Aging of connective tissue is of special interest, not only because connective tissue is a constituent of almost every organ but also because a number of diseases of old age involve the connective tissue and its main component, collagen. Since collagen comprises 72 to 79 per cent by weight of the human dermis, this tissue holds great promise for studies designed to reveal changes that occur with age.

Within the past few years it has become fairly well established that with advancing age collagen becomes increasingly stable. This is demonstrated by the findings that with aging collagen becomes less soluble (1–5), exhibits less capacity for swelling (1, 6), acquires more resistance to digestion by collagenase (7), shows less base-binding (4) but a higher calcium-binding capacity (8), shows a lower ratio of hexosamine to collagen (2, 9, 10), and shows a higher hydroxyproline content (11) but a lower content of extractable hydroxyproline (12–15). Also urinary hydroxyproline decreases in amount with age (16). The physiochemical causes of these changes are not precisely known, although most investigators agree that the increased stability results from a change in the number and type of cohesive bonds.

One property of collagen which has recently created some medical interest is that of hydrothermal shrinkage. This property relates gross tissue changes to events at the molecular structural level, which result from a phase transition or “melting” of the ordered crystalline structure of the collagen molecule to the preferred random coiled state (17, 18). Some investigators think that it is analogous to the process which goes on very slowly during the whole life of the collagen fiber (4, 8). A study of this property can be expected to throw considerable light on the aging process, although up-to-date results of studies using shrinkage temperature have been somewhat conflicting (11, 14, 19–23). Recent methods have been devised, however, which give new insight into the shrinkage phenomenon; the most significant of these is a method for the study of isometric contraction, a more sensitive indicator of tissue change (14).

Using a new technic suitable for the study of specimens of human skin (24), we studied normal human dermal collagen and the changes occurring with age in an attempt to provide an index to changes in collagen, which are not easily detected by other means.

**METHOD**

Samples of normal skin were taken from 124 individuals ranging in age from 32 weeks of gestation to 88 years. No selection of cases was made other than to eliminate from the study any skin involved in a disease process. Tissue was obtained by means of surgical excision and from necropsies performed soon after death before any preservatives were used. All samples were removed so that the long axis of the specimen was parallel to the natural body skin creases. The epidermis and adipose tissue were mechanically removed, and the dermal samples were cut into uniform strips measuring 2 by 15 mm. No attempt was made to alter the natural thickness of the dermis; instead the dermis was accurately measured on two different occasions so that in the final analysis tension could be recorded in grams per millimeter of dermal thickness.

For each experiment two dermal strips were used, one being attached to the arm of a kymograph and the other to a strain gauge. By means of a shrinkage technic previously described (24), the shrinkage temperature, the amount of contraction, and the tension—both the total and in relation to the shrinkage temperature—could be measured and recorded simultaneously (fig. 1), as the temperature of a waterbath was slowly raised from 50° to 90° C.
RESULTS

Shrinkage Temperature.—The shrinkage temperature, taken as the point where rapid shrinkage began, varied from 59.0° to 64.0° C. with a slight but definite increase with age (fig. 2). This increase, while demonstrating no clear-cut pattern, was a little more rapid in skin from young patients. There was a rise of about 2 degrees (60.0° to 62.0°) from birth to the early thirties as compared to a rise of less than 1 degree from the thirties to the late eighties. The total increase from birth to old age was not more than 2.5 to 3.0 degrees. No obvious age-related change in shrinkage temperature was found in the tissue from individuals less than 2 years of age (fig. 2).

Amount of Contraction.—The total contraction of normal collagen, as measured on the recording graph after being magnified 9.5 times by the kymograph arm, varied from 6.0 cm (42 per cent of 9.5 times initial length of specimen) to 11.7 cm (82 per cent) (fig. 3). From birth to old age the amount of contraction increased about 14 per cent; the average at birth was 8 cm (56 per cent) and that at old age, 10 cm (70 per cent). This rise seemed to follow a fairly straight line, although, considering the amount of normal variation found, it was difficult to make more than an approximation. The
The rate of tension development and the temperature at which the maximum tension was reached varied with the age of the individual (fig. 4). In individuals up to about 8 years of age, the maximum tension was reached before the temperature had reached 90° C. The tension in most of these cases dropped down after reaching a maximum, and samples from six of the nine patients 1 year of age or younger broke before reaching the 90° C. end point. The maximum tension for skin from individuals more than 8 years of age was not reached until 90° C. In general, the older the person the more rapidly the tension of his skin was rising at the 90° cut-off point. Undoubtedly, if the temperature could have been raised still higher, further differentiation with age by this method would have been possible.

A somewhat different age-related change was found when the maximum tension and the tension per unit of dermal thickness were compared. In figure 5 it can be seen that the maximum tension rose quite rapidly from 45 gm at birth to 150 gm by age 1½ to 2 years; subsequently, there was a steady rise to well over 200 gm. by the late eighties. The tension of the one sample from a premature infant was lower than any of the others; maximum tension measured only 24 gm. Samples from persons less than 2 years of age showed a fairly uniform age-related rise when plotted on a monthly scale (fig. 5). The somewhat higher results found in skin from individuals around age 50 probably resulted from the greater number of necropsy specimens of thick abdominal skin used for this age group.

