis	derivatograph
	6. Inorganic components	
bones, dental scales, dental calculi, urinary calculi	composition	derivatograph and thermometry and X-ray diffraction

tached of a protein core. The main fibrous elements of connective tissue are collagen and elastin fibres (Fig. 1).

The advantage of the application of thermal methods for the investigation of connective tissues was that the basic molecular structure of the material could be preserved, as no previous preparation procedures were needed. Typical thermoanalytical curves of connective tissue samples are shown in Fig. 2. The developed method enabled us to determine the structural water content and the GAG content and GAG composition of the ground substance [1] and to characterize the structural stability of the fibrous proteins [2] within the connective tissue of the same sample of biological material.

In an attempt to arrive at biologically interesting conclusions, we studied the changes in the extracellular components of various connective tissues with time

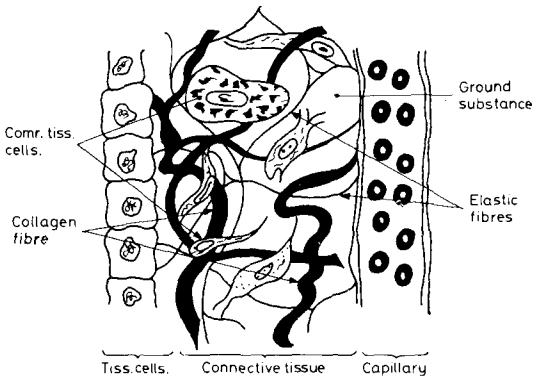

Fig. 1. The structure of connective tissues

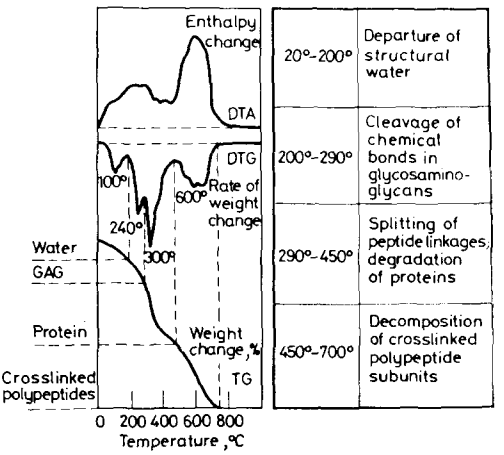

Fig. 2. Typical thermoanalytical curves of connective tissue samples

and continued use, as well within experimentally induced and human pathological conditions.

The structurally bound water content and the concentration of GAG-s were found to decrease significantly with age in various types of connective tissues, and the GAG composition gradually altered as well. A correlation could be demonstrated between the accumulation of covalent cross-linkages in the ageing structural proteins and the thermostability of the tissue samples [3, 4].

The maturation of newly synthesized fibrillar proteins can be inhibited by the administration of certain compounds, called lathyrogens, to experimental animals. In our experiments the synthesis of defective collagen and elastin in the connective tissues of growing animals receiving lathyrogens could be demonstrated by means of thermal analysis [5].

Similarly to all the other tissues of the organism, the connective tissues show considerable changes as the body passes through to senility. In addition a variety of different pathological conditions can result in the involvement of one or other component of connective tissue. Of the changes which may be considered as being of this type, we studied structural alterations taking place in the two most frequently occurring pathological conditions of connective tissue: osteoarthrosis of the joints and atherosclerosis attacking vascular tissues.

In an examination of the early osteoarthrotic degenerative alterations of articular cartilage and the subsequent reparative processes, progressive changes were induced in the cartilage of the knee joints of rabbits in a series of in vivo experiments. Independently of the type of the model experiment, the action of the damaging agent resulted in a nonspecific reaction consisting of degradation and loss of the proteoglycans in the first stage. There was a difference in the repair capacity of the young and the aged animals: in young rabbits repair of the ground substance took place up to normal level, while in aged animals the synthesizing activity was not sufficient to cope with the loss of the ground substance and as a result significant structural degradation could be observed in the "denuded" fibrous proteins [6–8]. It is of interest that the changes measured in human knee joints following meniscus unjury resembled those found in the animal experiments [8, 9]. Furthermore, this method could be used to study the effect of drugs at the tissue level [10].

In the pathogenetic process leading to the manifestation of atherosclerosis the arterial wall shows a series of discontinuously distributed changes in its ground substance and fibres, associated with lipid deposition. There is now overwhelming evidence to suggest that the lipid originates from the plasma in the form of low-density lipoprotein (LDL) and that changes in the vascular ground substance components are of crucial importance in the trapping mechanism. In our previous studies [11–14] we could prove that GAG-s of the aortic wall have the ability to form specific complexes with serum LDL-s and we considered the formation of these complexes as the beginning of the process of lipid deposition in the arteries. With the aid of the derivatograph we succeeded in demonstrating the existence of GAG-LDL complexes within the atherosclerotic aortas [15].

