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THE MEASUREMENT OF ELASTIN IN HUMAN SKIN AND ITS QUANTITY IN RELATION TO AGE*

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Although the literature contains many references to subjective observations on the state of the elastic fibers in the dermis (1) in various pathologic conditions, actual quantitation of elastin has to our knowledge been recorded in only a few selected conditions. That such quantitation may be important is attested to by the diversity of opinions previously expressed about the quantity of elastic fibers in such conditions as cutis laxa versus cutis hyperelastica. The present study represents an attempt to evaluate two methods for the quantitation of elastin in human dermis and to apply these methods to skin from persons of different ages. Results of application to skin from different anatomical sites, and from a variety of diseases of the dermis will be reported separately.

PART I—METHODS

Elastin was quantitated by two methods:

1. A new microphotometric method employing readings on orcein-stained serial sections of skin, either frozen cryostat-sectioned, or paraffin-blocked formalin fixed tissues.

2. A modification of the Scarselli method (2) in which orcein-stained elastin extracted from fresh frozen skin by enzymatic digestion was quantitated in a colorimeter.

I. Photometric Technic

Paraffin blocked or cryostat sections are cut at 10 microns, and 5 sections mounted on each of 2 slides. All sections are stained simultaneously according to the following procedure:

a. 1% orcein + 1% HCl in 70% ethanol solution for 30 minutes.
b. 0.57% HCl in 95% ethanol for 7 minutes.
c. Borate buffer (pH 8.6) for 5 minutes.

The ethanol-HCl rinse removes all orcein not bound to elastin, while the borate rinse results in full color development. This reaction between orcein and elastin is irreversible. The production of chemical bonding has been demonstrated spectrophotometrically by Scarselli (3), employing the principle of conjugations bathochromy, and the maximal absorption in the spectrum of the complex found to be at 5890 Å. This wave length was checked in a Beckman DU recording spectrophotometer in our laboratory, and found correct for the synthetic orcein (Chroma) used by us.

The elastin-orcein complex present in the histologic sections is quantitated by means of a microphotometer. This is a conventional Lison type apparatus (4), as modified by Sandritter, *et al* (5) for use in the visible light range. We use a Zeiss GFL trinocular microscope, the center tube being coupled to a Brinkmann-Faross microspectrophotometer head, an amplifier and galvanometer (Photovolt). Measurements are made through a Zeiss neofluar 10× objective and Zeiss 1.42 achromatic-aplanatic condenser, adjusted for Köhler illumination. Constancy of illumination from the built-in tungsten lamp is maintained by a constant voltage transformer.

Between the light source and condenser the following filters are inserted:

—Corning 3307, 25 mm thickness
—Bausch & Lomb interference filter, 2nd order, maximum transmission at 5890 Å, band width + or − 2.0 Å
—Neutral density filter

This filter system provides a sufficiently narrow band of light, almost monochromatic at 5890 Å. The second wave length employed, according to the requirements of the 2 wave length method to be discussed below, is 6400 Å.

A diaphragm with a 2 mm aperture, is inserted in the photometer head. With the magnification employed, this results in a field size of approximately 60 microns diameter—small enough to provide sufficient selectivity in choice of field so that all non-dermal structures can be avoided.

Because of the highly heterogeneous distribution of elastin in the dermis, it is necessary to take a large enough number of readings to obviate sample error. This is accomplished by moving the slides by means of the mechanical stage of the microscope. Readings are taken from the adjacent fields, either parallel or perpendicular to the skin surface. If quantitation of amounts of elastin in different layers of the dermis is desired, readings are taken from 30 adjacent fields in each of the upper, mid and deep portions of the corium. If measurement of total elastin is desired, sections are moved vertically, and as many adjacent fields between epidermis and hypoderm as possible are measured. In either case, results are then averaged to obtain a representative figure for the elastin in the areas examined.

