

# Effect of Topical Hormones on Aging Human Skin\*

CHRISTOPHER M. PAPA, M.D.†

*Presented September 20-21, 1966, Seminar, New York City*

**Synopsis**—The male hormone, testosterone, has a rejuvenating or ameliorative effect when applied to aging human skin. Clinically evident changes, such as effacement of wrinkles, hair growth, and augmented sweating, are present but modest, particularly when compared to the improvement in the microscopic architecture of the skin. Progesterone and pregnenolone produce similar but more diminutive alterations. The female hormone, ethinyl estradiol, was without effect, while the corticosteroids accentuated the degradative changes of senescence.

The aged comprise an ever-increasing proportion of the population. The chronic internal disorders of old age are under intensive study because they threaten life and lessen the enjoyment of living. The age-dependent changes of the vital organs have been reasonably well appraised, but the skin is a special case. No one dies of aged skin; appearance spoils life only indirectly as suggested by the statement that people feel as young as they look. The desolate appearance of truly senescent skin can impair mental and physical well-being. The importance of looking and feeling attractive may be easily submerged in the concern over the other traditional scourges. In light of recent medical advances against serious ailments, the time may be ripe to turn attention to the relatively minor skin problems. Indeed, if the medical utopia of the future could supply us with spanking new inner organs, what a dismal package it would still be, wrapped in old skin!

\* Supported in part by U. S. Public Health Service Grant.

† Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104.

Research activities, carried out in these laboratories have centered about the biology of aging skin. Dissatisfied with merely cataloging the alterations of senescence, a decision was made to try to do something about them. A strictly empirical mode of attack was based on the clinical impression of waning endocrine function with advancing age and utilized pharmacologic doses of topically applied hormones. Representative steroids from the male, female, and adrenocortical hormones were included in the study. The following report is a brief, composite picture of published work, as well as some still in progress (1, 2).

### METHODS

Approximately 200 residents of the Riverview Home for the Aged have participated in the research program over the past five years. Almost all subjects manifested obvious cutaneous deterioration; the median age of the group is 65 years (range 57-88 years). Although equal numbers of men and women were included in each particular project, the sex of the aged subject is irrelevant with regard to the effect of the hormones on the skin. Biopsy samples obtained since the onset of the work amount to more than 1500 individual specimens.

It is important to emphasize that only areas of skin with clear-cut aging alterations were used. The face, extensor forearm, and back of the hand, sun exposed sites which graphically illustrate the changes of senescence, were included. The axilla was studied since it also demonstrates recognizable aging, yet it is protected from solar radiation and so suffers less deterioration.

The following hormones, compounded either into hydrophilic ointment base or alcoholic solutions, were used: testosterone propionate 1.0%, progesterone 1.0%, pregnenolone acetate\* 0.5%, ethinyl estradiol 0.5%, triamcinolone acetonide† 0.5%, and fluocinolone acetonide 0.2%‡. Each unilateral test site received daily topical treatment with approximately 0.5 g of preparation while the contralateral side had the corresponding vehicle similarly applied.

### GROSS PROPERTIES OF AGED SKIN

The weather beaten, unsheltered face and hands are familiar landmarks on our aged population. Laced with fine and coarse furrows, such skin possesses a general laxness, sagging, and loss of resiliency.

\* Supplied by Revlon, Inc., as Eterna 27 Cream.

† Supplied by E. R. Squibb & Sons as Kenalog Cream.

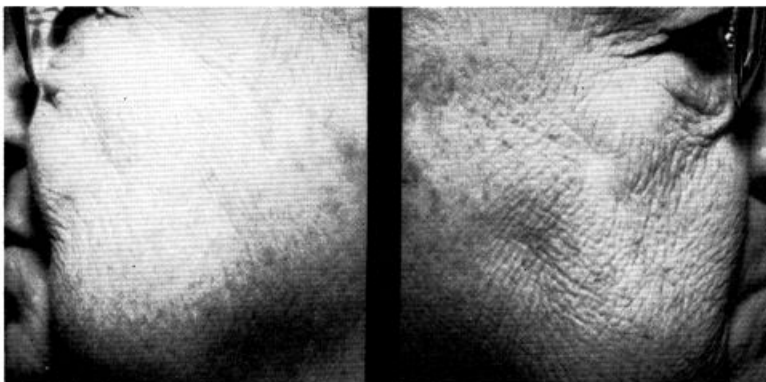
‡ Supplied by Syntex Laboratories, Inc., as Synalar Cream.

