

COLLAGEN, HEXOSAMINE AND TENSILE STRENGTH OF RABBIT SKIN DURING AGING*

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The process of aging is accompanied by changes in the chemistry of connective tissue. As a general rule collagen seems to accumulate with increasing age. Analysis of this material on the basis of its solubility indicates that what is being accumulated is insoluble fibrous material, whereas the soluble collagens, present in significant amounts in young skin, drop to low values in older age. These observations have been reviewed in detail by Harkness (1).

We have recently reported striking quantitative and qualitative changes in the collagen content of rabbit skin at different stages of the animal's development (2). Similar findings were observed in growing rats (3). Dunphy and Udupa found a very good correlation between the gain of wound tensile strength and the increase of local concentration of collagen (4). The especially large change in insoluble collagen which occurs between birth and adulthood suggested the possibility that the skin of these animals would show corresponding changes, possibly of a physical nature, which could adequately correlate with this observation. In addition, it appeared to be of interest to measure the amount of hexosamine present, at these different time intervals, as an index of mucopolysaccharide and glycoprotein materials. It has been shown that during aging the hexosamine/collagen ratio drops in tissues (5).

METHODS

For the purpose of these experiments albino rabbits of different ages were used. The skin was shaved, and after killing the animals, cleaned from the underlying connective, muscular and adipose tissues. The salt-soluble collagen was obtained by extracting the skin with 0.5 M NaCl in the cold. The residue was converted to gelatin

by autoclaving. Aliquots from both extractions were dialyzed, acid hydrolyzed, and analyzed for hydroxyproline as previously described (6). Hexosamine was determined in an acid extract of a skin aliquot by the method of Rondle and Morgan (7). Tensile strength was measured using an Instron Push-Pull testing machine. Strips of skin, 5 mm wide, were pulled at a rate of 5.1 cm/minute and the force required to rupture the specimen automatically recorded.

RESULTS

Figure 1 shows the changes in 0.5 M NaCl soluble collagen and of total hexosamine in the skin of rabbits of different ages. A sharp rise in hexosamine content occurred between the 2nd and 4th week, at which time its concentration reached its maximum. From there on hexosamine content in skin began to fall gradually. The salt-soluble collagen followed a similar pattern of events, but its maximum concentration in skin was reached 8 weeks after birth. It is to be noted that this period of rapid synthesis of both hexosamine as well as soluble collagen is preceded by very low values. These low values appear to be characteristic of very young animals. In the course of performing the biophysical measurement, namely tensile strength, we observed, confirming the findings of other investigators (8), that the deviations between measurements were quite large. We found that littermates showed much closer agreement among each other than did animals of the same age, but otherwise unrelated. Also the observed tensile strength differed significantly depending on both the direction and the area from which the specimens were cut. In our experiments as well as the ones previously noted, the long axis of the animals vertebral column was used as a guide.

Figure 2 shows the changes in the insoluble collagen present in the skin of these same animals, as well as its tensile strength. The total amount of insoluble collagen increased throughout the period of observation, but showed the greatest rate of increment between birth and the 6th week of life. The tensile strength of the

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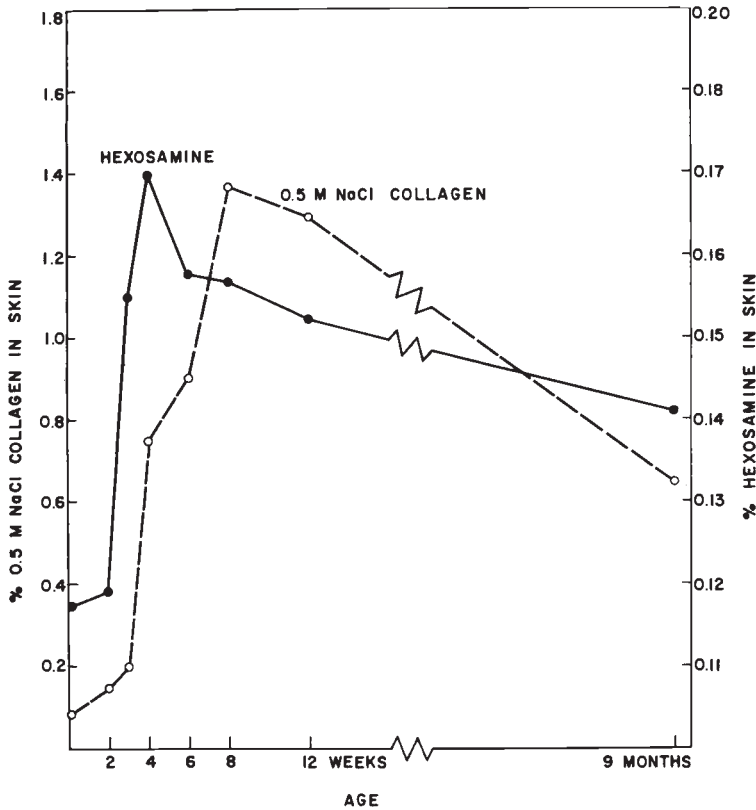


