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Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Humphries, W. T. and Wildnauer, R. H.(1974) 'Characterization of Aging Skin via Thermal Analysis', Journal of Macromolecular Science, Part A, 8: 1, 65 — 72
To link to this Article: DOI: 10.1080/00222337408065814
URL: http://dx.doi.org/10.1080/00222337408065814

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J. MACROMOL. SCI.—CHEM., A8(1), pp. 65-72 (1974)

Characterization of Aging Skin via Thermal Analysis

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ABSTRACT

Samples of full-thickness rat skin from birth to 3 years were characterized by thermal analysis. The techniques involve differential scanning calorimetry, thermomechanical analysis, and thermogravimetry.

In the dry state the skin samples exhibit thermally induced transverse dimensional changes at 33, 130, 192, and 250°C. The 130 and 192°C expansions are complimented by enthalpy changes. As the animal ages, the degree of the individual expansion decreases as the energy associated with the transition increases. This observation is consistent with the theory of increased cross-linking with age. The relationship observed in the dry state is also present in hydrated samples.

Thermogravimetric results indicate an initial weight loss up to 100°C, which is due to dehydration, followed by a major weight loss between 250 and 450°C. The decomposition products in the higher temperature range also varied with age. Work with recast collagen films and elastin aid in the interpretation of the thermal transitions observed in the native rat skin.

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INTRODUCTION

The major biophysical properties of mammalian skin are mainly determined by its dermal layer. This connective tissue is a complex fibrous network held together by collagenous and elastin-type fibers. These fibers have been shown to undergo distinct thermally induced structural transitions (e.g., shrinkage phenomena of collagen) and have been the subject of numerous investigations over the past decades [1]. With the aid of x-ray diffraction [2], this shrinkage process in collagen has been assigned to a phase transition involving the conversion of the crystalline helical collagen structure to an amorphous random coil form.

The objective of the present work is twofold: 1) characterization of this biopolymeric system by thermal analytical techniques, and 2) to determine if these thermal characterizations are a function of the age of the animal.

The high degree of organization at both the morphological and molecular level suggests that the dermis should be amenable to thermal techniques commonly used to characterize polymeric systems. A similar approach has been taken to better understand the structure property relationships of other biorelated tissues such as hair and stratum corneum [3-5].

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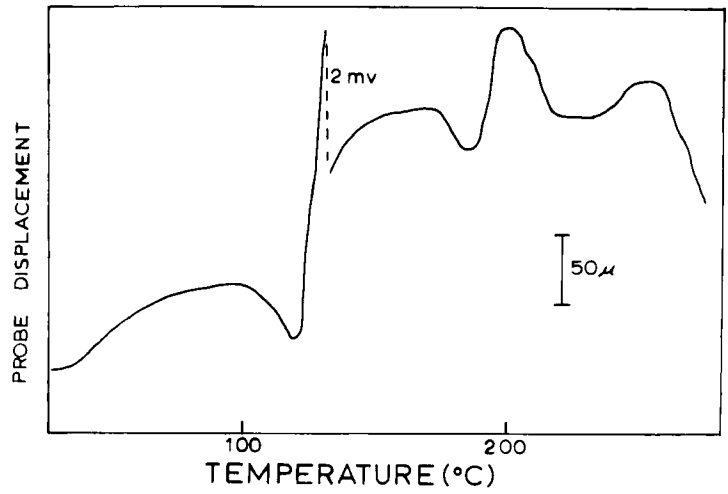

FIG. 1. Thermomechanical analysis of 10-day old rat skin.

