J. Soc. Cosmetic Chemists, **19**, 829–840 (Dec. 9, 1968)

Changes in Human Skin in the Light of Current Theories of Aging

WILLIAM R. TROY, M.S.*

Presented April 3, 1968, New York City

Synopsis—The four major theories of aging are discussed, as is the expression of these changes in the body's cutaneous covering. An increase in the insoluble collagen fraction of aged dermis is seen, representing an over-all total collagen increase. Reports of elastin changes in the aged dermis are found to be equivocal.

The best known changes in the epidermis include a decrease in the number of cell layers (in those parts of the skin that are not exposed to continual friction) and an increase in the turnover time of senile epidermis.

Introduction

Although the problem of aging began with the evolution of the first living organism on earth, its serious, scientific study has been neglected until only very recently. The wealth of theories proposed to explain aging, however, indicate a desire to make up for lost time. In the following discussion, the term "aging" will be definitively limited to those changes in the individual which occur over a time interval that is long in comparison with the individual's life span, and which are generally thought of as being undesirable to him, such as greying hair, drying skin, decline of mental acuity, etc. This will distinguish aging changes from changes which, although they may resemble aging, are precipitous in nature; that is, they occur during a very short time interval in relation to the individual's life span. The first part of this examination of the aging problem will, then, be concerned with the major hypotheses of aging. In the second section, changes in the skin due to the aging process will be discussed.

* Division of Basic Research, Revlon Research Center, Inc., 945 Zerega Ave., Bronx, N. Y. 10473.

Wear-and-Tear Theory

The first theory to be considered is the so-called wear-and-tear theory. It postulates that the organism depreciates more and more, the longer it is alive, much like an automobile, and that after a number of years (i.e., approaching old age) the organism becomes worn out due to the continual use of its component parts. If this theory were true, the organism's life expectancy would be regulated to a great extent by the metabolic rate at which its cells function. In line with this concept, Johnson *et al.* (1) found that rats kept at 10°C, a temperature at which they had to increase their rate of metabolism to maintain their body temperature, had a life span which was considerably reduced when compared to that of rats kept at 28°C. In addition, the cold-acclimatized rats were found to die of the same diseases as the warm-acclimatized ones, however much sooner they did die. Work with rats using a completely different type of stress (2), however, has produced entirely different results. Feeding studies in which the animals were given an adequate amount of vitamins, minerals, and proteins, but a greatly reduced carbohydrate diet, have also shown a higher rate of metabolism per gram of body tissue. The rats on this controlled diet, however, were found to have extended life spans when compared with the animals fed *ad libitum,* so that the underfed animals lived in several cases as long as 1400 days, whereas the nonrestricted rats seldom lived longer than 965 days. It should be noted here, however, that in both of these studies the only parameter studied was life span, which is not a true indication of the aging process. The stress of sacrificing an animal by exsanguination also has the effect of shortening the life span, but has nothing to do with the aging process.

Another version of this wear-and-tear theory is that senescence is caused by a summation of stresses which the organism receives throughout its lifetime. It is known, for example, that ionizing radiation does have a dramatic effect in shortening the life span of the exposed organism, as well as in producing aging-like effects. In his investigation using an irradiated group of mice, and groups which had been stressed with large doses of nitrogen mustard and typhoid vaccine, Curtis (3) found no shortening of the life span in the latter groups, but a definitely higher death rate among the irradiated. This could only mean that radiation is not the same kind of stress-producer as nitrogen mustard and typhoid vaccine. To investigate further the effect of chronic doses of certain stressors, Curtis gave large, nonlethal doses of typhoid toxin, typhoid toxoid, nitrogen mustard, and turpentine at regular intervals and in increasingly larger doses (to compensate for the animals' increased tolerance). He was unable to note any effect on the life span of the animals. The evidence presented here would indicate, then, that there is no *conclusive* evidence of a causative link between stress and the phenomenon of biological senescence.

Somatic Mutation Theory

The next theory to be dealt with is that of somatic mutations. Its proponents feel that the changes in the aging organism are due to changes or mutations in the chromosomes, or genetic material, of the cell. The longer an organism lives, the more mutations it is liable to accumulate (due to various causes) and the more inefficient the functioning of the cell will become, since it is assumed that practically all mutations are deleterious. After a time, the body cells will be functioning so inefficiently that either some internal metabolic disorder will result, or, because of an impaired immunologic defense system, the body will succumb to an invasion of some pathogenic agent.

