TRANSFORMATION OF COLLAGEN TO "ELASTIN" IN DERMAL COLLAGENS WITH VARYING SENSITIVITY TOWARDS COLLAGENASE

BY

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It has been reported previously (Keech, 1955) that the dermal collagen from eight out of sixty individuals failed to show any evidence of digestion by collagenase under the electron microscope, and that two others reacted excessively for their particular age groups. Indications of variable reactivity towards collagenase were also obtained from nitrogen determinations of the material digested (Keech, 1954a). It was also shown that in one case of dermatomyositis the rash-bearing skin was collagenase-resistant, whereas the abdominal skin from the same case digested normally. All these findings remained unexplained and appeared to be independent of the disease, therapy, and all the experimental procedures.

A small quantity of eight out of the ten abnormally-reacting substrates still remained, so a more detailed electron microscope investigation was undertaken to find out whether the samples shown to be resistant to the action of collagenase would also prove resistant to the action of alkali and periodate which apparently transforms collagen to elastin-like structures (Burton, Hall, Keech, Reed, Saxl, Tunbridge, and Wood, 1955; Hall, Keech, Reed, Saxl, Tunbridge, and Wood, 1955). Microshrinkage temperature experiments were performed as well as analytical studies on the amino acid composition of the various samples. More detailed characterization was accomplished by chromatography and hydroxyproline determinations.

Materials and Methods

Prepared Collagen.—Six of the eight collagenase-

resistant substrates and the two exhibiting excessive digestion which were used in previous work (Keech, 1955), together with five controls of comparable age showing average collagenase digestion, constituted the starting materials. Each was examined as described below and then treated in one of the following ways:

- (a) Suspended in sterile distilled water pH 5.6, heated at 37° C., and samples taken after 1½, 3, and 24 hrs.
- (b) Suspended in 0.2 M potassium hydrogen phthalate buffer, pH 5.0, heated at 37° C., and samples taken after 1½ and 3 hrs.
- (c) Suspended in a mixture of 1 per cent. sodium metaperiodate in phthalate buffer, pH 5.0, incubated at 37° C., and samples taken at 1½ and 3 hrs.
- (d) Suspended in 0.2 M borate buffer, pH 8.8, and incubated at 37° C. for 24 hrs.

Antibiotics.—0.05 ml. of a penicillin and streptomycin mixture was added to each test tube in the above experiments. This successfully prevented bacterial contamination.

Samples for Electron Microscopic Examination were ground gently in a glass tissue grinder until a milky suspension was obtained; drops were placed on collodion-covered 200-mesh copper grids, drained with filter paper, allowed to dry, washed with distilled water, shadowed with chromium, and examined in a Siemens electron microscope, type UM 60 C.

Counts of the different elastin-like structures were made by carefully scanning two grids from each sample. The specimen holder had been adjusted to visualize 25 squares of the grid, and each part of each square was examined in sequence. As each square was scanned at a magnification of 11,000 and as about 25 fields were needed to cover each square, a *minimum* of 2 × 625 fields were scrutinized from each specimen.

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Attempts were made to obtain approximately the same amount of deposit on all the grids from all the samples, so that valid comparisons could be made. This proved difficult, but the majority of the grids were classified as bearing a "heavy" deposit, and where variations in density affect comparisons in the counts, this has been indicated in both Tables and text.

The above experiments were performed in large batches, treating several different substrates from each group in each of the ways described at the same time. This ensured that exactly the same conditions (chemical solutions, incubation temperature, etc.) appertained to the reactive and resistant materials alike. Again, electron microscope examination of all the experiments on the same substrate were undertaken within 2 to 3 days, for ease of comparison.

Terminology.—The presence of several types of "elastin" in the materials used in this study is somewhat confusing. For clarity they were classified as follows:

(a) Naturally-occurring elastin or fully-formed elastin (Fig. 14) as described previously (Keech, Reed, and Wood, 1956) comprising three variants (skin-type, filamenting, and natural networks) which are found in fresh whole dermis and prepared dermis from all age groups, aorta, tendon, and ox ligamentum nuchae. This appears in the text without quotation marks. The term *fully-formed elastin* bears no genetic implication, but simply indicates that it is found in all fresh and prepared starting materials.

(b) Morphologically similar structures, seen after a variety of procedures, are referred to as *fully-formed "elastin"* or *elastin-like structures*. There is much evidence that these structures resemble the naturally-occurring elastin described under (a) in chemical properties as well as in morphology (Burton and others, 1955; Hall and others, 1955). However, until this point is fully established, quotation marks are used.

(c) A larger form, composed of a very thick, dense portion splaying out into typical skin-type or filamenting elastin (Figs 26-35). These frequently stretched over several microscopic fields, measured 20-30 μ in length, and formed a distinct group which proved the most sensitive index of a true transformation picture (see below). The term *transformation structure* (TS) is used for this group, implying that they exhibit one or more of the various morphological characteristics of "elastin". As already reported (Keech and Reed, 1957), large, dense, square-ended fibres are present in all the starting materials of both fresh whole dermis as well as the prepared substrates. As shown in the published illustrations, prolonged heat at or below body temperature gradually converted some of these fibres into TS, their appearance depending on the amount of electron-opaque amorphous material coating the elastin filaments. However, they were found in far greater numbers following the reagents (borate, periodate and phthalate) used in the present study.

A *transformation picture* (Figs 36 and 37) denotes,

therefore, a quantitative and qualitative change in the whole deposit, characterized by:

- (i) A smaller amount of collagen, the majority of which showed evidence of degeneration;
- (ii) An increase in skin-type "elastin" above that which could be accounted for by heat alone;
- (iii) An increase in TS (or in large networks in the substrate from the infant D12: see Table I).

Qualitatively, the appearance of the deposit was quite distinctive, the elastin-like material covering most of the grid in the samples recorded as presenting a marked transformation picture.

The word "transform" is thus used to denote the apparent transformation of collagen to elastin-like structures reported previously (Burton and others, 1955; Keech and Reed, 1957) as well as in the present study. The term "transformation structure" (TS) was first used to describe the conversion of the large, dense fibres present in the starting materials to "elastin" (Keech and Reed, 1957). The same name is now used for the identical structures found in greater numbers in the present investigation. A "transformation picture" (defined above) refers to a change in the whole deposit, the mainly collagenous starting material being replaced by elastin-like structures. Part of this change was accomplished by the conversion of the large, dense fibres to TS.

It is important to make it quite clear that the terms defined above are used descriptively and as an aid in classifying the range of structures counted and recorded in Tables I and II. They are *not* intended to imply any underlying mechanism or biological process.

Results

The results are summarized in Tables I to III, where Group I comprises substrates exhibiting average collagenase digestion, Group II comprises collagenase-resistant substrates, and Group III comprises those showing excessive digestion by collagenase.