FIG. 1. 0.5 M NaCl extractable collagen and total hexosamine content of rabbit skin of different ages.

individual skin specimens paralleled very closely their content of insoluble collagen.

DISCUSSION

It is evident that during the early stages of post-natal growth there is a very rapid accumulation of hexosamine. This increase in hexosamine concentration is followed within the next few weeks by a rise in the collagenous material that can be extracted with 0.5 M NaCl. This collagen fraction contains the newly synthesized material which should subsequently aggregate to form insoluble fibers. All these changes occur during the period of maximal rate of growth of the animal. After the 8th week the concentration of soluble collagen begins to decline, presumably correlating with the decreased rate of collagen synthesis as well as to the decreased growth rate of the animal (9).

In the rat, the rate of conversion of the soluble collagen material into collagen fibers is independent of age. On the other hand, the rate

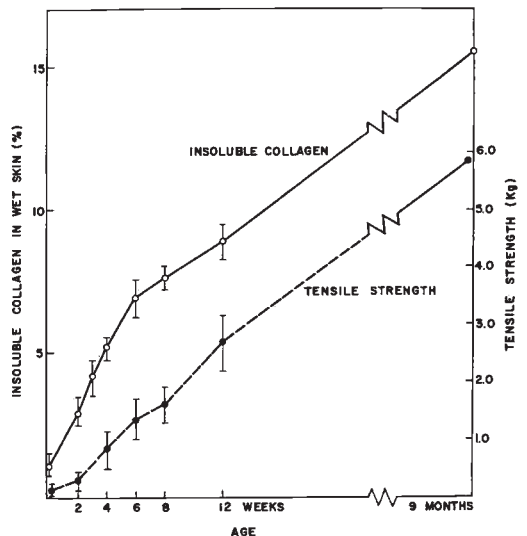


FIG. 2. Insoluble collagen and tensile strength measured in the skin of rabbits at different stages of development. Bars represent the standard error of the determinations performed on each group of animals.

of turnover of insoluble collagen is strictly age-dependent. During periods of rapid growth the turnover time ($T_{1/2}$) was 28 days whereas as the animal ages it reached 300 days (6). This progressive insolubilization of collagen may explain, at least in part, the progressive accumulation of collagen occurring in spite of a decreased rate of synthesis. With advancing age, collagen becomes tougher, more crystalline, and more difficult to dissolve.

The tensile strength of the skin, as measured in these experiments, parallels the increase in insoluble collagen. There is an indication, however, that during the period of rapid growth the rate of increase of insoluble collagen proceeds at a slightly faster pace than the increase in tensile strength. It is generally believed that aging is accompanied by increased hydrogen bonding and cross-linking resulting in further insolubilization of collagen.

SUMMARY

The hexosamine content of skin has been shown to rise very rapidly after birth and reach a maximum concentration in the skin of the 4 week old rabbit. The soluble collagen extractable by 0.5 M NaCl showed a similar rise but reached its maximum at the age of 8 weeks. Both values began to decline as the animals aged. The insoluble collagen in skin rose continuously over the experimental period (9 months). Measurement of skin tensile strength revealed a

very marked correlation with age. The force required to rupture a strip of skin from a newborn rabbit was of the magnitude of 150 gm and increased to almost 6.0 Kg in the adult animal. The changes in tensile strength show good agreement with the amount of insoluble collagen present in skin.

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