In the above explanation it has been implied that the genetic material of the cell exerts an influence over the cell's behavior long after the union of the gametes derived from the parental organisms. Indeed, within the past decade it has been shown that the function of the genes does not lie solely in determining hair and eye color, height, and other characteristics of the developing organism, but in the very intimate control which the genes exert over the biochemical machinery of the cell's metabolism. At this point, it would be instructive to make brief mention of this control as a background for the further explanation of the mutation theory.

Gene Control

It is known that the chromosomes which are present in the nuclei of all animal cells are composed of deoxyribonucleic acid or DNA. It is further believed that there are specific areas on the chromosome which are responsible for certain manifestations and activities in the organism; these areas are called genes. It is under the direction of the basic chemical structure within the gene, the so-called genetic code, that the cellular proteins are manufactured; and the most important group of proteins, perhaps, is the enzymes which catalyze most of the metabolic reactions in the living cell. Thus the genes of the organism exert direct control over the metabolic machinery of that organism by directing the synthesis of the major cellular catalysts.

To relate this back to the mutation theory should not be too difficult now. If a mutation caused by irradiation occurred, and a part of a chromosome were broken off, then the enzymes whose manufacture was involved with that portion of the chromosome would be affected. The practical problem, however, of how to detect possible mutations of this kind arises. Many of the somatic cells of the body do not usually form a second generation and so these chromosomal abnormalities would not be manifest in the daughter cells. Albert (4), however, has devised a technique of dosing mice with carbon tetrachloride and thus destroying about 60% of the normally nondividing liver cells. He reasoned that the ability of this organ to regenerate lost portions would then provide a method of recording chromosome abnormalities in second generation cells. Using this technique, Stevenson and Curtis (5) found a steady increase in chromosome aberrations as a function of increasing age; the number of mutations reached a peak value of 22% of all the liver cells when the animals were 12 months old.

The cells of the body which divide frequently, however, present quite a different situation than do the infrequently dividing cells. In the former types of cells, a mutation can arise much more easily and does not need radiation insult for its cause. In the process of replication of the nuclear DNA, if a wrong base lined up opposite its complementary half and were incorporated into the DNA, this would first cause misreading of the genetic code and then substitution of the wrong amino acid in the enzyme protein structure. In many cases, the substitution of one wrong amino acid in an enzyme leads to its inactivation, since much of an enzyme's activity depends upon its stereochemistry. While it is also true that in the frequently dividing body cells replication errors will tend to be eliminated by the process of natural selection, several faulty enzymes may be synthesized before this elimination takes place; these enzymes will then be unavailable to mediate the reactions for which they were originally intended. Mutations, therefore, of both nondividing and dividing cells of the body play a very real role in the process of senescence, since they affect the fundamental control of cell function.

Cross-Linkage Theory

A third theory of aging concerns the possible cross linking of various of the body's most important molecules as it grows older. Bjorksten (6), one of the most vigorous proponents of this theory, has postulated that the ester linkages which are present within the three strands of the triple helical molecule of collagen may shift to other strands within the same molecule. These linkages, he says, can also form between two different molecules of collagen and, as the body ages and this process continues, the collagen becomes more and more rigid. Verzar (7) has described in detail how this process may occur in collagen. In addition to this molecule, however, Bjorksten extends his theory to include other cellular proteins and nucleic acids. Arranged as they are in long, linear strands, he feels that DNA and RNA are perfect targets for cross linking by agents in the cell which are capable of such action. He lists the dibasic acids formed in the tricarboxylic acid cycle, quinones, aldehydes, silicon, and various metals as possible cross-linking agents. One can imagine the consequences if two strands of DNA became linked in such a way that cellular replication or guided protein synthesis could not take place. The manufacture of faulty enzymes has already been suggested above in the explanation of mutation theories; such useless, giant molecules might well have a clogging effect on the cell's machinery if not used, but instead are allowed to accumulate.