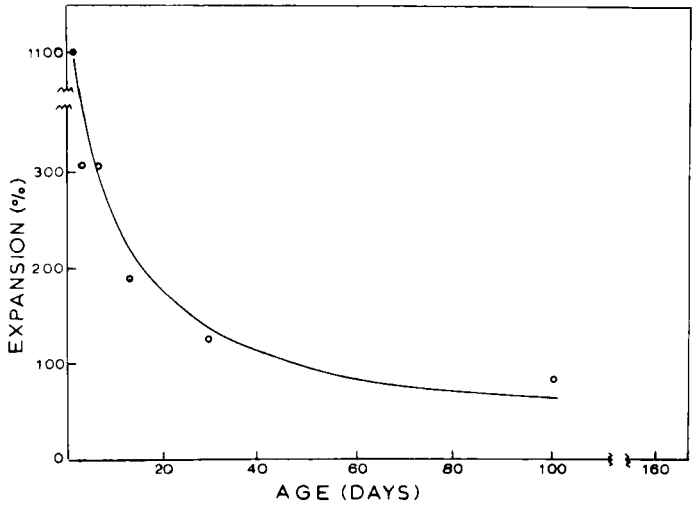

FIG. 2. Transverse expansion at 190°C vs age for dry material.

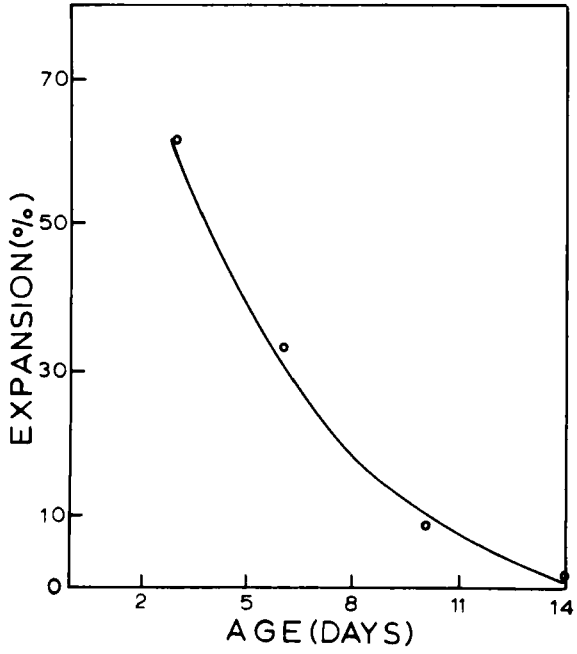

FIG. 3. Degree of 33°C transition vs age.

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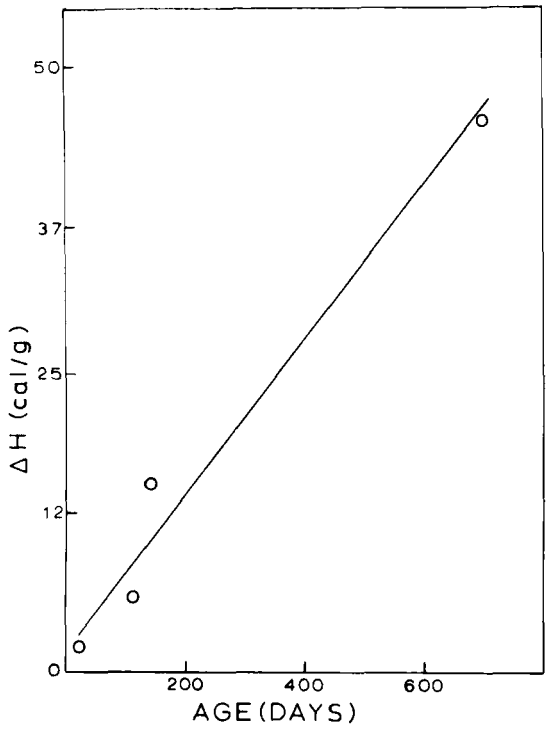

FIG. 4. Relationship between ΔH and age for dry samples.

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MATERIALS AND METHODS

The skin samples were obtained from a controlled colony of Charles River CD strain rats. Animals were sacrificed at various times from birth up to ages approaching their normal life span (2 to 3 years). The hair from the skin was removed mechanically without the aid of a depilatory agent. The samples were stored in a 0.9% by weight NaCl solution at 35°F or dried to room temperature prior to examination. Storage in the NaCl solution for periods of up to 5 days did not alter the results. The recast collagen films were prepared from a ficin digested Bovine tendon.