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Galvanometer readings may be made from either absorption or transmission scales, since these are related when Beer's law applies, as it does in this case. For convenience, most readings are taken from the transmission scale.

II. Enzymatic Extraction Technic: (modified from Scarselli) (2)

Fresh or frozen, unfixed skin specimens are defatted in ethanol-ether mixture for 24 hours, then dried in 37°C incubator for 24 hours.

20 mg samples of the dried skin are immersed in 10 ml 0.IN NaOH for 5 hours at room temperature, with occasional stirring.

Samples are recovered by centrifugation, and chopped as finely as possible with a razor blade. Resulting samples are rinsed for 30 minutes each at 37°C in two changes of 95% ethanol + 0.5% HCl.

Samples are stained for 24 hours in 10 ml stock orcein solution, diluted 1:10 with 95% ethanol + 1% HCl. The stock solution contains 1% synthetic orcein, 1% HCl (Chroma) in 70% ethanol. Samples are washed in 95% ethanol-HCl until no more free orcein is released, as many changes of wash as necessary being used. Samples are recovered from the last wash by centrifugation and suspended in 10 ml of borate buffer (0.2 M, pH 8.6) for 30 minutes, recentrifuged, and resuspended for another 30 minutes.

Elastin is then removed by digestion by bacterial elastase (Calbiochem), 2 mg in 10 ml borate buffer. Digestion is carried out for 15 hours at 37°C., with occasional stirring.

The sample is removed by centrifugation and the color intensity in the supernatant read at 4890 Å in a Beckman B colorimeter.

DISCUSSION

Neither of the two technics described permit absolute quantitation of elastin in any specimen of tissue. Both methods have inherent limitations which will probably always make necessary the use of appropriate controls. A limitation common to both is that they depend on specificity and full intensity staining of elastin by orcein. There is little reason to doubt the specificity of orcein staining of elastin, but the intensity of staining depends somewhat on the source of the orcein, even different lots from the same manufacturer producing somewhat different results. Furthermore, in the photometric method there is some variation, however slight, between formalin-fixed paraffin-blocked sections and cryostat-sectioned frozen tissue, the latter giving somewhat greater values for elastin, presumably because of greater uptake of orcein on unfixed fibers.

Several other possible sources of error, inherent in the photometric method, will probably always prevent attainment of absolute quantitation by this technic. Since measurements are carried out on histologic sections, a source of error of at least theoretical importance is introduced by variation in thickness of these sections. This can be largely avoided by taking readings from a sufficient number of sections. Bahr (6) evaluated such variation as a source of error, and found it to be relatively unimportant if sections are carefully prepared. By taking readings from a number of different sections from the same specimen, we did not find important differences arising from variation in thickness of the sections.

A small positive error is introduced into photometric transmission readings by light refraction and absorption by tissue elements other than the elastin, even though these remain unstained, and by slide, coverslip and mounting medium. To compensate for this, as well as that of negative error introduced by incidence of stray light into the instrument, the 2 wave-length method, as described by K. Patau (7) is employed. The wave lengths are chosen so that their percentage transmission through the elastin-orcein conjugate stands in a ratio of 1:2. In this case, 1 = 6400 Å. Then, if I_0 represents the flux of light through a "blank" (*i.e.* slide, coverslip and mounting medium, as well as unstained tissue elements) and I_1 is the flux of light through field A, containing slide, coverslip, mounting medium, unstained tissue, and in addition, elastin-orcein complex to be measured, then:

$I_1/I_0 = T =$ transmission through field A, determined by the amount of elastin-orcein alone

The transmittance is determined for a blank field, and for a field containing elastin-orcein conjugate, at each of two wave lengths ($\lambda_1 =$ 6400 Å, $\lambda_2 =$ 5890 Å)

λ_1 yields readings $I_1 + I_0$

and

λ_2 yields readings $I_1' + I_0'$

This results in 2 transmission ratios, $T_1 = T_2$

$$T_1 = I_1/I_0 \qquad T_2 = I_1'/I_0'$$

and:

$$T_1/T_2 = Q = \text{relative amount of elastin-orcein conjugate.}$$

Note again that Q is an expression only of an arbitrary ratio, and does not represent an absolute value for the quantity of conjugate.