Coloring is uneven, with hyperpigmented macules appearing on the susceptible individual in ever-increasing numbers with age. Coarse hairs grow from the rim of the nose and ears of men, while women often acquire stubble on the chin and mustache areas. The sebaceous glands may attain unusual prominence and are easily visible along with sprays of superficial vessels beneath the thinned integument. Fairly large, irregular black and blue splotches are present on the extensor forearm caused by spillage of blood into innocently traumatized skin which barely cushions its vasculature. Characteristic sequelae of these events are seen as roughly linear, small ivory scars.

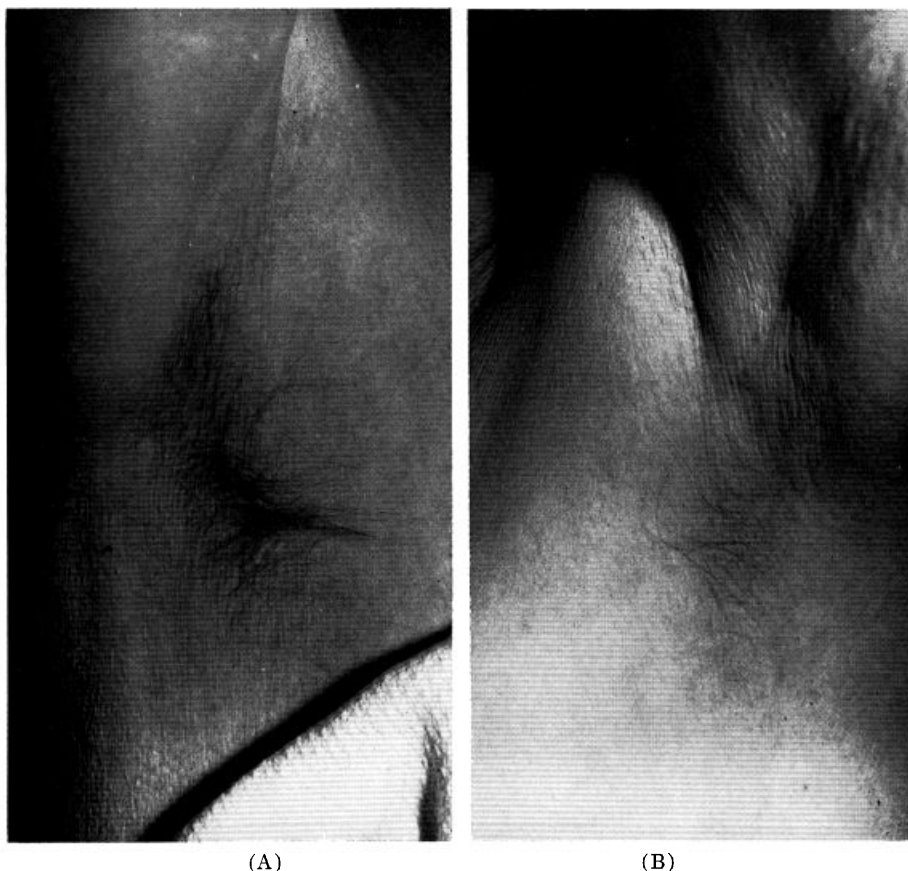
The aged axilla fares somewhat better with the passage of time. There is, however, considerable loss of hair, and that which remains tends to be thin, wispy, and depigmented. The underarms are drier than those of the young adult, an observation which correlates well with qualitative studies of eccrine sweating which is considerably diminished. In short, aging skin appears to be a degraded structure which bears little resemblance to its neonatal predecessor. It hardly seemed likely that further alterations were possible.