The thermal techniques involved the use of a Du Pont 990 thermal analysis system equipped with a differential scanning calorimeter cell as well as the Du Pont 951 Thermogravimetric Analyzer. The thermomechanical analysis was performed with a Perkin-Elmer Model TMS-1

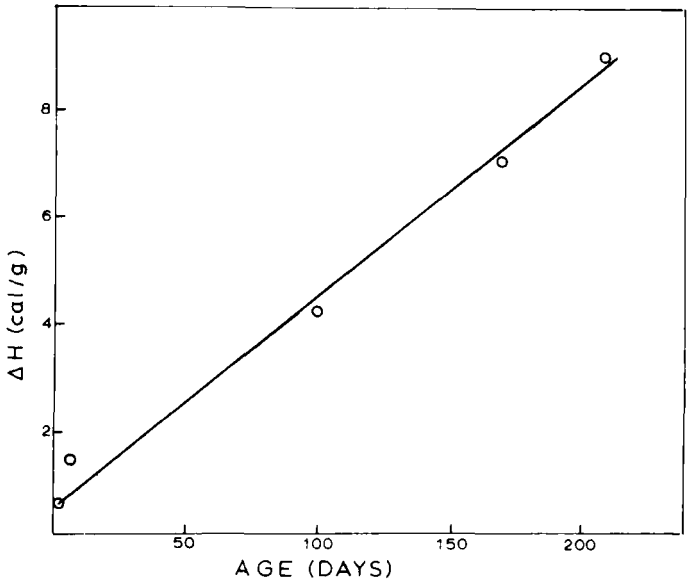

FIG. 5. Agreement between ΔH and age for wet samples.

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Thermomechanical Analyzer. The TMA and DSC evaluations were performed in a helium atmosphere while the TG was in nitrogen.

In the TMA, the expansion mode was utilized with a zero applied force. The magnitude of the transverse expansion was calculated based on the initial sample thickness.

EXPERIMENTAL RESULTS

In the thermomechanical analysis, the dried skin samples, exhibited four thermally induced transverse dimensional changes: 33, 130, 192, and 250°C. A TMA thermogram for a 10-day old rat is shown in Fig. 1. In this case the total degree of expansion at 250°C is 300% of the sample's original thickness. In previous work with a recast collagen film, only the 190°C transition was observed in the dry state. If the recast collagen film was exposed to a diluent such as silicone oil, the 190°C transition was reduced to 137°C. This lowering of melting temperature of collagen by diluents was observed by Flory [6]. By altering the diluent concentration, Flory was able to extrapolate to the melting point of the dry material.

These two collagen transitions (130 and 190°C) are both present in the rat skin. The magnitude of the total expansion of both transitions does decrease considerably with the age of the animal. The relationship between the expansion and the age is shown in Fig. 2.

The transition observed at 33°C was absent in the recast collagen film and may be a result of some other component (e.g., lipids) or a low molecular weight fraction of the collagen which is not present in the recast film. The relationship between age and the degree of this 33°C transition is shown in Fig. 3. This initial transition decreased more rapidly than the 190°C transition and was completely absent by the twentieth day. A pellet prepared from isolated elastin extracted from ligamentum nuchae exhibited only one thermally induced transverse expansion at 250°C. This transition temperature corresponds to the high temperature expansion observed in the rat skin.