The extractive method also presents several limitations on attainment of complete accuracy in determination of the absolute quantity of elastin in any given specimen of skin. Since values are dependent on weight of tissue samples used, significant error is introduced by the heterogeneity of skin. While considerable effort is made toward accurate separation of epidermis from dermis, the presence or absence of adnexal structures in tissue samples of the small size employed introduces considerable variation in the ratio of dermis to epidermal or adnexal structures. This potential source of error can be compensated for in part by averaging results from a sufficiently large number of samples of any given skin, the number of samples needed to produce an accurate result depending, among other factors, upon the anatomic site of origin of skin specimen.

Another possible source of error in this method is that which may be introduced by variation in susceptibility to elastase digestion of elastins from different skin conditions. That there is considerable variation in such susceptibility is indicated by results from our studies. These will be reported in detail in other publications. It should be noted that elastase of bacterial rather than pancreatic origin was used. Bacterial elastase is a more uniformly active product than pancreatic elastase, which is probably a mixture of enzymes acting on more than one site of the protein core of elastic fibers (8). Furthermore, pancreatic elastase is subject to considerably greater variation from lot to lot than is bacterial elastase. It was therefore decided that the bacterial product would give more reproducible results, and it was used throughout these measurements.

After comparison of the photometric method of elastin quantitation to the enzymatic extraction technic, the former appears to offer several advantages. Thus formalin-fixed, paraffin-blocked specimens, readily available in collections in histopathology laboratories, can be used, while the enzymatic method depends on availability of an adequate supply of fresh or frozen tissue. Secondly, much smaller samples can be used for the photometric method and they are not destroyed during the process of measurement, but remain available for future use of the same tissue samples. An important advantage, of course, is that while the extractive technic measures only total elastin in a skin sample, the photometric method allows measurements of elastin content in separate dermal layers. In elastotic skin this may be a most important factor. Furthermore, because it permits selectivity of field, errors introduced by the presence of adnexal structures in skin samples are greatly reduced in the photometric method, as compared to the extractive method. The photometric method also avoids the possible error introduced by variation in elastase employed, and differences in susceptibility of elastic fibers to enzymatic digestion. Lastly, the photometric method is faster and simpler. An experienced worker can in the same amount of time quantitate elastin in many more specimens by the photometric than by the rather tedious extractive method, with statistical accuracy being correspondingly enhanced because a larger number of readings were available for averaging.

Adequate control specimens should be run simultaneously with both methods. If this is done, these methods, used either separately, or better, concurrently, offer opportunity for determination of at least relative quantities of elastin in normal and pathological skin.

PART II—MEASUREMENT OF ELASTIN IN SKIN FROM PERSONS OF DIFFERENT AGES

The effect of age on elastin has been a subject of interest to many investigators. A number of authors have considered that age results in degeneration of elastic fibers (9). On the other hand, Scarselli (10), using his enzymatic procedure, observed an apparent increase in the elastin content of pulmonary tissue with advancing age. An alternative interpretation of Scarselli's findings is that age makes elastic fibers more susceptible to the enzymatic digestion and so results in higher readings in a method depending on elastase digestion. In order to test this hypothesis, both the photometric and enzymatic methods described in

Part I were applied to bisected samples of skin taken from the side of the abdominal incision made during autopsy of 18 men.

Results of these determinations are shown in Table I. It will be seen that 10 of these males were less than 45 years of age at time of death, the ages ranging from 15 to 45 years, while 8 had attained more than 70 years of age. In performing the microphotometric determinations on tissue sections, readings were taken both vertically in relation to the plane of the epidermis, and at 3 different levels in the dermis, parallel to the epidermis. There is a high degree of correlation between results obtained by these two different methods, thus confirming the reliability of either method of sampling. A feature of interest is the lack of variation in the elastin content of the various dermal layers in skin from such an area as the abdomen, which has not been exposed to the effects of actinic radiation.