#### GROSS RESULTS OF STEROID APPLICATION

Approximately six months of treatment are necessary before the hormonal effects become apparent. Ameliorative alterations resulted primarily from androgen application but were clearly defined in only 20% of the subjects with regard to changes in the sun-damaged skin.



*Figure 1.* Cosmetic effect of topical progesterone therapy on the face of a 75-year-old woman. The left side has been treated with the hormone and the right side with the cream vehicle for three years. There is considerable effacement of the wrinkles on the progesterone-treated side and some stimulation of downy hair growth



*Figure 2.* Comparison of hair growth in testosterone treated (A) versus control axilla (B) of 77-year-old woman. The untreated site typifies the great regression of axillary hair in old age. Note that the testosterone effect is strictly limited to the hormone treated side, where there is remarkable regrowth of hair

Fully three quarters of the individuals, however, showed unmistakable axillary responses. The wrinkled appearance of the face and dorsum of the hand was considerably smoothed, and the skin felt firmer and thicker (Fig. 1). The lax integument of the back of the hand which formerly tented for a finite time when pulled up and stretched now sprang back quickly. A remarkable stimulation of terminal hair growth was found in the axilla (Fig. 2) and to a lesser extent on the forearms and face (Fig. 3). Similar augmentation was produced on the abdomen, back, and thigh. Eccrine function of the axilla, assessed using Wada's iodine-starch-castor oil procedure, was also enhanced. This effect is due



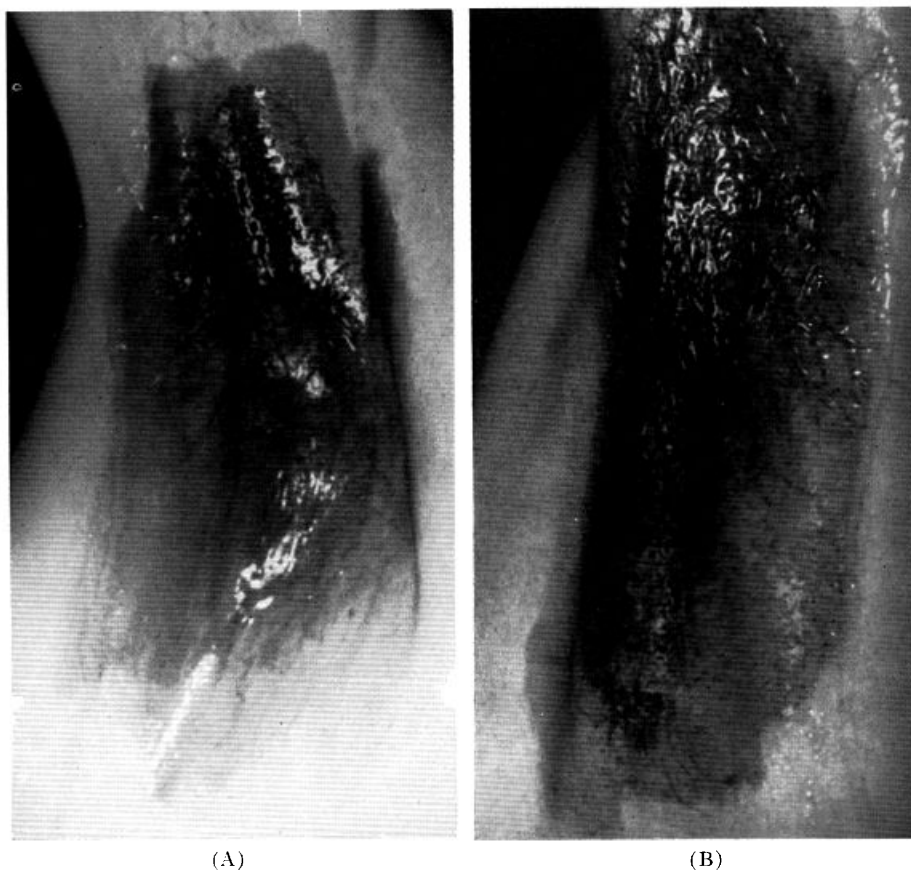
*Figure 3.* Unilateral beard growth by topical testosterone. The left side of the face of this 84-year-old white woman was treated with the hormone for  $1\frac{1}{2}$  years, while its cream vehicle was similarly applied to the right side. The mustache was present beforehand, as were a few wisps of hair on the chin. This again shows the purely local action of the topically applied steroid