Samples of fresh rat dermis examined in 0.9% NaCl solution exhibited only one transverse expansion at 70°C. This temperature corresponds to the normally reported shrink temperature of

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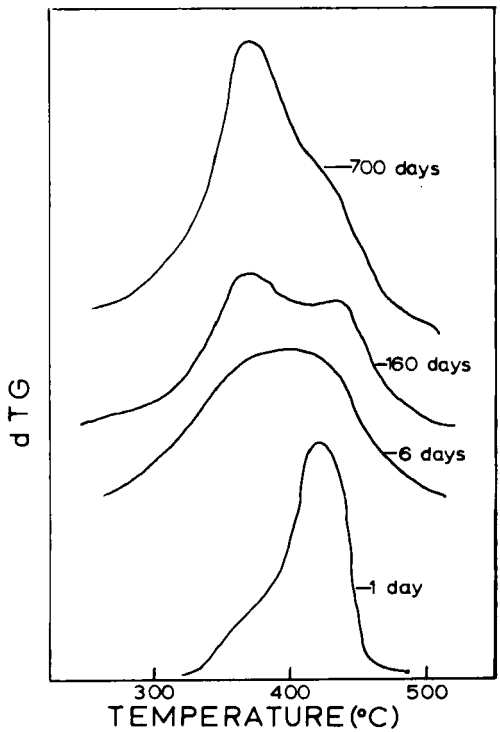

FIG. 6. dTG as a function of temperature for dry samples.

collagen. The magnitude of the expansion decreased with age but not as significantly as the dried material. The rat dermal samples contained equilivate amounts of water as determined by drying the samples to room conditions followed by thermogravimetric analysis.

In the differential scanning calorimeter, the dried skin samples exhibited a broad endotherm with a minimum at 70°C, which is probably the loss of water, followed by sharper endotherms at 130 and 180°C. The high-temperature endotherms agree with the dimensional changes observed in the thermomechanical analysis. The ΔH associated with the 130 and 180°C endotherms increased dramatically with age. The agreement between ΔH and age of the animal is shown in Fig. 4. The relationship between ΔH and age is considerably more linear than the TMA data. As in the TMA, the recast collagen film only exhibited the 190°C transition.

The animal samples stored in 0.9% NaCl solution were run in hermetically sealed pans to prevent the loss of solvent. The DSC thermogram showed two distinct endotherms with minimums at 62 and 75°C. As with the dry sample, the total ΔH observed increased with age. The ΔH as a function of age is shown in Fig. 5.

The thermogravimetric analysis on the dried animal skin exhibited a gradual weight loss from 25 to 100°C representing the loss of water (~10%) and a more substantial loss between 250 and 450°C. A plot of the differential of the weight loss vs temperature between 250 and 450°C did expose some variation with age. The derivative thermogravimetric (dTG) curves are shown in Fig. 6. The younger samples contained only a higher temperature component. As the animal aged, a second component at a lower decomposition temperature appeared. A further analysis of the evolved gas is needed to distinguish between these components.

CONCLUSION

Modern thermal analytical techniques are useful in the study of the structure property relationship of these biopolymeric systems. The techniques can accommodate materials in the dry or wet state as well as only requiring very small samples, which is usually a major problem in examining these biopolymers.

This work is highly descriptive but does illustrate that thermal analysis is a tool which reveals changes in macromolecular structure with age. The reduction in the degree of expansion in the TMA and the increase in the ΔH associated with the transitions are consistent with the theory of increased cross-linking with age. Since the dermal system is a complex mixture, further biochemical work in connection with these thermal techniques is necessary to establish the macromolecular events occurring during the observed transitions and what type of cross-links, if any, are changing with age.

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REFERENCES

[1] A. Veis, Treatise on Collagen (G. N. Ramachandran, ed.), Academic, London, 1967.
[2] D. Puett, A. Ciferni, and L. V. Rajagh, Biopolymers, 3, 439 (1965).
[3] W. T. Humphries and R. H. Wildnauer, J. Invest. Dermatol., 57, 32-37 (July 1971).
[4] W. T. Humphries and R. H. Wildnauer, Ibid., 58, 9-13 (January 1972).
[5] W. T. Humphries, D. L. Miller, and R. H. Wildnauer, J. Soc. Cosmet. Chem., 23, 359-370 (May 1970).
[6] P. J. Flory and R. R. Garrett, J. Amer. Chem. Soc., 80, 4836 (1958).

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