When the maximum tension was corrected for dermal thickness a more definite pattern was found and a more meaningful curve produced (fig. 6). From 26 gm per millimeter at
birth, the average tension rose rapidly to reach a peak of about 67 gm per millimeter by the age of 1½ to 2 years after which it leveled off, with a suggestion of a dip during the teens, and then rose steadily to about 100 gm per millimeter in old age. The period of rapid rise from birth to age 2 when plotted on a monthly scale (fig. 6) showed a uniform increase with the 12 gm of tension developed by the 8-week premature infant fitting well into the beginning of the curve.

**COMMENT**

That collagen becomes increasingly stable with age has been well documented in the literature. This aging process is independent of the growth rate and reflects the biologic age of the individual (14). The present study adds to the evidence, suggesting an increase in the stability of collagen by demonstrating, with advancing age, a definite change in the hydrothermal shrinkage qualities of collagen from human skin. This is most accurately measured by isometric contraction which shows an increase in the amount of tension and in the rate of its development, but these findings also can be seen in a cruder way by the increase in the shrinkage temperature and in the amount of shrinkage. Both the isometric and isotonic measurements show the same type of age-related change.

The marked difference in sensitivity of the shrinkage-temperature and tension studies points out the inadequacy of shrinkage temperature if used alone to measure the stability of collagen. This undoubtedly accounts for much of the existing confusion in dealing with
the shrinkage temperature, and it has led Verzar (14) to make the statement that the shrinkage temperature does not change at all with age. He said that the previously unrecognized fact that young collagen develops a maximum tension at a lower temperature has led to an error in reporting this as a lower shrinkage temperature in the young tissue. From our studies, however, this would not seem to be the case since no correlation was found between the shrinkage temperature and the rate at which the tension developed. In fact, the tension if anything began to rise sooner in the older individuals even though it did not reach a maximum as soon. In addition, from the work of Gustavson (25) and of Rigby and co-workers (26), which showed that adding stress to the collagen fibers increases the shrinkage temperature, the small weights used in this experiment to keep the specimen in an extended position would be expected, if anything, to increase the shrinkage temperature of the young tissue more than that of the old.

Probably the most unique and significant finding from our study is the two-stage rise in collagen stability, best demonstrated by the tension curve when corrected for dermal thickness. This begins with a rapid increase from gestation to early childhood, continues with a leveling off from about the age of 2 years to the late twenties or early thirties, and ends with a slow rise beginning in the early thirties and continuing until death. Verzar (14), in a study of the tension developed in rat and frog tendons, did not find this variation in the rate of collagen increase; instead he found a roughly parallel rise in tension with aging of the animals. The fact that he did not show the initial rapid rise found here may have been because his youngest rat was 2 months old, an age which would roughly correspond to about 5 years of age in human beings.

Brown and Consden (19) and Brown and co-workers (27), on the other hand, found a 2-stage rise in the shrinkage temperature of human dura mater with the largest increases occurring from birth to adolescence and again after middle age. They were unable, however, to find any change in the shrinkage temperature of seven human fetuses from 12 to 40 weeks of gestation, a finding which led them to believe that the new collagen of the fetus has a certain amount of stability, when or shortly after it is laid down, which does not change appreciably until after birth. From our study this would seem to be most unlikely, since the collagen from our 32-week-old fetus was found to be less than half as stable as that of full-term infants. Also, Gross (28) has recently shown that the shrinkage temperature of reconstituted collagen fibers increases by 4° to 6° C. when aged for 1 year at 37° C. Considering that the newly formed collagen molecules in the fetus would be somewhat similar to the reconstituted fibrous gels used by Gross, a similar rise in fetal collagen stability would be expected.

Many attempts have been made to explain the physiochemical changes which occur in collagen with advancing age. Gross (28, 29), who worked primarily with collagen solubility and reconstituted collagen, attributes the aging process to the highly specific structure of the molecule alone. He thinks that, as the molecules become packed closer and closer together, the electrical forces between their surfaces rise sharply, creating secondary bonds which act like glue binding the surfaces together. Slow increases in the concentration of the intrafibrillar protein and in the mechanical restraints of the non-collagenous ground substance and of the circumferential fibril networks are thought to contribute. Along the same line, Banga and co-workers (30) believe that the aging of the fibers results from stabilization of linkages between procollagen and metacollagen, and Verzar (14) thinks that the changes result from the spontaneous accumulation of covalent and ester intermolecular bonds resulting from their random contact with body ions and substances such as mucopolysaccharides and mucoproteins.