Starting Materials (Figs 1-8)

These showed marked differences between each group, as well as age differences within Group I, the latter giving the typical pictures of child and adult prepared dermis. In the younger subjects the slightly thinner collagen fibrils were usually found in clumps, mixed with, or partially obscured by, the plentiful amorphous material and dense bits. In contrast, the collagen in a 56-year-old adult was in long, winding bundles of clean fibrils with very little associated amorphous material or dense bits. The substrate from the infant (D12) contained nearly as much filamenting as skin-type elastin (a common finding in very young subjects), whereas filamenting formed only a small fraction of the total

fully-formed elastin in the rest. In addition many structures were intermediate between the four variants of elastin listed in Table I, but (as has been noted in previous work) the action of heat alone, or

heat plus other reagents, rapidly rendered them typical of one particular variant and hence easy to categorize. The 56-year-old dermis (D15) had scanty, long, thick pieces of filamenting elastin which

TABLE I

RESULTS OF VARIOUS TREATMENTS TO PREPARED DERMAL SUBSTRATES WHICH DIFFERED IN SENSITIVITY TOWARDS COLLAGENASE

AT LEAST 1,250 MICROSCOPIC FIELDS WERE EXAMINED AT EACH TIME-INTERVAL

GROUP I. SUBSTRATES EXHIBITING AVERAGE COLLAGENASE DIGESTION (CONTROL GROUP)

Substrate and Treatment (hrs)		Fully-Formed Elastin			TS	Fine Threads	Dense Bits	Amor-phous Material	Grid De-posit	Collagen	Elastin	Remarks	
		S	F	N									
D12 20 mths (Normal Control)													
D12 ₁ Starting Material in H ₂ O		53	39	4	34	V.Sc.	Num.	Mod.	H	Typical child substrate. In clumps with AM and DB	Difficult to categorize as many intermediate forms. Mostly small pieces counted		
D12 ₅ Heat only in H ₂ O at 37° C.	1½	138	13	11	38	Mod.	Num.	Mod.	H	Mostly unaltered	Majority counted were tiny pieces	No TP	
	3	259	2	3	19	V.Sc.	Num.	Mod.	H	Mostly unaltered	Majority counted were tiny pieces Only small TS	No TP	
	24	637	10	28	86	Sc.	Num.	Mod.	VH	Fair amount starting to degenerate	Large TS V. large networks and long pieces F	Marked TP	
D12 ₃ Buffer (pH 5·0)		3	301	20	85	90	Sc.	Num.	Mod.	MH	Fair amount starting to degenerate	* See footnote	Marked TP
D12 ₄ Periodate (pH 5·0)		3	397	16	55	93	V.Sc.	Num.	Mod.	H	Fair amount starting to degenerate	* See footnote	Marked TP
D12 ₂ Buffer (pH 8·8)		24	286	52	3	91	V.Sc.	Num.	Inc.	H	Some starting to degenerate	No large "networks". Some mod. large TS	Mod. TP. Not as marked as D12 ₃ and D12 ₄
D13 9 yrs (Normal Control)													
D13 ₁ Starting Material in H ₂ O		86	6	7	29	V.Sc.	Num.	Mod.	H	Typical child substrate. In clumps with AM and DB	N of mod. size		
D13 ₅ Heat Only in H ₂ O at 37° C.	1½	130	14	7	39	Sc.	Num.	Mod.	H	Mostly unaltered		No TP	
	3	160	7	—	14	Sc.	Num.	Mod.	H	Mostly unaltered	Nearly all small or mod. in size	No TP	
	24	168	16	1	53	Sc.	Num.	Mod.	H	Mostly unaltered		No TP	
D13 ₃ Buffer (pH 5·0)		3	469	10	21	108	Sc.	Num.	Mod.	H	Some starting to degenerate	Many large TS. Most of the N were of mod. size	Marked TP
D13 ₄ Periodate (pH 5·0)		3	470	15	8	56	V.Sc.	Num.	Mod.	H	Some starting to degenerate	Nearly all small or mod. in size	Not such a marked TP as D13 ₃
D13 ₂ Buffer (pH 8·8)		24	321	32	20	109	Mod.	Num.	Inc.	H	Some starting to degenerate	Several large TS and a few networks as in D12	Mod. TP

continued—

TABLE I (continued)

D14 19 yrs (Normal Control)												
D14 ₁ Starting Material in H ₂ O	219	23	24	87	V.Sc.	Num.	Mod.	H	In clumps mixed with AM and DB	Nearly all small or mod. in size	A few large TS	
D14 ₅ Heat Only in H ₂ O at 37° C.	1½	208	11	8	66	Sc.	Num.	Mod.	H	Mostlyunaltered	Nearly all small or mod. in size	Like starting material. No TP
	3	268	10	3	57	Mod.	Num.	Mod.	H	Mostlyunaltered	Nearly all small or mod. in size	No TP
	24	183	5	4	96	V.V.Sc.	Num.	Mod.	H	Mostlyunaltered	Nearly all small or mod. in size	No TP
D14 ₃ Buffer (pH 5·0)	1½	Not done										
D14 ₄ Periodate (pH 5·0)	1½	Not done										
D14 ₃ Buffer (pH 5·0)	3	380	7	5	135	V.Sc.	Num.	Mod.	H	Some starting to degenerate	Inc. in large TS	Marked TP
D14 ₄ Periodate (pH 5·0)	3	361	3	5	95	V.Sc.	Num.	Mod.	H	Some starting to degenerate	Not so many large TS	Mod. TP
D14 ₂ Buffer (pH 8·8)	24	362	14	3	123	Mod.	Num.	Inc.	H	Some starting to degenerate	Inc. in mod. sized TS	Mod. TP
D5 43 yrs (Normal Control)												
D5 ₁ Starting Material in H ₂ O	67	1	1	18	None	Few	Mod.	VH	In clumps mixed with AM	Majority counted were tiny pieces		
D5 ₅ Heat Only in H ₂ O at 37° C.	1½	176	2	—	28	None	Mod.	Mod.	VH	Mostlyunaltered	Majority counted were tiny pieces	No TP
	3	350	2	—	35	None	Mod.	Mod.	VH	Mostlyunaltered	Majority counted were tiny pieces	No TP
	24	109	—	—	21	V.Sc.	Less	Less	MH	Mostlyunaltered	Majority counted were tiny pieces	No TP Lighter deposit
D5 ₃ Buffer (pH 5·0)	1½	243	2	1	36	None	Mod.	Mod.	H	Mostlyunaltered	Some large TS	Mod. TP
D5 ₄ Periodate (pH 5·0)	1½	254	1	—	60	None	Mod.	Mod.	H	Mostlyunaltered	Some large TS	Mod. TP
D5 ₃ Buffer (pH 5·0)	3	403	11	1	86	V.Sc.	Mod.	Mod.	H	Mostlyunaltered	Some large TS	Mod. TP
D5 ₄ Periodate (pH 5·0)	3	121	1	—	17	None	Mod.	Mod.	H	Mostlyunaltered	Like starting material	Unexpected picture
D5 ₂ Buffer (pH 8·8)	24	348	6	2	49	V.Sc.	Mod.	Mod.	VH	Mostlyunaltered	Many tiny pieces Some v. large TS	Mod. TP
D15 56 yrs (Normal Control)												
D15 ₁ Starting Material in H ₂ O	128	16	6	28	Sc.	Few	Sc.	H	"Adult type" unaltered collagen often in bundles	Tiny pieces S. Long thick pieces F. Scanty, small atypical TS		
D15 ₅ Heat Only in H ₂ O at 37° C.	1½	181	9	4	41	V.Sc.	Mod.	Mod.	H	Mostlyunaltered	Small or mod. sized typical TS	Like starting material. No TP
	3	202	4	3	47	V.Sc.	Mod.	Mod.	H	Mostlyunaltered	A few mod. large TS	No TP
	24	244	21	9	57	Sc.	Mod.	Mod.	H	Mostlyunaltered	Inc. in small and mod. sized S	No TP
Buffer (pH 5·0)	1½	Not done										
Periodate (pH 5·0)	1½	Not done										
D15 ₃ Buffer (pH 5·0)	3	296	10	1	27	V.Sc.	Mod.	Mod.	H	Mostlyunaltered	Inc. in small and mod. sized S	No TP
D15 ₄ Periodate (pH 5·0)	3	318	4	3	39	V.Sc.	Mod.	Mod.	H	Mostlyunaltered	Mod. large typical TS	No TP
D15 ₂ Buffer (pH 8·8)	24	280	17	3	34	Sc.	Inc.	Inc.	H	Mostlyunaltered	Inc. in small and mod. sized S	No TP