It will be noted that there is little difference in elastin content of skin in the younger group compared to the older, as determined by microphotometric observations. This is in marked contrast to results obtained by the method employing enzymatic digestion of elastin and colorimetric quantitation *in vitro*. By the latter method the readings were more than twice as high in the men over 70 than in those less than 45 years of age. For the over-70 group, the mean values for reading from 8 individuals was 484 (arbitrary) units, while the mean for 10 persons less than 45 years of age was only 211. Expressed as a percentage of soluble elastin in dry weight of whole skin, the mean value in the over-70 group was 9.4%, while for the younger men, the mean value was 4.2%.

TABLE I

Age of Patient	*ENZYMATIC METHOD*		*PHOTOMETRIC METHOD* Transmission expressed as arbitrary units				
	Colorimeter Readings (arbitrary unit)	% Soluble Elastin per Dry Weight of Skin	Vertical Readings	Transverse Readings			
				Upper Corium	Mid Corium	Lower Corium	Mean
15	.170	3.45%	64.51	73.75	63.71	68.44	68.63
20	.243	4.8	56.75	61.07	56.85	59.85	59.26
22	.244	4.85					
25	.152	3.05	55.08	60.42	55.85	58.77	58.39
33	.274	5.4	52.23	62.91	49.63	60.93	57.87
36	.231	4.7	63.38	71.28	59.74	65.14	65.38
36	.189	3.75	66.45	71.80	67.60	68.00	69.13
40	.181	3.6	65.86	69.14	65.00	68.00	67.38
42	.256	5.1	67.93	80.54	66.80	74.69	74.01
44	.193	3.85	64.82	67.26	55.90	63.22	62.12
44	.189	3.8					
Mean for Patients Under 45	.211	4.16%	61.89				64.70
71	.329	6.6%	54.98	66.94	54.39	60.49	60.60
71	.551	9.9	68.65	72.43	65.57	68.21	68.73
72	.617	11.7	69.30	76.50	64.64	68.90	70.01
72	.424	8.5	68.10	75.00	64.30	68.60	69.31
73	.426	8.52	68.34	68.50	64.00	74.00	68.83
73	.670	13.4	57.99	67.61	54.77	62.19	61.52
74	.452	9.0	52.86	66.20	52.30	57.84	58.78
81	.401	8.05	57.30	74.65	57.03	64.07	65.25
Mean for Patients Over 70	.484	9.4%	62.19				65.37

Thus, if only the enzymatic technic had been used, the erroneous interpretation might have been made that there is more elastin in senile than in young skin, whereas the differences are probably due only to an increased susceptibility of senile elastic fibers to elastase digestion. Therefore, Scarselli's findings in pulmonary tissue should be reconsidered in the light of these new observations.

SUMMARY AND CONCLUSIONS

Two methods for determination of relative quantities of elastin in skin specimens have been described. One depends on microphotometric measurements of orcein-stained elastin in histologic sections, the other on extraction of elastin from skin samples through enzymatic digestion, with colorimetric quantitation of extracted material. While both methods permit determination of relative elastin content of the dermis, it is concluded that the microphotometric method has important advantages over the enzymatic technic. Both technics require the use of adequate controls and statistical averaging of a number of determinations.

These methods were applied to quantitation of elastin in abdominal skin obtained at autopsy from men of varying ages. Comparison of the results from the microphotometric method indicated no higher measurements in skin of men over 70 than in those less than 45 years of age. The extractive method, on the other hand, produced readings more than twice as high in the over-70 group. It is concluded that the higher readings found by the extractive method in the older age group indicates an increased susceptibility of senile elastin to elastase digestion, rather than a real increase in its quantity, with age.

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