both to earlier recruitment and an increased density of functioning glands (Fig. 4).

Progesterone-treated sites demonstrated effects qualitatively similar to those of testosterone but of a lower order of magnitude. Pregnenolone acetate, while also working in the same direction, was considerably weaker in effect. Estrogens produced no observable improvement in the aged skin and, if anything, tended to suppress both hair growth and eccrine sweating in the axilla.

The corticosteroid hormones unequivocally accentuated the defects of senescence. The skin became thinner, atrophic, and even more lax. Spontaneous petechiae developed in the axilla, while the forearm displayed the identical likeness to senile purpura (Fig. 5). Hair growth in the axilla diminished. Triamcinolone acetonide suppressed eccrine function, and fluocinolone acetonide stimulated sweating.

Thus, in regard to gross structure and function, testosterone, progesterone, and pregnenolone, in that order, have a general rejuvenating effect on senescent skin. On the other hand, estrogen mildly accentuated some of the aging characteristics, while the corticosteroids profoundly increased the degradative alterations.



*Figure 4.* The effect of topical testosterone on eccrine function as demonstrated by use of Wada's iodine-starch-castor oil technique. Sweat droplets appear as black puncta trapped beneath the oil. The control side (A) clearly demonstrates the diminished activity seen in the aging axilla. (Subject is a 75-year-old white man.) The contralateral, testosterone treated area (B), however, has greatly increased sweating

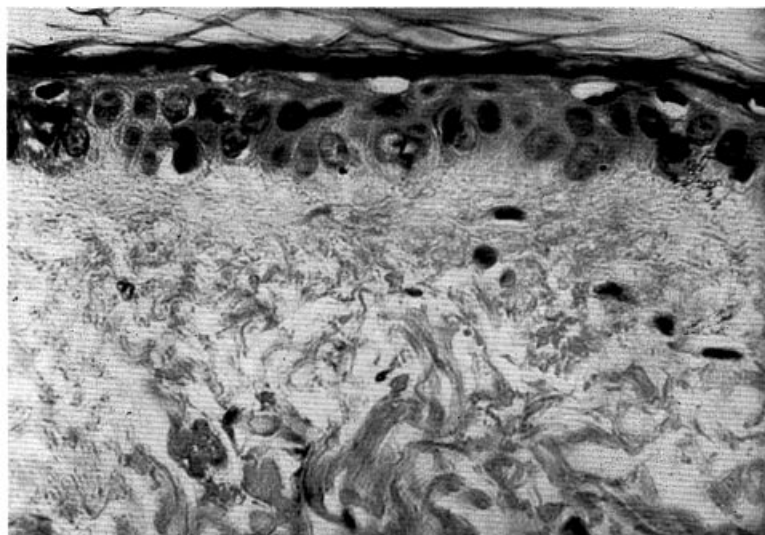
#### HISTOLOGIC CHANGES IN AGING SKIN

Most impressive of the structural changes are the marked thinning and flattening of the epidermis. It is composed of cells which display great irregularity with marked discrepancy in size, shape, and staining properties. The cytoplasm of the prickle cells often condenses in the periphery, leaving perinuclear halos. The nucleus is often small and darkly stained, and the nucleoli are obscured. The orderly progression of cells from the basal layer to the surface is often disrupted, with considerable loss in cell polarity (Fig. 6A). Under the light microscopy,