Free-Radical Theory

A fourth theory which is directly connected to the cross-linkage proposal is the free-radical theory of aging. It states that the degenerative changes which occur in old age are due to the accumulation of free radicals in the body which may also act as cross-linking agents. Harman (8) believes that two of the more common free radicals involved are the hydroxyl radical (OH) and the hydroperoxide radical (HO_2), which can be formed by the interaction between oxidative enzymes and oxygen, by the enzymatic degradation of hydrogen peroxide, or by radiation. Norins (9) has also observed the production of free radicals in the skin following exposure to ultraviolet light. To investigate his own theory, Harman included in the diet of a special breed of short-lived mice such compounds as 2-mercaptoethylamine and cysteine, which would react rapidly with free radicals. He then studied the life spans of these mice as compared to those receiving a normal diet. He found that the half-survival time of the animals on the special diet was extended to 10 months, as compared to the 8 months of the control animals.

Consideration of Theories

The preceding review of four of the most popular theories of aging indicates that there is no want for opinions on this topic. There does seem to be, however, an unfortunate tendency to compact the phenomenon of aging and attempt to have it fit one of these four theories to the exclusion of all others. From our viewpoint, it would seem that the theories mentioned here are actually very closely related. As has been shown, a mistake in the genetic material, the most basic yet most important controlling mechanism in the cell, might lead to inactive molecules which would be perfect candidates for creating a log jam in the cell. In addition, the wear and tear on an organism may be due to increased defects and inefficiency in cell function as such metabolic clogs and cross-linked compounds are formed. Metabolic by-products such as free radicals add to the impairment of the cellular operation. Thus, the problem of aging may be thought of as a steadily increasing cell disfunction at the molecular level, terminating in cell death.

Age-Related Skin Changes

After discussion of *why* externally manifest changes with age may occur, the next consideration should properly be the changes themselves. Some of the most readily apparent consequences of aging in the body are found in the area with which we are all so intimately concerned—the skin. In the following sections, then, the qualitative and quantitative age-related changes in the biochemistry and histology of the body's cutaneous covering will be examined.

Dermal Changes: Collagen

Most of the investigations to date into skin aging have centered on the dermis. Unfortunately, even as late as the first part of this decade, aging changes in the skin were being confused, and often equated, with changes brought about by exposure to ultraviolet light. Actually, it has been shown that specific differences exist between young and old skin, young and ultraviolet-exposed skin, and old and ultraviolet-exposed skin. Lorincz (10) has therefore suggested that the skin changes which were formerly classified as "senile elastosis" are actually a result of chronic ultraviolet exposure and should more properly be termed "solar elastosis."

The epidermal and dermal layers of the skin represent, respectively, 5% and 95% by weight. The dermis contains 11% lipid, 1% carbohydrate, 7% nonfibrous protein, 79% collagen, and 2% elastin. Obviously, to quantitate any changes in normal or elastotic skin, the components believed to have changed must be separated from the skin. Sams and Smith (11) have described a representative procedure in which the different fractions of collagen and elastin are separated by acid digestion and centrifugation. The collagen fractions are quantitated by measuring the amount of hydroxyproline in each fraction; this amino acid is present in fairly constant amounts in the peptide linkages of each of the various types of collagen and, as such, is used as a marker for collagen.

When the skin from the back or abdomen of a stillborn infant is compared with the skin from the "Y" incision at autopsy of adult individuals, a decrease is found in the soluble fraction, and an increase in the insoluble fraction, of collagen from the adult skin; in addition, there is an increase in total collagen per gram of wet weight tissue over the value for infant skin (11). That this is a true increase in collagen, and not just an apparent change due to water loss from the skin, is borne out by the findings of both Rothman (12) and Flesch (13) who reported an *increase* in the water content of senile skin as compared to infant skin. It should be noted that both of the above sites of tissue biopsy are not usually exposed to any great extent to the sun, so that the effects observed here are due to age alone. These changes could be explained by a phenomenon which was described earlier—namely, the cross linkage of collagen molecules.

When the forearm skin of an aged donor is examined, it is found that there is no change in the soluble collagen fraction, but a remarkable decrease in the insoluble fraction, representing a decrease in total collagen over the value for the normal adult (11). These effects are commonly seen in the skin of the neck, forearm, face, etc., which has had chronic exposure to the ultraviolet rays of the sun; this is usually referred to as sun-damaged skin. Smith and Finlayson (14) have advanced a theory which may explain these changes. They propose that collagenase is released from subcellular organelles located in the dermal fibroblasts called lysosomes when these lysosomes are labilized by ultraviolet rays below 3100 A. Therefore, upon repeated and chronic exposure to sunlight, the liberated collagenase digests and thereby reduces the dermal content of collagen.