TABLE 1 (continued)

GROUP II. SUBSTRATES EXHIBITING COLLAGENASE RESISTANCE

Substrate and Treatment (hrs)		Fully-Formed Elastin			TS	Fine Threads	Dense Bits	Amorphous Material	Grid Deposit	Collagen	Elastin	Remarks
		S	F	N								
D8 19 mths (Indigestible)												
D8 ₁ Starting Material in H ₂ O ..	70	7	—	12	None	Few	Mod.	H	"Adult type", mostly clean, in bundles and unaltered	Nearly all L	Very unlike usual baby substrate	
D8 ₅ Heat Only in H ₂ O at 37° C.	1½	Not done										
	3	99	9	5	34	V.Sc.	V. few	Sc.	MH	Same as starting material	Inc. in large TS and some large N	No true TP
	24	87	3	—	29	None	V. few	Sc.	MH	Same as starting material	Some mod. large TS	No true TP
D8 ₃ Buffer (pH 5·0)	3	169	2	2	115	None	Few	Sc.	H	Some in clumps or starting to degenerate	Marked inc. in large TS	Marked TP
D8 ₄ Periodate (pH 5·0)	3	64	2	2	24	V.V.Sc.	Few	Sc.	MH	Some in clumps or starting to degenerate	Less than D8 ₃	Slight TP
D8 ₂ Buffer (pH 8·8)	24	35	3	—	27	V.Sc.	Inc.	Mod.	MH	Some in clumps or starting to degenerate		No TP
D11 5½ yrs (Indigestible)												
D11 ₁ Starting Material in H ₂ O ..	57	—	—	36	None	Few	Mod.	H	"Adult type", mostly clean, in bundles and unaltered	Mod. number of TS		
D11 ₅ Heat Only in H ₂ O at 37° C.	1½	Not done										
	3	88	2	—	60	V.Sc.	Few	Mod.	H	Same as starting material	Inc. in mod. large TS	No true TP
	24	114	3	—	65	V.Sc.	Few	Mod.	H	Same as starting material		No true TP
D11 ₃ Buffer (pH 5·0)	3	58	1	—	28	None	Few	Mod.	H	Same as starting material		No true TP
D11 ₄ Periodate (pH 5·0)	3	85	2	—	44	None	Few	Mod.	H	Same as starting material	Nearly all tiny pieces	No true TP
D11 ₂ Buffer (pH 8·8)	24	132	1	1	71	None	Inc.	Inc.	VH	Same as starting material	Inc. in large TS	VH deposit No true TP
D9 9½ yrs (Indigestible)												
D9 ₁ Starting Material in H ₂ O ..	41	—	—	16	None	V. few	V.Sc.	H	"Adult type", clean, in bundles and unaltered		Very similar to D10	
D9 ₅ Heat Only in H ₂ O at 37° C.	1½	Not done										
	3	87	5	—	30	V.Sc.	V. few	V.Sc.	H	Same as starting material		No TP
	24	51	1	—	43	V.Sc.	V. few	V.Sc.	MH	Same as starting material	Increase in TS some quite large	Lighter deposit No TP
D9 ₃ Buffer (pH 5·0)	3	101	2	—	64	V.V.Sc.	V. few	Sc.	H	Same as starting material	Inc. in mod. sized TS and S	Mod. TP
D9 ₄ Periodate (pH 5·0)	3	57	3	—	25	V.V.Sc.	V. few	Sc.	MH	Same as starting material		Lighter deposit Slight TP
D9 ₂ Buffer (pH 8·8)	24	60	1	—	28	V.Sc.	Few	Sc.	MH	Same as starting material		No TP

continued—

TABLE 1 (continued)

D10 13 yrs (Indigestible)												
D10 ₁ Starting Material in H ₂ O	23	3	—	10	None	V. few	V.Sc.	H	"Adult type", clean, in bundles and unaltered			Very similar to D9
D10 ₅ Heat Only in H ₂ O at 37° C.	1½	Not done										
	3	27	2	—	15	None	V. few	V.Sc.	H	Same as starting material		No TP
	24	51	3	—	19	None	V. few	V.Sc.	H	Same as starting material	Inc. in S	No TP
D10 ₃ Buffer (pH 5·0)	3	28	—	—	19	None	V. few	V.Sc.	H	Same as starting material	Nearly all tiny pieces	No TP
D10 ₄ Periodate (pH 5·0)	3	21	—	—	15	None	V. few	V.Sc.	MH	Same as starting material		No TP
D10 ₂ Buffer (pH 8·8)	24	79	—	—	27	None	Few	Sc.	H	Same as starting material	Nearly all L.	No TP
D4 18 yrs (Indigestible)												
D4 ₁ Starting Material in H ₂ O	21	1	—	13	None	V. few	V.V.Sc.	H	"Adult type", clean, in bundles and unaltered			Almost identical to D3
D4 ₅ Heat Only in H ₂ O at 37° C.	1½	40	2	—	7	None	V. few	V.V.Sc.	H	Same as starting material		No TP
	3	39	6	—	9	None	Few	V.Sc.	H	Same as starting material		No TP
	24	177	—	—	51	V.Sc.	Few	V.Sc.	VH	Same as starting material	Inc. in mod. or large pieces of L	VH deposit No TP
D4 ₃ Buffer (pH 5·0)	1½	28	1	—	10	None	V. few	V.V.Sc.	H	Same as starting material	Nearly all tiny L	No TP
D4 ₄ Periodate (pH 5·0)	1½	40	1	—	11	None	V. few	V.Sc.	H	Same as starting material	Nearly all tiny L	No TP
D4 ₃ Buffer (pH 5·0)	3	38	2	—	17	None	V. few	V.Sc.	VH	Same as starting material	Some L	No TP
D4 ₄ Buffer (pH 5·0)	3	32	—	—	10	None	V. few	V.Sc.	H	Same as starting material	Some L	No TP
D4 ₂ Buffer (pH 8·8)	24	73	1	—	10	None	V. few	V.Sc.	H	Same as starting material	Mostly L	No TP
D3 51 yrs (Indigestible)												
D3 ₁ Starting Material in H ₂ O	22	—	—	3	V.Sc.	V. few	V.V.Sc.	H	"Adult type", clean, in bundles and unaltered	Nearly all L		Almost identical to D4
D3 ₅ Heat Only in H ₂ O at 37° C.	1½	50	—	1	25	V.V.Sc.	V. few	V.Sc.	H	Same as starting material	Nearly all L	No TP
	3	64	1	—	22	V.V.Sc.	V. few	V.Sc.	H	Same as starting material	Nearly all L	No TP
	24	113	—	1	18	V.V.Sc.	Few	Sc.	H	Same as starting material	Inc. in L	No TP
D3 ₃ Buffer (pH 5·0)	1½	50	5	—	8	V.Sc.	V. few	V.V.Sc.	VH	Same as starting material	Nearly all L	No TP
D3 ₄ Periodate (pH 5·0)	1½	27	1	—	13	V.V.Sc.	V. few	Sc.	VH	Same as starting material	Nearly all L	No TP
D3 ₃ Buffer (pH 5·0)	3	135	2	—	31	None	V. few	V.Sc.	VH	Same as starting material	Inc. in L	No TP
D3 ₄ Periodate (pH 5·0)	3	53	1	—	5	None	V. few	V.Sc.	H	Same as starting material	Lighter deposit	No TP
D3 ₂ Buffer (pH 8·8)	24	127	3	—	20	Sc.	V. few	Sc.	VH	Same as starting material	Nearly all L	VH deposit No TP