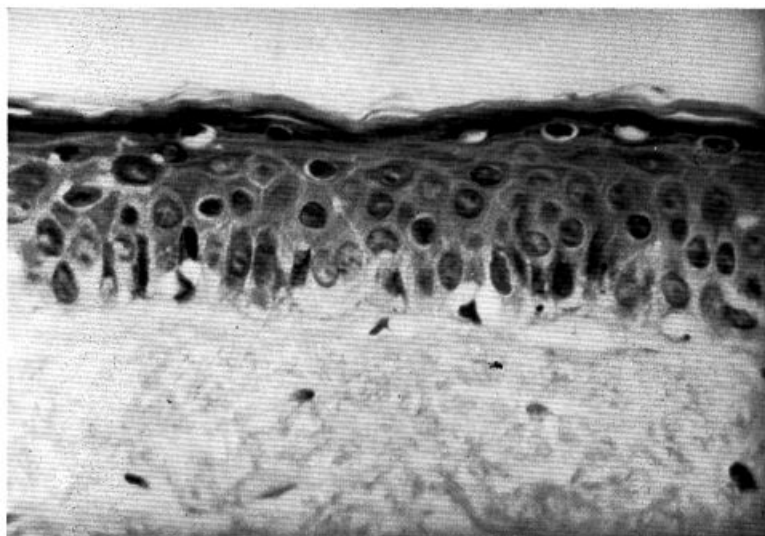


*Figure 5.* Senile purpura-like lesions produced by topical application of corticosteroids. The left extensor forearm has been treated with triamcinolone acetonide cream for one year, the right with its control vehicle

the basement membrane, which normally is a sharp, serrated structure inserting into the basal layer at the dermo-epidermal junction, appears as a blunted, hazy, attenuated, and discontinuous structure (Fig. 7A). Dermal degradation is most prominent in the sun-exposed sites. Murky masses of dense, basophilic, elastotic material occupy the major portion of the dermis, sparing but a thin subepidermal zone. Elastic staining, particularly in thick sections, reveals a tangled skein of coarse fibers, while the delicate, finely branched subepidermal strands are reduced. The acid mucopolysaccharide (AMP) ground substance, defined by Hale staining, is greatly diminished, surviving in small quantity in the papillary dermis. Correspondingly, there exists a depletion of fibro-



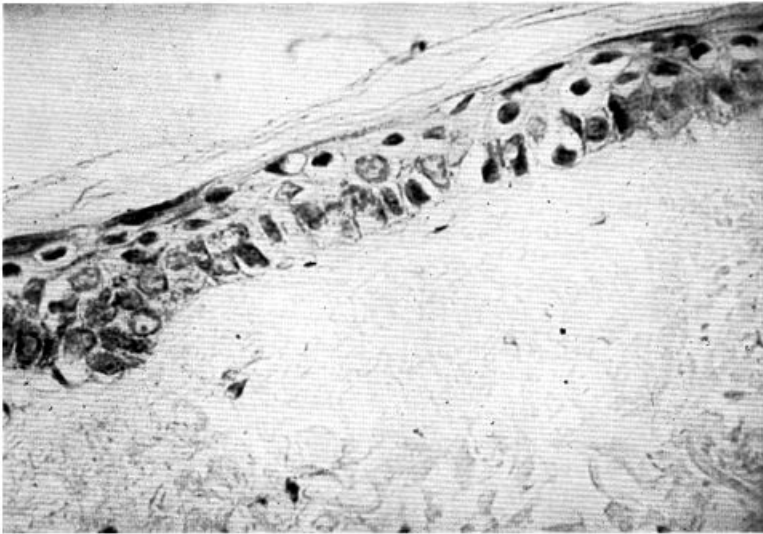
(A)