Other investigators, however, think that the increased stability of collagen is best explained by a slow denaturation of the collagen molecules. This theory stems from the work of several investigators and has to do with the possible in vivo tanning of collagen. Gustavson (31) and others (8, 32–34) have shown that collagen denaturation can occur very slowly at physiologic temperatures, with a resultant increase in the hydrogen-bonding capacity as shown by increased tanning with chromium and vegetable tannins. This, combined with the work of Verzar (14), who found that the shrinkage temperature and tension production from thermal contraction increased when incubated with formaldehyde, and the work of Milch and Murray
(32) and of Milch and co-workers (35), and of others (6, 36), who found that collagen becomes more stable when incubated with glyceraldehyde and other aldehydes normally occurring in human metabolic pathways, has led to the conclusion that the increased stability of collagen is largely due to its slow denaturation with the resultant increase in hydrogen-bonding capacity and to its combination with in vivo tanning agents as glyceraldehyde.

The two distinctly different age-related changes found in this study suggest that both of these processes are involved in increasing the stability of collagen. The initial rapid rise, seen up to and through infancy, most likely results from a simple maturation of the collagen fibers with the newly synthesized soluble collagen molecules becoming tightly aggregated to form insoluble native fibers during this time. This is probably a function of the highly specific structure of the molecules, which results from the increasing perfection of fit between them as they gradually pack together in a "lock and key" type of association as described by Gross (29). Secondary electrical bonds and covalent and ester intermolecular linkages from the ground substance may contribute in binding these collagen molecules together. The collagen turnover studies of Lindstedt and Prockop (37) support this concept, since these authors have shown three different rates of collagen turnover in young rats, the more metabolically active of which disappears as aging occurs.

The slower rise in collagen stability, appearing in the late twenties and continuing for the life of the individual, could possibly be due to a continuation of the above maturation process, but more likely it is due to the slow denaturation of the collagen molecule, which provides new binding sites, and to the combination with in vivo tanning agents such as glyceraldehyde as proposed by Milch and Murray (32) and Milch and co-workers (35). This is supported by the work of Kohn and Rollerson (6) who found a marked decrease in the swelling properties of human diaphragm tendon from patients more than 30 years old, which they interpreted to indicate the formation of new cross links between polypeptide chains, possibly on the basis of slow irreversible thermal denaturation. It is also consistent with the turnover studies of Lindstedt and Prockop (37) who found, in addition to the three rates of collagen turnover in young tissue, a much more stable collagen in adults, with a half life approaching the life span of the animal.

Thus, it is possible that two seemingly opposed processes, one a process of maturation and the other a degenerative one, could account for the increase in collagen stability through increases and changes in the intramolecular and intermolecular bonding. Both probably occur simultaneously and independently for the life of the individual, varying, according to the age of the tissue, in the degree to which they contribute to the increased stability. It is unlikely that denaturation would account for much of the aging seen in the infant, since denaturation would not be expected to occur at such a rapid rate in young tissue. Likewise, one would not expect to see the abrupt halt in the rise of tension seen in this study at the age of 2 years, nor in the following period would one expect the tension to remain nearly static, if simple maturation of the collagen fibers were the only cause for the increased stability.

The lower tension values found in tissue from individuals around the age of puberty most likely reflect the increased hormonal levels known to occur during this period. This is consistent with the findings of Stringer and Highton (22), who found the shrinkage temperature of skin from the abdomen of pregnant women to be lower than that from normal women, and those of Brown and Consden (19) and Brown and co-workers (27), who found the shrinkage temperature of uterine collagen to be lower in premenopausal than in postmenopausal women. In the present study, it is interesting that even though the maximum tension of tissue from the adolescents was about the same or even lower than that from patients from 2 to 8 years old, the temperature of maximum tension production in adolescent skin was characteristic of adult collagen and not of young collagen. Thus endocrine factors, while influencing collagen to the extent of lowering the tension production, do not alter the temperature at which this tension is maximal. This along with the work of Neldner (38), who found a lower collagen hydroxyproline content in patients on steroids and aspirin, might provide an interesting tool for the investigation of the effects of certain drugs on human collagen.
THERMAL CONTRACTION OF HUMAN DERMIS: AGE-RELATED CHANGES

SUMMARY
The shrinkage temperature, amount of contraction, and rate and amount of tension developed during isotonic and isometric hydrothermal contraction of dermal collagen were determined on samples of normal skin taken from 124 individuals ranging in age from 32 weeks of gestation to 88 years.

Measurement of total tension corrected for dermal thickness was found to be the most sensitive indicator of collagen change. This increased rapidly from 26 gm at 32 weeks of gestation to 60 to 70 gm by age 2 years. This was followed by a leveling off with a slight dip at puberty and then a slow steady rise beginning in the late twenties or early thirties to 100 to 120 gm by old age. Both the shrinkage temperature and the amount of contraction showed a small nonspecific increase with age.

The findings suggest that normal collagen becomes increasingly stable with age and that this increase may result from both a maturation and a degenerative process, acting at different rates on the collagen molecules. The dip at puberty is most likely a reflection of the high hormonal levels known to occur during this period.

REFERENCES
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