TABLE I (continued)

GROUP III. SUBSTRATES EXHIBITING EXCESSIVE COLLAGENASE DIGESTION

Substrate and Treatment (hrs)		Fully-Formed Elastin			TS	Fine Threads	Dense Bits	Amorphous Material	Grid Deposit	Collagen		Elastin	Remarks
		S	F	N									
D6 20 yrs (Overdigestible)													
D6 ₁	Starting Material in H ₂ O	65	1	—	3	Sc.	Few	Mod.	H	In clumps mixed with AM and DB. Some degeneration		Nearly all tiny pieces Only three TS	Resembled a control child substrate
D6 ₅	Heat Only in H ₂ O at 37°C.	1½	157	4	5	34	Sc.	Few	Mod.	MH	Same as starting material	Inc. in mod. large TS	No true TP
		3	138	3	6	29	Sc.	Few	Mod.	H	Same as starting material	A few large TS	No true TP
		24	136	4	4	32	Mod.	Few	Mod.	MH	More collagen starting to degenerate	A few large TS	No true TP
D6 ₃	Buffer (pH 5.0)	1½	Not done										
D6 ₄	Periodate (pH 5.0)	1½	Not done										
D6 ₃	Buffer (pH 5.0)	3	298	1	—	56	Sc.	Marked inc.	Marked inc.	VH	More collagen starting to degenerate	Inc. in large TS	Very marked TP
D6 ₄	Periodate (pH 5.0)	3	456	3	1	33	Sc.	Slight inc.	Slight inc.	H	Some starting to degenerate	Some large TS	Mod. TP
D6 ₂	Buffer (pH 8.8)	24	171	7	—	38	Many	Slight inc.	Slight inc.	MH	Some starting to degenerate	Some large TS	Lighter deposit Mod. TP
D7 52 yrs (Overdigestible)													
D7 ₁	Starting Material in H ₂ O	102	—	2	12	Sc.	Few	Mod.	H	Both separate and in clumps with AM. A little degen.		Nearly all tiny pieces	Resembled a young adult control substrate
D7 ₅	Heat Only in H ₂ O at 37°C.	1½	119	1	1	24	None	Few	Mod.	VH	Same as starting material	Nearly all tiny pieces	No TP
		3	268	7	2	36	None	Inc.	Inc.	VH	More coll. degen.	Several large TS	No true TP
		24	82	1	2	18	Sc.	Few	Mod.	MH	More coll. degen.	Lighter deposit	No true TP
D7 ₃	Buffer (pH 5.0)	1½	445	5	1	51	None	Marked inc.	Marked inc.	H	Some coll. degen.	Inc. in TS and S	Marked TP
D7 ₄	Periodate (pH 5.0)	1½	311	5	2	39	Sc.	Mod. inc.	Mod. inc.	H	Some coll. degen.	Inc. in TS and S	Mod. TP
D7 ₃	Buffer (pH 5.0)	3	265	8	1	51	V.V.Sc.	Mod. inc.	Mod. inc.	H	Some coll. degen.	Some large TS	Mod. TP
D7 ₄	Periodate (pH 5.0)	3	235	2	1	30	V.V.Sc.	Mod. inc.	Mod. inc.	H	Some coll. degen.		Mod. TP
D7 ₂	Buffer (pH 8.8)	24	252	9	2	47	Sc.	Mod. inc.	Mod. inc.	H	Some coll. degen.	Nearly all tiny pieces of S	Mod. TP

* The N recorded were very large "networks" representing the completed conversion of the transformation structures (TS) into "Elastin". The division between some of the TS and these large "networks" was arbitrary.

L = tiny pieces of elastin presenting a stippled or lumpy appearance (as in Fig. 13).

S = skin-type elastin; F = filamenting elastin; N = natural elastin network; Num. = numerous; Mod. = moderate; TS = transformation structure; TP = transformation picture; DB = dense bits; AM = amorphous material; H = heavy; VH = very heavy; MH = moderately heavy; V.Sc. = very scanty; degen. = degenerating; Inc. = increased.

Total "elastin" counts	{	from the two substrates in Group III	=	4,124	} 21,576
		from the six substrates in Group II	=	4,006	
		from the five substrates in Group I	=	13,446	

Substrate and Treatment (hrs)	Group I Average Collagenase Digestion									
	Fully-Formed Elastin			TS	Grid Deposit	"Elastin" Increase	Transformation Picture	Fully-Formed Elastin		
	S	F	N					S	F	N
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24	D12 20 mths 2-3 5 12	— — —	— — 7	— H H VH	Unexpected picture. VH deposit Sig. inc. S, N, and TS Sig. inc. S, N, and TS No inc. over unexpected 24-hr heat-only picture.	Marked Marked Marked Mod.	D8 19 mths Not done 1-1½ 1-1½	— — —	— — —
Buffer (pH 5.0)	3	6	—	21	3			2-3	—	—
Periodate (pH 5.0)	3	8	—	13	3			—	—	—
Buffer (pH 8.8)	24	5-6	1½	—	3			—	—	—
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24							D11 5½ yrs Not done 1½ 2	— — —	— — —
Buffer (pH 5.0)	3							—	—	—
Periodate (pH 5.0)	3							1½	—	—
Buffer (pH 8.8)	24							2-3	—	—
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24	D13 9 yrs 1-2 2 2	2 — 2	— — —	— H H H	Sig. inc. S, N, and TS Sig. inc. S and TS Sig. inc. S, F, N, and TS	Marked Mod. Mod.	D9 9½ yrs Not done 2	— — —	— — —
Buffer (pH 5.0)	3	5-6	—	3	3-4			2-3	—	—
Periodate (pH 5.0)	3	5-6	2	—	2			1-2	—	—
Buffer (pH 8.8)	24	4	5	3	3-4			—	—	—
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24							D10 13 yrs 2 — 3	— — —	— — —
Buffer (pH 5.0)	3							—	—	—
Periodate (pH 5.0)	3							—	—	—
Buffer (pH 8.8)	24							—	—	—
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24	D14 19 yrs — — —	— — —	— — —	— H H H	Unusually high counts Slight inc. S and TS Slight inc. S and TS Slight inc. S and TS	Marked Mod. Mod.	D4 18 yrs 2 2 8	— — —	— — —
Buffer (pH 5.0)	1½	Not done	—	—	—			2	—	—
Periodate (pH 5.0)	1½	Not done	—	—	—			2	—	—
Buffer (pH 5.0)	3	1-2	—	—	1-2			1-2	—	—
Periodate (pH 5.0)	3	1-2	—	—	1-2			3-4	—	—
Buffer (pH 8.8)	24	1-2	—	—	1-2			—	—	—
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24	D5 43 yrs 2-3 5 1-2	— — —	— — —	1-2 2 —	Mod. inc. S and TS (H deposit) Mod. inc. S and TS (H deposit) Mod. inc. S and TS (H deposit) No sig. inc. (unexpected picture) Mod. inc. S and TS (VH deposit)	Mod. Mod. Mod. — Mod.			
Buffer (pH 5.0)	1½	3-4	—	—	2					
Periodate (pH 5.0)	1½	3-4	—	—	3					
Buffer (pH 5.0)	3	6	—	—	5					
Periodate (pH 5.0)	3	1-2	—	—	—					
Buffer (pH 8.8)	24	5	—	—	3					
Starting Material Heat only in H ₂ O at 37° C.	1½ 3 24	D15 56 yrs — 1-2 1-2	— — —	— — —	— 1-2 2	Slight inc. small pieces S Slight inc. small pieces S Slight inc. small pieces S	None None None	D3 51 yrs 2 3 5 3	— — — —	— — — —
Buffer (pH 5.0)	1½	Not done	—	—	—			6	—	—
Periodate (pH 5.0)	1½	Not done	—	—	—			2-3	—	—
Buffer (pH 5.0)	3	2-3	—	—	—			5-6	—	—
Periodate (pH 5.0)	3	2-3	—	—	—			—	—	—
Buffer (pH 8.8)	24	2-3	—	—	—			—	—	—

Figures represent number of times "elastin" content increased over that present in starting material.