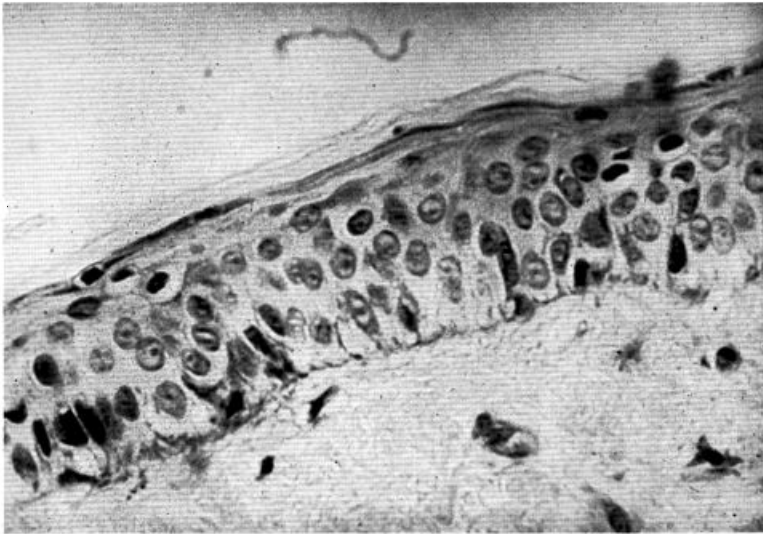
(B)

**Figure 6.** Enhancement of epidermal structure by testosterone. The control specimen (A) from the face of an 80-year-old white woman typifies the chaotic, atrophic epidermis of exposed aged skin. Some of the degradative dermal changes are also evident. The testosterone-treated side (B) has undergone remarkable "rejuvenation." Both specimens are photographed at the identical magnification. (H & E  $\times 400$ )





(A)

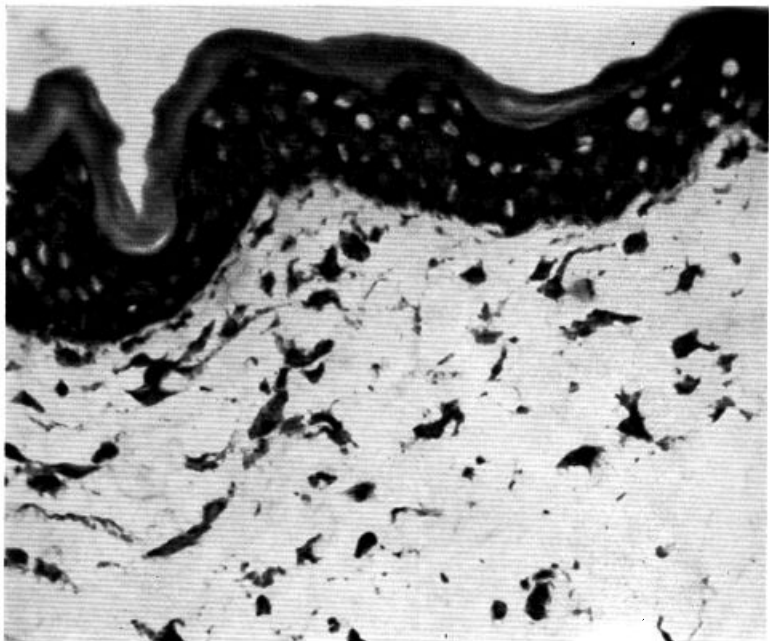


(B)