In addition to quantitative changes in dermal collagen with age, qualitative changes have also been recorded. Nimni *et al.* (15) have reported that there is an increase in the tensile strength of collagen with age, and that this increase in strength is in direct correlation with the known increase in insoluble collagen. A possible explanation for these results might again be the theory of cross linking between collagen molecules. In addition, Rasmussen *et al.* (16) found an age-related increase in the shrinkage temperature of collagen—that is, the temperature at which rapid shrinkage of an isolated collagen strip began. Other findings of a decreased capacity for swelling (17), an increased resistance to collagenase (18), a lowered hexosamine: collagen ratio (19), and a higher calcium-binding capacity (20) have also been reported for aged collagen.

Another dermal component often studied in investigations into aging is the acid mucopolysaccharides (AMPS). These are long-chain, protein-bound polymers of high viscosity containing repeating units of hexosamine and uronic acid or galactose (21). Although their exact function is unknown, they may serve as a template for laying down fibrous connective tissue such as collagen.

Upon examination of aging skin, it has been found that the total AMPS decrease, while in sun-damaged skin they increase. Likewise, the hexosamine component of AMPS isolated from aged skin decreases, and in actinic skin it increases. Nimni *et al.* (15) have shown a striking relation between dermal hexosamine and neutral salt-soluble collagen in aging skin. In rabbit skin a few weeks after birth there was an initial sharp increase in hexosamine with a lag in collagen formation. After four weeks, when the amount of hexosamine reached a peak and began to decline, collagen exhibited a sharp increase. After about eight weeks, the soluble collagen followed the hexosamine in a gradual, age-related decline as the insoluble fraction began to replace both the ground substance and the soluble collagen.

With age, then, there is an increase in insoluble collagen and a decrease in AMPS; with sun damage, there is a decrease in insoluble collagen and an increase in AMPS.

Dermal Changes: Elastin

It was mentioned previously that the protein elastin represented about 2% by weight of the dermis of normal skin. Elastin fibers are not present in premature infants of less than four months' gestation, but in older subjects are seen as delicate, wavy, freely branching fibers, as compared to the dense, coarse bundles of collagen. It is agreed that there is a tremendous increase in the elastin content of skin which has been chronically exposed to the sun—described previously as solar elastotic skin—so that the elastin then represents 13% by weight. It was initially noted that concomitant with this large increase in elastin was the striking decrease in collagen. Several investigators therefore offered the theory that the collagen was being converted into an elastin-like compound. However, Smith (22) and others have reported that this increase is due to true elastin build-up, basing their beliefs on the morphology, solubility, enzyme susceptibility, tinctorial and physical properties, and amino acid composition of the elastin formed.

In quantitation of elastin from aged skin, diverse results are presented. Sams and Smith (11), after chemical and physical separation of elastin from adult skin, concluded that there is a slight, quantitative increase with age. Weinstein and Boucek (23) subjected the elastin fraction to enzymatic digestion with elastase and found no increase in aged skin. Hult and Goltz (24) employed a procedure whereby they investigated the elastin content of human aged skin by two methods: the first, a microphotometric examination of orcein-stained skin sections, and the second, an enzymatic digestion. This was done to test whether there might be an increased susceptibility of elastin in aged skin to enzymatic digestion, rather than an increase in the quantity of elastin. They showed that there were indeed higher values for aged skin as opposed to young skin when the enzymatic method was used. The theory of age-related increased susceptibility might therefore appear to be true. But it would also seem that a positive answer has yet to be arrived at.

Epidermal Changes

Turning now from the dermis to the epidermis of the skin, a first impression might be that the knowledge of the changes in protein and mucopolysaccharide content in the dermis with age has been gained at the expense of investigation of aging effects on the epidermis. Although some of the most apparent changes which accompany aging—skin wrinkles—are perceived in the outer layer of the skin, their true cause lies in the changes in dermal collagen and, possibly, elastin, which take place beneath the epidermis. Another common sign of aging is dry and flaking skin. At first this might seem hard to reconcile with the finding that there is an increased water content in human skin with increased age (13). However, it must be remembered that the outer horny layer of a healthy epidermis normally forms a barrier against the exchange of water, and that hydration of the skin *surface* is not influenced to any great extent by the water present in the *deeper* layers.