S = Skin-type elastin.

F = Filamenting elastin.

N = Natural elastin network.

TS = Transformation structure.

Mod. = Moderate.

LE II

TIN" COUNTS FROM ALL GROUPS

Group II Collagenase-Resistant				Group III Excessive Collagenase Digestion						
TS	Grid Deposit	"Elastin" Increase	Trans- formation Picture	Fully-Formed Elastin			TS	Grid Deposit	"Elastin" Increase	Trans- formation Picture
				S	F	N				
3 2-3	H MH MH									
10 2 2	H MH MH	Sig. inc. S and TS No sig. inc. No sig. inc.	Marked None None							
2 2 — — 2	H H H H VH	No sig. inc. No sig. inc. No sig. inc.	None None None							
2 2-3 4 1-2 1-2	H MH H MH MH	Sig. inc. TS No sig. inc. (MH deposit) No sig. inc. (MH deposit)	Mod. None None							
1-2 1-2 1-2 1-2 2	H H H H MH H	No sig. inc. No sig. inc. V. slight inc. S	None None None							
— — 3-4 — — — —	H H H VH H H VH H H	No sig. inc. No sig. inc. No sig. inc. No sig. inc.	None None None None None	D6 20 yrs 2-3 2 2 Not done Not done 4-5 7 2-3	— — — — — — — —	— — — — — — — —	11 9 10 18 11 12	H MH H MH VH H MH	Sig. inc. S and TS Sig. inc. S and TS Mod. inc. S and TS	V. marked Mod. Mod.
8 7 6 2 4 10 — 6	H H H H VH VH VH H VH	No sig. inc. (VH deposit) No sig. inc. (VH deposit) Slight inc. only (VH deposit) No sig. inc. No sig. inc.	None None None None None	D7 52 yrs — 2-3 — 4-5 3 2-3 2-3 2-3	— — — — — — — —	— — — — — — — —	2 3 — 4 3 4 2-3 4	H VH VH MH H H H H H	Sig. inc. S and TS Sig. inc. S and TS Sl. inc. S and TS (H deposit) Sl. inc. S and TS (H deposit) Mod. inc. S and TS (H deposit)	Marked Mod. Mod. Mod. Mod.

"Elastin" increase column estimated by subtracting relevant heat-only increase and allowing for variations in density of grid deposit.

Sl. = Slight.
H = Heavy.
VH = Very heavy.
MH = Moderately heavy.
Sig. inc. = Significant increase.

TABLE III
SUMMARIZED ELECTRON MICROSCOPIC CHARACTERISTICS OF THE THREE GROUPS

Group	I Average Collagenase Digestion	II Collagenase- Resistant	III Excessive Collagenase Digestion
Starting Material	Usual age differences In young subjects collagen often in clumps with abundant amorphous material and dense bits In older subjects cleaner collagen in bundles with less amorphous material and dense bits Elastin tended to increase with age	Very little age difference All very similar, consisting of bundles of clean collagen and hardly any amorphous material and dense bits Elastin tended to decrease with age	Resembled Group I specimens from younger age groups; e.g. 20-yr-old was similar to substrate from a child control
Elastin		Much scantier than Groups I and III after all treatments High proportion of L* Many small bundles of collagen resembling elastin (Fig. 9)	
Response to Heating in H ₂ O up to 24 hrs at 37° C.	Slight increase in skin-type "elastin" No transformation picture	Slight increase in skin-type "elastin" and transformation structures No transformation picture	Slight increase in skin-type "elastin" and transformation structures No transformation picture
Response to Borate Buffer (pH 8.8) 24 hrs at 37° C.	Moderate transformation picture in children and young adults Increase in amorphous material and dense bits	No effect except slight increase in amorphous material and dense bits	Moderate transformation picture equal to younger control subjects
Response to Phthalate Buffer (pH 5.0) 3 hrs at 37° C.	Marked transformation picture under 20 yrs, moderate in 43-yr-old, and absent in 56-yr-old	Marked transformation picture in infant and moderate in 9½-yr-old Otherwise no effect	Transformation picture very marked in 20-yr-old and marked in 52-yr-old
Response to Periodate in Phthalate Buffer (pH 5.0) 3 hrs at 37° C.	Less effect in same substrates as responded to phthalate buffer alone	No effect	Less effect than to phthalate buffer alone, giving moderate transformation picture

* L = tiny pieces of elastin presenting a stippled or lumpy appearance as illustrated in Fig. 13.

were seen throughout all the experiments performed on this particular substrate.

A totally different picture was found in the group that did *not* digest after incubation with collagenase (Group II). The starting materials were very similar to each other (Figs 2, 3, 4, and 7). Even the substrate from the infant (D8, Fig. 2) closely resembled that found in the adults, with bundles of clean, unaltered collagen fibrils. There was also a notable absence of amorphous material and dense bits, which caused small bundles of collagen to resemble filamenting elastin on the electron microscopic viewing screen. Continual examination through the magnifying eyepiece was necessary to get a correct "elastin" count throughout this group (Fig. 9).

The two substrates exhibiting excessive collagenase digestion (Group III) resembled younger specimens of the control group: the 20-year-old (D6, dead of fulminating acute rheumatic carditis) could be mistaken for a child's dermis, and the 52-year-old (D7, who had received intravenous nitrogen mustard 4 days before death as therapy for

carcinoma of the lung) resembled the dermis of a young adult.

In all three groups, the majority of the "elastin" counted was in tiny pieces, and frequently presented a stippled or lumpy appearance in Group II (Fig. 13a-c). As already mentioned, occasional large, dense, square-ended fibres, similar to those found in untreated, fresh whole dermis (Keech and Reed, 1957) were seen in all the prepared substrates used in the present study (Figs 10-12). The transformation structures (TS), described above and present in all the prepared starting materials, appear to be an altered form of these naturally-occurring dermal components.

In Groups I and III the elastin content of the starting material showed a tendency to increase with age: an exception was the unexpectedly high count in the 19-year-old substrate (D14). In Group II not only was the elastin scanty but the trend appeared to be in the reverse direction.

Treatment in H₂O at 37° C.—Table I shows that heat alone produced a progressive increase in

The scale marked on all the figures represents 1μ and all the preparations were shadowed with chromium

Fig. 1.—Prepared abdominal skin collagen from an infant aged 20 months (Control group D12). This starting material shows the striated collagen fibrils mixed with, and partially obscured by, the plentiful amorphous material and dense bits.

Fig. 2.—Starting material from an infant aged 19 months (Collagenase-resistant group D8). In contrast to Fig. 1 the substrate consists of bundles of clean collagen fibrils with a notable absence of amorphous material and dense bits.

skin-type "elastin" counts in all groups, directly proportional to the length of incubation. This effect, therefore, must be taken into account when assessing the action of any chemical treatment. Group II reacted least, whereas Group III and the youngest member of Group I gave the greatest reactions. In fact, the 24-hr picture in D12_s was unexpected and gave a much higher count than any other treatment, aided by a very heavy grid deposit. Another substrate (the 20-year-old D6 from Group III that resembled a control child dermis) reacted in a similar fashion, together with a marked increase in TS, in spite of only moderately heavy grid deposits. Apart from those just mentioned, the majority of the substrates showed an increase in skin-type "elastin" two or three times greater than that present in the starting material (Table II), this increase consisting almost entirely of tiny or moderately-sized pieces and *not* constituting a transformation picture as defined above.