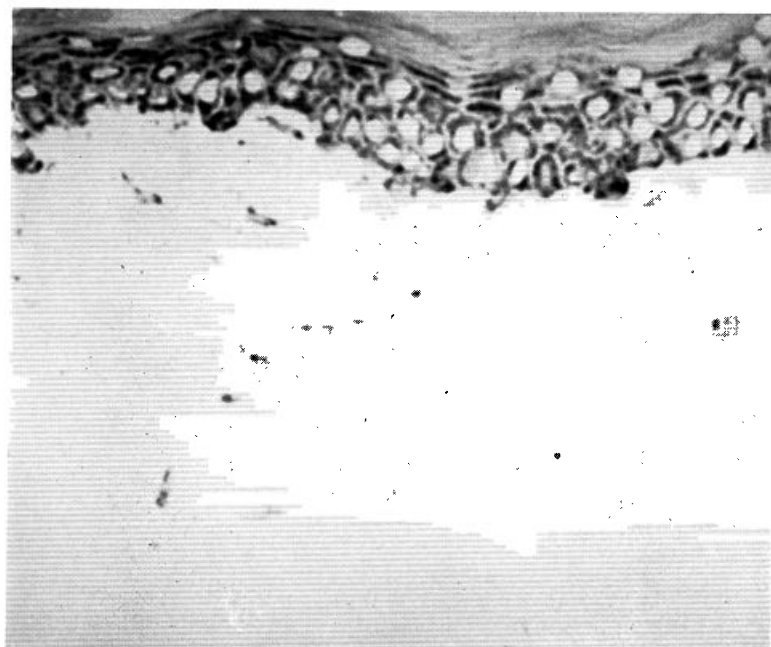
**Figure 7.** Effect of topical testosterone on the basement membrane. The control site (A) from the face of an 80-year-old white woman shows a much attenuated, discontinuous structure that is barely visible. The hormone-treated skin (B) demonstrates a fine, serrated band of PAS-positive material along the length of the dermo-epidermal junction. (Original magnification 400 $\times$ )



(A)



(B)



(C)

*Figure 8.* Hormonal effect on dermal cells. The control specimen (A) exhibits the characteristic bipolar, spindle shaped fibroblasts, packed in parallel bundles at the upper portions of the dermis. Testosterone treatment (B) causes the cells to assume a more blast-like, stellate form, and they are found more uniformly distributed throughout the dermis. Topical corticosteroids cause a marked diminution on the cell population (C). Either the fibrocytes are not staining or their numbers have been reduced. The relative diphosphopyridine nucleotide diaphorase (DPND) activity in the epidermis is also seen with this enzyme preparation. (Original magnification 200X)

cytes, which are shrunken to skimpy, bipolar forms packed in parallel bundles within the Hale staining areas (Fig. 8A).

#### HISTOLOGIC RESULTS OF STEROID APPLICATION

The microscopic structural improvements following the application of hormones are far more impressive than those appreciated with the unaided eye. These alterations are readily apparent in the majority of subjects (about 75%), including many individuals who showed no clinical modifications. The tenor of the testosterone effect, and that of progesterone and pregnenolone to a lesser degree, was toward a more youthful architecture. Thickening of the treated epidermis (through increase in cell size and number of cell layers) occurred to an extent which made it appear as though it had been viewed at a higher

magnification than its control specimen. Regularity in cell size and shape was restored, nuclear and cytoplasmic content seemed plumper, and nucleoli became distinct. Stratification of cells throughout the epidermis was restored to the normal pattern (Fig. 6B). The basement membrane thickened perceptibly, stained more vividly, and became sharply defined and unbroken (Fig. 7B).

The dermis showed increased AMP staining of deeper hue and broader distribution. There was an accompanying alteration in the morphology of the fibrocytes, which resumed a plump stellate form and were found dispersed throughout the dermis (Fig. 8B). The heavy material of basophilic degeneration appeared diluted or washed out by the increased AMP. In a similar fashion, the coarse tangles of elastic tissue appeared more separated and were pushed further away from the papillary dermis. This subepidermal zone now contained numerous fine elastic fibers which ran perpendicularly to the dermo-epidermal junction.

Estrogen-treated tissues could not be distinguished from the controls. As with the clinical results, the corticosteroids produced degradative changes easily seen by microscopic examination. The epidermis became uniformly atrophic and was often reduced to a structure of only several cell layers' depth. The basement membrane suffered further washout and became more indistinct. Decimation of the cell population of the dermis (Fig. 8C) was accompanied by diminished AMP staining. The subepidermal orcein staining fibers were little changed following corticosteroid treatment.

#### DISCUSSION

These studies indicate that topically applied steroid hormones can produce definite alterations in aging human skin. Androgens primarily tend to ameliorate the degradative changes, while the corticosteroids further exaggerate the deterioration. Exactly how these modifications develop is not yet known, and perhaps study of the mechanisms may provide insight into the aging process itself. Cutaneous senescence at least appears to have been conveniently bracketed by this divergent action of structurally related chemicals.