Brief mention should also be made here of the effects that hormones can have on the aging skin. Although the role of the hormones in the body's homeostasis is all-encompassing, just one of the effects of the so-called sex hormones on the skin is presented here. In later life, and especially in the female, who undergoes a rather abrupt decline in hormonal output at the menopause, the epidermal sweat and sebaceous glands become much less active, almost to the point of degeneration. Although the exact method of action of the sex hormone on the cell layers of the skin is not known, it is recognized that the "female" hormones and hormone-like steroids, when topically applied to aged skin, effect a histological picture in the epidermis which is strikingly similar to that of a young person.

One area of investigation concerning the epidermis and aging that has been studied to some degree is that of rate of turnover of the cells of the epidermis. The turnover time, or renewal time, is usually defined as the average time for all the cells in the basal layer of the epidermis to reach the horny layer. Although several studies have been undertaken to measure this turnover time, various sources have been used for epidermal sections, and many different areas of the body have been used for tissue sampling. This lack of coordination between donor and site, coupled with infrequent recording of the donor's age, has unfortunately led to an unclear presentation of the relation of epidermal turnover to aging.

Weinstein (25) has deduced values for turnover time using skin from the back of an adult pig. The morphology of the pig epidermis has been found to be strikingly similar to human epidermis. He found a transit time of 14 days through the viable cell layer, and 16 days through the horny layer, giving a total turnover time of 30 days. In working with the rat, Storey and Le Blond (26) reported a turnover time of 19 days in plantar epidermis taken from an adult animal. Bertalanffy (27), also using adult rats, found that the renewal time for the abdominal epidermis was 19 days. In a second study, Bertalanffy *et al.* (28) compared epidermal growth in young, adult, and senile rats. They found that when the total thickness of the epidermis from different age groups was compared, the senile skin showed a reduction by about two to three layers in the epidermis taken from ear or abdomen. In areas such as the bottom of the foot, there was an *increase* by one or two in the number of cell layers. As regards the mitotic rates, it was found that there was a general increase by about 50% in the rate of turnover of the senile epidermis as opposed to the young. In a similar study using human abdominal skin samples, Thuringer and Katzberg (29) observed an increase in the mitotic index such that the epidermis of the senile individuals was dividing twice as fast as that of the young group.

The cause of this increased epidermal turnover is not yet positively known. However, one might be tempted to postulate a feedback control mechanism operative in the epidermis. As was mentioned, the adult and senile skin show increased desquamation, possibly because of increased keratinization, modification of dermal vascularity, or insufficiency of epidermal glandular secretion. This cell loss may act as a trigger to signal an increase in the replacement of cells. As cell loss is accelerated, then, so also is cell replacement.

While there are but few techniques presently available for the treatment of aging skin, the ultimate method will probably involve the use of agents which will be able to act directly on the controlling mechanisms of the body's cells—the genes—either to correct errors (which, as we have seen, can evolve with time), or to initiate some desired activity (such as induction of a specific enzyme). When man is able to achieve this kind of control, there will be practically no limit to his ability to regulate the functioning of his body even to stemming the tide of aging.

(Received April 17, 1968)