N (Fig. 14), mentioned in the elastin columns of Table I, denotes the medium-sized, compact structures previously described (Keech, Reed, and Wood, 1956). However, heat alone on the substrates from both the infants (D12 and D8) produced large networks, sometimes stretching over several microscopic fields (20-30 μ in length), and composed of loosely-knit, winding bundles of filamenting "elastin" (Fig. 15). This was much more marked in D12 than D8.

Effect of Phthalate Buffer (pH 5.0) Alone at 37° C. (Tables I and II).—3-hr incubation samples were examined throughout all groups, but only four substrates were examined at 1½ hrs, as it was found that a better response occurred with longer incubation. A significant increase (above that which could be accounted for by heat alone) was found in both skin-type and TS throughout Groups I and III, and in two of the younger subjects gave the

Fig. 3.—Starting material from an individual aged 18 years (Collagenase-resistant group D4). Again the substrate is typical of Group II. Compare with Figs 2, 4, and 7.

Fig. 4.—Starting material from a 13-year-old substrate (Collagenase-resistant group D10). This is again typical of Group II. The arrow indicates part of a collagen fibril under tension, as illustrated and described previously (Keech and others, 1956).

Fig. 5.—Starting material from an individual aged 19 years (Control group D14). The collagen is mixed with a moderate amount of amorphous material and dense bits.

Fig. 6.—Starting material from a 20-year-old substrate (Group exhibiting excessive collagenase digestion D6). Compared with the control of the same age (Fig. 5), a fair number of the collagen fibrils are starting to degenerate, and the general picture on the electron microscopic viewing screen resembled younger specimens from the control group.

Fig. 7.—Starting material from an individual aged 51 years (Collagenase-resistant group D3), showing the substrate typical of this group. Compare with Fig. 8.

Fig. 8.—Starting material from a 56-year-old adult (Control group). The collagen is mixed with a small amount of amorphous material, and a few dense bits. Compare with Fig. 7.

Fig. 9.—Two examples of bundles of collagen, partially coated with a thin layer of amorphous material which resembled filamenting elastin on the electron microscopic viewing screen. These were characteristic of Group II.

Figs 10 and 11.—Examples of square-ended, large, dense fibres from the fresh whole dermis of a 66-year-old adult. These were seen in both fresh and prepared dermis from all age groups, and sometimes splayed out into a bundle of collagen fibrils.

Fig. 12.—Starting material from a 9½-year-old child (Collagenase-resistant group D9), showing a branched example of the large dense fibres illustrated in Figs 10 and 11.

Fig. 13(a), (b), (c).—Examples of tiny pieces of elastin presenting a stippled or lumpy appearance which characterize Group II.

Fig. 14.—A typical compact, ordinary network from the starting material of the 56-year-old adult (Control group D15). This is one variant of the naturally occurring or fully-formed elastin described previously (Keech and others, 1956).

Fig. 15.—In contrast to Fig. 14, when the substrates from both the infants (D12 and D8) were heated in water at 37° C., large networks were produced, sometimes stretching over several microscopic fields. These networks measured 20-30 μ in length, and were composed of loosely-knit, winding bundles of filamentous "elastin".

highest readings, *e.g.* D12 and D13, in which the network counts were also significantly raised. In contrast, the 56-year-old control exhibited a slight increase in small pieces of skin-type only, and the

majority of Group II were unresponsive. In the substrates which responded, a marked transformation picture was produced in the younger subjects (Fig. 37) and in Group III, but only a moderate

transformation occurred in the 43-year-old adult control and the 9½-year-old indigestible.

Effect of Periodate in Phthalate Buffer (pH 5.0) at 37° C. (Tables I and II).—Samples were examined after the same periods of incubation as were used

in the experiment with buffer alone. A definite, but less marked, response was found in all the substrates in Groups I and III that reacted to buffer alone to give a moderate transformation picture (Figs 16 and 17), but *no* effect was produced in the group resistant to collagenase.

Fig. 16.—Effect of phthalate buffer (pH 5.0) alone on the 19-year-old substrate (D14) from the Control group. After 3 hrs' incubation at 37° C., a transformation picture was found, with a significant increase in the "elastin" counts. This shows degenerating collagen mixed with amorphous material and elastin-like structures. Compare with Fig. 17.

Fig. 17.—Effect of periodate in phthalate buffer (pH 5.0) on the 20-year-old substrate exhibiting excessive collagenase digestion (D6). A transformation picture was found after 3 hrs' incubation at 37° C. with collagen (some of which was starting to degenerate) mixed with amorphous material and elastin-like structures.

Compare with Fig. 16.

Effect of Borate Buffer (pH 8.8) for 24 hrs at 37° C. (Tables I and II: Figs 18-25).—The usual increase in amorphous material and dense bits was found in all substrates, being most marked in Groups I and III. A significant increase in "elastin" counts was seen in all the control substrates (with the exception of D12 owing to the gross increase after heat alone for 24 hrs), being of moderate degree and accompanied by a moderate transformation picture. However, in the 56-year-old adult, there was a slight increase in skin-type only and *no* transformation picture. Both the 20- and 52-year-old substrates in Group III gave a moderate increase in skin-type and TS with a moderate transformation picture. In contrast, *none* of Group II had "elastin" counts significantly raised above that which could be accounted for by heat alone. The markedly different responses to alkali by substrates of the same age but belonging to different groups, is illustrated in Figs 18-25.

Collagen (Table I)

The variation in the starting materials has already been described (Figs 1-8). In general, the younger the substrate, the more easily could fairly mild agents induce fibril degeneration, whereas the older substrates remained relatively unaltered. For

example, heat alone for 24 hrs caused a small proportion of the infant collagen D12 to exhibit the early stages of degeneration, and the only substrate in Group II to show any collagen alteration after any treatment was the infant D8. The liability of fibril degeneration also varied between the three groups, being most marked in Group III and nearly absent in Group II.

Elastin

The different variants have been described above under "Terminology", and the effect of treatment is shown in Table I. The examples of the so-called transformation structures illustrated in Figs 26-35 underline the wide range of variants seen, and Figs 36 and 37 show small areas of the almost continuous transformation picture found in the substrate from the infant (D12) after treatment either with phthalate buffer alone or with periodate in buffer.

Amorphous Material and Dense Bits

These were plentiful in the younger subjects of Group I and scarcer in the adults. They were strikingly absent in Group II. Incubation with alkaline buffer produced an increase in all substrates, being least noticeable in Group II.

Figs 18 and 19.—Effect of incubation in borate buffer (pH 8.8) for 24 hrs at 37° C. The collagenase-resistant substrate from the infant (D8) illustrated in Fig. 18 was practically unaltered, whereas the control substrate from the infant (D12) illustrated in Fig. 19 produced a transformation picture. There was an increase in amorphous material and dense bits in both, but this was less marked in D8.

Figs 20 and 21.—Effect of incubation in borate buffer (pH 8.8) for 24 hrs at 37° C. The 9½-year-old collagenase-resistant substrate (D9) remained unaltered (Fig. 20), whereas the 9-year-old control (D13) produced a transformation picture. Fig. 21 illustrates part of a sheet of “elastin networks”.