One fact which is clear is that the observed results are entirely local. Similar effects on distant or neighboring untreated skin do not occur. Systemic administration of the hormones in therapeutic amounts over an extended time are also ineffective (3). Of all the steroids used, only the estrogen showed significant absorption as such, with general effects

such as gynecomastia in men and resumption of uterine bleeding in postmenopausal women.

Apparently the application of pharmacologic quantities of hormones to the skin yields unique results which cannot be predicted from knowledge of their usual physiologic and systemic effects. It is becoming evident that cutaneous pathways of metabolism for steroids exist and that much remains to be learned of their integumentary fate (4-8). It is clear, however, that hasty generalizations drawn from "expected results" can be misleading. The reliance on topically applied female hormones to alleviate the defects of aging skin undoubtedly draws support from this type of reasoning.

With regard to the practical implications of our studies, it is of course most encouraging to have achieved even these modest changes on skin which was already markedly damaged. This strongly suggests that prophylaxis might well yield even more satisfactory results. It is conceivable that application of the appropriate steroid begun rather early in life, particularly in light skinned, susceptible individuals, might not only enhance their appearance in later years but also afford protection against the development of cutaneous epitheliomas.

#### SUMMARY

The characteristic structural changes which typify aged skin, both in protected and actinically damaged areas, can be modified by the topical application of steroidal hormones. Androgenic compounds exert an ameliorative effect, restoring the gross and microscopic architecture toward a more youthful integument. Hair growth is stimulated, sweat secretion increased and, in some instances, the sagging and fine wrinkling of senescence effaced. The microscopic improvement is even more uniform and striking, particularly in the epidermis where the disorderly cytologic and histologic alterations are reversed. The "beneficial" effects of topical estrogen have not been confirmed, and such substances exert no appreciable activity on human skin. Corticosteroid action is diametrically opposite to that of the androgens, and topical administration accentuates the degradative changes.

#### ACKNOWLEDGMENT

Particular appreciation is acknowledged to the administration (Raymond S. Groller, Director) and the residents of Riverview Home for the Aged who participated in this study.

(Received November 14, 1966)

## REFERENCES

- (1) Montagna, W., Formisano, V., and Kligman, A. M. Hormonal factors in aging. *Hormonal Steroids, Biochemistry, Pharmacology and Therapeutics: Proceedings of the First International Congress on Hormonal Steroids*. Vol. 1, pp 559-568, Academic Press, New York, 1964.
- (2) Papa, C. M. and Kligman, A. M. The effect of topical steroids on the aged human axilla, in Montagna, W., *Advances in Biology of Skin-Aging*, Pergamon Press, New York, 1965.
- (3) Formisano, V., and Kligman, A. M., Modification of human aged skin by sex steroids. (In press)
- (4) Wotiz, H. H., Mescon, H., Doppel, H., and Lemon, H. M., The *in vitro* metabolism of testosterone by human skin, *J. Invest. Dermatol.*, **20**, 113 (1956).
- (5) Baillie, A. H., Calman, K. C., and Milne, J. A., Histochemical distribution of hydroxysteroid dehydrogenases in human skin, *Brit. J. Dermatol.* **77**, 610 (1965).
- (6) Gomez, E. C., and Hsia, S. C., Studies in cutaneous metabolism of testosterone 4-C<sup>14</sup> *Federation Proc.*, **25**, 282 (1966).
- (7) Hsia, S. L., Witten, V. H., and Hao, Y. L., *In vitro* metabolic studies of hydrocortisone-4-C<sup>14</sup> in human skin. *J. Invest. Dermatol.* **43**, 407 (1964).
- (8) Frost, P., Weinstein, G. D., and Hsia, S. L. Metabolism of estradiol-17<sup>B</sup> and estrone in human skin. *Ibid.*, **46**, 584 (1966).