References

(1) Johnson, H. D., Kintner, L. D., and Kibler, H. H., Effects of 48°F and 83°F on longevity and pathology of male rats, *J. Gerontol.*, **18,** 29 (1961).
(2) McCay, C. M., Magnard, L. A., Sperling, H., and Barnes, L. L., Retarded growth, lifespan, ultimate body size and age changes in the albino rat after feeding diets restricted in calories, *J. Nutr.*, **18,** 1 (1939).
(3) Curtis, H. J., Biological mechanisms underlying the aging process, *Science*, **141,** 686 (1963).
(4) Albert, M. D., X-irradiation induced mitotic abnormalities in mouse liver regenerating after CCl_4 injury. I. Total body irradiation. *J. Nat. Cancer Inst.*, **20,** 309 (1958).
(5) Stevenson, K. G., and Curtis, H. J., Chromosome aberrations in irradiated and nitrogen mustard treated mice, *Radiation Res.*, **15,** 774 (1961).
(6) Bjorksten, J., Chemical causes of the aging process, *Proc. Sci. Sect. Toilet Goods Assoc.*, **41,** 32 (1964).
(7) Verzar, F., The aging of collagen, *Sci. Am.*, **208,** 104 (1963).
(8) Harman, D., The free radical theory of aging: effect of age on serum copper levels, *J. Gerontol.*, **20,** 151 (1965).
(9) Norins, A. L., Free radical formation in the skin following exposure to ultraviolet light, *J. Invest. Dermatol.*, **39,** 445 (1962).
(10) Lorincz, A. L., Physiology of the aging skin, *Illinois Med. J.*, **117,** 59 (1960).
(11) Sams, W. M., Jr., and Smith, J. G., Jr., Alterations in human dermal fibrous connective tissue with age and chronic sun damage, in Montagna, W., *Advances in Biology of the Skin—Volume VI—Aging*, Pergamon Press, New York, 1965, pp. 199–210.
(12) Rothman, S., Water and electrolytes, in *Physiology and Biochemistry of the Skin*, The University of Chicago Press, Chicago, 1961, pp. 493–514.
(13) Flesch, P., The chemistry of the aging skin, *J. Soc. Cosmetic Chemists*, **6,** 377 (1955).
(14) Smith, J. G., Jr., and Finlayson, G. R., Dermal connective tissue alterations with age and chronic sun damage, *Ibid.*, **16,** 527 (1965).

(15) Nimni, M. E., deGuia, E., and Bavetta, L. A., Collagen, hexosamine and tensile strength of rabbit skin during aging, *J. Invest. Dermatol.* **47,** 156 (1966).
(16) Rasmussen, D. M., Wakim, K. G., and Winkelmann, R. K., Isotonic and isometric thermal contraction of human dermis. II. Age-related changes, *Ibid.,* **43,** 341 (1964).
(17) Kohn, R. R., and Rollerson, E., Relationship of age to swelling properties of human diaphragm tendon in acid and alkaline solutions, *J. Gerontol.* **13,** 241 (1958).
(18) Keech, M. K., The effect of collagenase on the fixed and unfixed skin lesions of morphoea: An electronmicroscopic study, *J. Pathol. Bacteriol.,* **77,** 351 (1959).
(19) Houck, J. C., DeAngelo, L., and Jacob, R. A., Connective tissue. III. Dermal chemical response to age, *Proc. Soc. Exp. Biol. Med.,* **107,** 280 (1961).
(20) Verzar, F., The aging of connective tissue, *Gerontologia,* **1,** 363 (1957).
(21) Smith, J. G., Jr., Davidson, E. A., and Taylor, R. W., Human cutaneous acid mucopolysaccharides: The effects of age and chronic sun damage, in Montagna, W., *Advances in Biology of the Skin—Volume VI—Aging,* Pergamon Press, New York, 1965, pp. 211–8.
(22) Smith, J. G., Jr., The dermal elastoses, *Arch. Dermatol.,* **88,** 382 (1963).
(23) Weinstein, G. D., and Boucek, R. J., Collagen and elastin of human dermis, *J. Invest. Dermatol.,* **35,** 227 (1960).
(24) Hult, A. M., and Goltz, R. W., The measurement of elastin in human skin and its quantity in relation to age, *Ibid.,* **44,** 408 (1965).
(25) Weinstein, G. D., Autoradiographic studies of turnover time and protein synthesis in pig epidermis, *Ibid.,* **44,** 413 (1965).
(26) Storey, W. F., and Le Blond, C. P., Measurement of rate of proliferation of epidermis and associated structures, *Ann. N. Y. Acad. Sci.,* **53,** 537 (1951).
(27) Bertalanffy, F. D., Mitotic activity and renewal rate of sebaceous gland cells in rat, *Anat. Record,* **129,** 231 (1957).
(28) Bertalanffy, F. D., Pusey, V., and Abbott, M. D., Mitotic rates of rat epidermis, *Arch. Dermatol.,* **92,** 91 (1965).
(29) Thuringer, J. M., and Katzberg, A. A., The effect of age on mitosis in the human epidermis, *J. Invest. Dermatol.,* **33,** 35 (1959).