Fine Thread-like Structures

These were described by Keech and others, 1956. They were only found in very small numbers throughout this study, being virtually absent in Group II. Alkaline buffer produced an increase in most of the substrates in Groups I and III.

Manufactured Networks (MN)

Small and medium-sized structures closely resembling the MN described previously (Keech and others, 1956) were occasionally seen throughout the present study. They numbered one to twenty for each substrate (*i.e.* per 8-11,000 fields), and there was no correlation with either age, treatment, or “elastin” increase. However, though present throughout Groups I and III, they were only found in two of the youngest substrates in Group II.

Discussion

Several points arise out of the electron microscope part of this study (Table III):

(1) Treatment of the substrates in water at 37° C. could produce an increase of up to two or three times the amount of skin-type “elastin” at 24 hrs (Tables I and II). This was associated with relatively unaltered collagen fibrils and did *not* con-

stitute a transformation picture (the exception of D12 has already been mentioned). This finding confirms previous work (Keech and Reed, 1957) on fresh whole dermis, where such an increase was found in all age groups after prolonged incubation, in either water or normal saline, at or below body temperature. This increase was allowed for in estimating the effects of the various reagents, as explained in Table II. However, there was a group difference, Group II showing the least and Group III the greatest responses.

(2) The response to treatment with all the reagents used was dependent on age. The decrease in collagen breakdown as age increased paralleled the quantitative increase in “elastin” recorded in Groups I and III. This lessening reactivity to chemical agents with advancing years is reminiscent of the similar response obtained from identical substrates after incubation with collagenase (Keech, 1954a). However, Group II did not react to either collagenase or to alkali, suggesting that the enzyme may attack similar linkages within the collagen molecule.

(3) This age-dependent response was associated with an age-difference in the collagen fibrils themselves, both in the starting materials within the

Figs 22 and 23 show the unaltered appearances of the 13-year-old (D10) and 18-year-old (D4) collagenase-resistant substrates respectively.

Fig. 24 demonstrates the degenerate appearance of the 19-year-old control collagen (D14) with an increase in amorphous material and dense bits.

Fig. 25 illustrates part of one of the many large transformation structures found in the 20-year-old substrate exhibiting excessive collagenase digestion (D6).

Figs 22 to 25.—Effect of incubation in borate buffer (pH 8·8) for 24 hrs at 37° C.

Fig. 26.—Transformation structure (TS) from the 51-year-old collagenase-resistant substrate (D3) after heating in water for 24 hrs at 37° C. The two arrows indicate a striated collagen fibril which apparently changes to "elastin". The single arrow demonstrates part of a collagen fibril under tension, as illustrated and described previously (Keech and others, 1956).

Fig. 27.—Transformation structure (TS) from the 52-year-old substrate exhibiting excessive collagenase digestion (D7), after incubation in phthalate buffer (pH 5.0) for 1½ hrs at 37° C. The thick, elastin-like structure splays out into a bundle of collagen fibrils at the upper left corner of the picture. The arrow indicates part of a collagen fibril under tension and raised above the surface of the grid as demonstrated by the direction of the shadow (Keech and others, 1956).

Fig. 28.—Transformation structure (TS) from the 5-year-old collagenase-resistant substrate (D11) after heating in water for 24 hrs at 37° C. The dense bundle (a) splay out into several areas typical of "elastin" (b-b) finally merging into a bundle of collagen fibrils (c).

Fig. 29.—Transformation structure (TS) from the 19-year-old control starting material (D14). Each end of the dense bundle splay out into elastin-like material.

Fig. 30.—Part of a large transformation structure (TS) from the 51-year-old collagenase-resistant substrate (D3) after incubation with periodate in phthalate buffer (pH 5.0) for 1½ hrs at 37° C. The elastin-like material is intimately associated with the striated collagen fibrils.

Fig. 31.—Part of a large transformation structure (TS) from the 9½-year-old collagenase-resistant substrate (D9) after heating in water for 24 hrs at 37° C. Each end of the dense bundle splays out into "elastin".

control group and *between* groups (see Table III). The appearance of the fibrils reflected the *reactivity* of each particular substrate: when they showed

evidence of degeneration, the "elastin" counts were increased and transformation pictures were found. On the other hand, the *unaltered* fibrils in Group II

Fig. 32.—A small transformation structure (TS) from the 9-year-old control substrate (D13) after heating in water for 1½ hrs at 37° C. The smaller quantity of electron-opaque material at one end allows the underlying elastin-like nature of the structure to be revealed.

Fig. 33.—Transformation structure (TS) from the same substrate as Fig. 32 after incubation in phthalate buffer (pH 5.0) for 3 hrs at 37° C. Part of a long, dense bundle terminating as "elastin".

correlated with the lack of response which characterized this particular group.

(4) The same reagents had different effects on

different groups. The response of the three groups showed a striking similarity to their known reactivity towards collagenase: the collagenase-resistant sub-

Fig. 34(a, b).—Two small transformation structures (TS) demonstrating the appearance of elastin-like material where the dense coating of amorphous material is reduced in amount. Numerous examples were seen throughout this investigation.

strates were relatively inert, those exhibiting excessive enzyme digestion were highly active, and the control group was intermediate between these two. This is demonstrated by the total "elastin" counts recorded at the foot of Table I. The count from the five substrates in the control group (13,446) was nearly $3\frac{1}{2}$ times that for the six collagenase-resistant substrates (4,006). In contrast, the two substrates exhibiting excessive digestion by collagenase gave a slightly higher total (4,124) than the six indigestible derma.

(5) A 3-hr incubation with phthalate buffer alone (pH 5.0) gave a greater response in all the groups

Fig. 35.—Transformation structure (TS) from the 56-year-old control substrate (D15) after incubation with periodate in phthalate buffer (pH 5.0) for 3 hrs at 37° C. Most of the dense bundle can be visualized as "elastin".

than either periodate in phthalate buffer or borate buffer (pH 8.8) for 24 hrs at 37° C. These experiments in buffer alone were the *control* series for the periodate studies, and periodate was one of the first reagents described which apparently transforms collagen into elastin-like structures (Burton and others, 1955). However, the detailed scrutiny and exhaustive counting undertaken in the present investigation revealed that, though the periodate experiments *did* increase the "elastin" counts and produced a transformation picture, the buffer solutions *alone* gave a greater response in all cases. It is also noteworthy that this response occurred after only 3 hours' incubation, and that both periodate and phthalate buffer alone produced higher counts and more transformation pictures than the longer, 24-hr incubation with alkali. In addition, the phthalate buffer alone evoked a response even in two of the youngest subjects (D8 and D9), yet both periodate and alkali were

Figs 36 and 37.—Incubation of the control substrate from the infant (D12) for 3 hrs at 37° C. with periodate in phthalate buffer (Fig. 36) and in phthalate buffer (pH 5.0) alone (Fig. 37). These illustrate small areas from the almost continuous transformation picture seen after each of these treatments.

without effect. These as yet unexplained phenomena merit further study. It is possible that the addition of the 1 per cent periodate solution to the phthalate buffer produced a dilution effect or an alteration in ionic strength which decreased the ability of the reagent to break down collagen. Unfortunately insufficient dermal substrate remained to investigate this matter in detail.

The paucity of the fine thread-like structures (Table I) following the action of all the reagents used in this study is in conformity with previous work (Keech and others, 1956) which demonstrated an increase in numbers as the pH value rose above 7.0. Most of the present experiments were conducted at pH 5.0 and 5.6, but incubation with borate buffer (pH 8.8) produced an increase in most of the substrates in Groups I and III. The appearance of structures closely resembling the manufactured networks (MN) described previously, show that they *can* occur at 37° C. although they were far less in comparison with the numbers seen after heating *above* body temperature, treatment with acetic acid, etc., as already reported (Keech and others, 1956). In the present investigation, only 65 of these manufactured networks were seen in the process of scanning a minimum of 120,000 fields. Their occurrence was sporadic, and bore no apparent relationship to age, treatment, or increase in "elastin".