Structural studies on serum lipoproteins

It is known that LDL is a spherical particle: the polar constituents of the molecule are located at the surface, whereas cholesterol esters and triglycerides are located in the central lipid core (Fig. 3). Cholesterol esters may exist in a completely disordered liquid state or in the form of smectic liquid crystals, and it has been reported that an order-disorder liquid crytalline phase change of cholesterol esters within the LDL particle also occurs [16]. Smectic crystals have been identified in the atherosclerotic aorta as well, but nothing was reported on the possible underly-

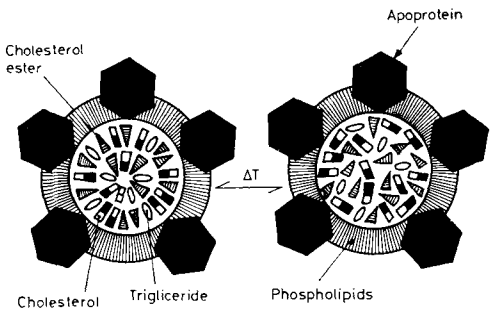

Fig. 3. The structure of the LDL molecule

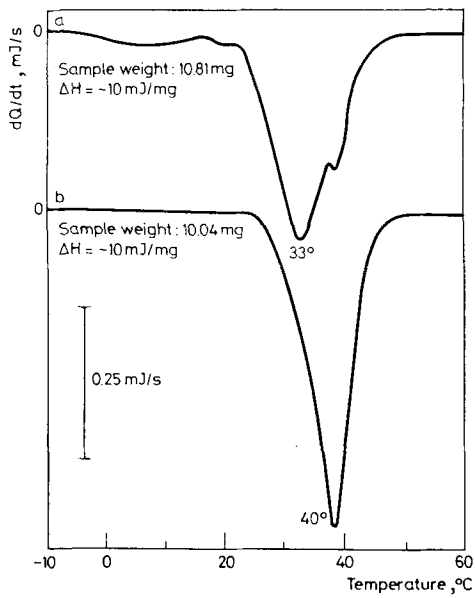

Fig. 4. Typical DSC curves of human serum LDL (a) and of aortic — GAG — LDL complex (b) Heating rate: 5 degree · min^{-1}

ing mechanism. In our experiments the relationship between the structure of the LDL core and the complex formation between LDL and GAG-s have been studied by means of DSC [17]. In the LDL molecule a reversible endothermic transition took place with its peak at 33°. Cholesteryl esters within the LDL core existed as an isotropic solution above this temperature (i.e. around body temperature), and in the form of smectic liquid crystals below it (Fig. 4. a). When LDL was converted into GAG-LDL complexes, the DSC curve showed an elevation of the transition temperature: the peak value was found at 40° and under the polarizing microscope birefringence was observed, typical of smectic liquid crystals (Fig. 4. b). This phenomenon may occur in biological system as well. It is possible that certain GAG-s of the serum or of the arterial wall enhance the liquid crystalline phase transition within the core of the LDL molecule. The altered physical structure of LDL might initiate a sequence of events with further metabolic consequences (e.g. decreased mobility, altered rheological properties, increased resistance to enzymes, deposition in the arterial wall, etc.).

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ZUSAMMENFASSUNG — Die Gleichgewichte und die Thermodynamik einer Reihe von natürlich vorkommenden isolierten Verbindungen (z. B. Proteine, Aminosäuren, Kohlenhydrate) wurden in verschiedenen Laboratorien in verschiedenen Temperaturbereichen untersucht. Hierbei wurden thermoanalytische Methoden zum Studium der Strukturänderungen von biologischem Material, u. a. von menschlichem Gewebe, eingesetzt.

In unseren Untersuchungen gelang es den Derivatographen zur Prüfung von Glycosaminoglycanen und zur Charakterisierung der Stabilität quervernetzter Proteine in intakten menschlichen und tierischen Geweben einzusetzen. Durch diese Methode wurden altersbedingte und

pathologische Änderungen sowie Reaktionen zur Wiederherstellung in verschiedenen Binde- und Vasculärgeweben studiert.

Andere temperaturabhängige Techniken (DSC, Polarisationsmikroskopie) wurden in anderen Versuchsserien mit Erfolg eingesetzt.

Резюме — Недавно в нескольких лабораториях были изучены в различных областях температуры равновесие и термодинамические свойства природных соединений, напр. протеинов, аминокислот, углеводов, а термоаналитические методы были использованы для изучения структурных изменений биологических веществ, в том числе и человеческих тканей. В представленной работе авторы применили дериватограф для анализа гликоаминогликанов и для определения устойчивости сетчатых протеинов в неповрежденных человеческих и животных тканях. Посредством этого метода были изучены старение и патологические изменения, реакции восстановления в различных соединительных и сосудистых тканях. В других сериях экспериментов были использованы ДСК и поляризационная микроскопия.