It must be remembered that the starting materials used throughout comprised human dermis that had been "purified" by the method of Neuman (1949a, b) as shortened by Keech (1954a). This process involves prolonged extraction at 4° C. with 10 per cent. NaCl followed by N/15 Na₂HPO₄, to remove mucoproteins and interfibrillar material. It is unlikely that this would affect the original natural elastin, which is present in small quantities in all the starting materials. However, although Neuman claims that this procedure does not denature or alter the properties of collagen, recent work demonstrating the apparent transformation of collagen to elastin-like structures by alkali (Burton and others, 1955; Hall and others, 1955) throws doubt on this method of preparation. However, whatever the nature of the substrate after purification, does not invalidate the comparisons made in this study.

The lack of response to incubation with borate buffer (pH 8.8) exhibited by all the collagenase-resistant substrates, both biochemically (*vide infra*) and under the electron microscope, provides a clue to the uniform morphology of all the starting materials in this group (Tables I and III). The alkali used in Neuman's procedure may have removed the interfibrillar material without altering

the fibrils, thus accounting for the clean appearance of the collagen and the lack of amorphous material and dense bits (which are presumably derived from the products of collagen breakdown). This group's resistance to breakdown correlated with a lack of transformation into "elastin". However, in the two inert substrates in which collagen degeneration was induced by phthalate (D8 and D9 *vide supra*) the usual conversion to elastin-like material was found. This is in keeping with the hypothesis (Hall and others, 1955) postulating collagen breakdown as a prerequisite for the formation of "elastin" by rearrangement of the collagen components.

The following diagram offers an explanation for the morphological findings as based on our knowledge to date.

The starting materials of both fresh whole dermis and the "purified" dermis of all age groups contain collagen, a small quantity of naturally-occurring or fully-formed elastin, and large, dense, square-ended fibres. Prolonged heat alone, at or below body temperature, can produce evidence of early collagen degeneration in the substrates from children and young adults (Keech and Reed, 1957), the effect being markedly increased by the addition of reagents such as borate, periodate, or phthalate. Collagen degeneration is associated with an increase in amorphous material and dense bits, which are probably collagen breakdown products.

The amorphous material under the electron microscope may derive from two sources: the breakdown of the collagen fibrils themselves, or the interfibrillar material released from the bundles during the various treatments. It may vary, therefore, in chemical composition according to its origin. Since an increase in skin-type "elastin" only occurs in the presence of some degree of collagen breakdown, these structures could be formed from degraded collagen combined with varying amounts of amorphous material.

Another possible, though not probable, cause of the increase in fully-formed "elastin" would be the progressive fragmentation of the original elastin by

heat, breaking it up into a larger number of smaller pieces. This seems unlikely, as there was no noticeable decrease in the size of the structures counted after heat alone.

As mentioned above, it has already been reported that the large, dense, square-ended fibres present in all the starting materials are converted into transformation structures (Keech and Reed, 1957). Thus *two* pathways are postulated for the production of the elastin-like material which constitutes the transformation picture: the products of collagen degeneration combine with varying amounts of amorphous material to give fully-formed "elastin", and the large, dense, fibres give rise to the TS.

Both these pathways can occur after the application of heat alone (depending on the age of the dermis), but the process is greatly enhanced by reagents such as borate, periodate, and phthalate. That these two mechanisms need not necessarily react together or in the same degree, is illustrated in Group II. Heat alone produced a smaller increase in skin-type "elastin" in these collagenase-resistant substrates, but a greater increase in TS when compared with the control group (Table II). This difference in response is thus further evidence of the different reactivities of these two groups of collagen.

The results of biochemical investigations of the same dermal substrates were in general agreement with the electron microscope findings. Protein determinations after incubation with either borate buffer (pH 8.8) or potassium hydrogen phthalate buffer (pH 5.0) revealed a difference between the three groups. The substrates of average collagenase sensitivity gave results similar to those recently reported (Hall, 1956), *i.e.* a tendency for the amount of protein dissolved by alkali to decrease as the age of the subject increased. In contrast, the two collagenase-resistant substrates showed no significant breakdown by alkaline buffer, whereas the group which exhibited excessive digestion by collagenase gave higher readings than controls of comparable age. Similar results were found after incubation with phthalate buffer.

Chromatography showed that the total amino acid analysis was the same for all the groups, only the substrates in Group III took three times as long to hydrolyse as the others. The hydroxyproline content and shrinkage temperatures have recently been reported in detail (Hall and Reed, 1957). The hydroxyproline levels of all three groups were within the normal (mammalian) range, the values lying between 12.3-14.3 per cent. The micro-shrinkage temperatures (Borasky and Nutting, 1949), however, were quite distinct. The collagens

in the control group shrank over a range of 62 to 69.5° C., and the collagenase-resistant samples in Group II over a range of 72 to 79.5° C., whilst those in Group III, which were hypersensitive towards collagenase, shrank over the lower temperature range of 60 to 67° C. Thus, the distinct ranges of thermal shrinkage again reflected a difference between the three groups of collagen. In view of the drawbacks of Neuman's "purification" method already mentioned, no firm chemical explanation is possible. However, the most likely hypothesis is a difference in mucoid between the three groups.

The fact that the fibrils exhibit the morphological characteristics of normal collagen does not preclude a chemical difference. Morphological similarity does not necessarily mean chemical identity, as was recently demonstrated by Solomons and Irving (1956). They found that the availability of hydroxylysine and lysine polar side-chains was significantly greater in human dentin than in ox-hide collagen, and that this availability probably played a part in the combination of mineral material with the protein matrix of human dentin. This protein matrix has been shown under the light microscope (Widdowson, 1952) and under the electron microscope (Scott and Wyckoff, 1950; Yasuzumi and Obata, 1955) to consist of collagen morphologically identical with that found in human and animal skin. Thus, the collagenase-resistant collagens investigated in the present study may contain linkages which are either inaccessible to the action of collagenase and alkali, or, by some configurational variation from the normal collagen, are inaccessible to these two reagents. In the two collagenase-resistant substrates, which were mildly attacked by phthalate, one must assume that the linkages concerned in this type of reaction differ from those susceptible to collagenase and alkali.

The importance of the reactivity of collagen to collagenase has recently been shown by Ziffren and Hosie (1955), who demonstrated collagenase activity in canine pancreatic juice. Previous work (Keech, 1954b) suggested the presence of some substance capable of altering collagen in human dermis of all age groups. As mentioned at the beginning of this paper, rash-bearing and non-rash-bearing skin from a case of dermatomyositis differed in sensitivity towards collagenase. The answer to this riddle may be the key to the cause of so-called "collagen disease".

Summary

Further investigations were undertaken on six of the collagenase-resistant human dermal substrates and the two exhibiting excessive digestion by collagenase previously described by Keech (1955).

These were compared with five substrates showing average collagenase sensitivity.

Under the electron microscope, a significant increase in elastin-like structures was produced by phthalate buffer, periodate, and alkaline buffer, the degree of response depending on the age of the subject. The results paralleled the sensitivity of the substrate towards collagenase, the resistant group remaining unattacked by alkali and periodate, and only two of the six being mildly attacked by phthalate. In the remaining cases, phthalate buffer alone proved to be a more potent reagent for the apparent transformation of collagen to elastin-like structures than either periodate or alkali. Treatment in water at 37° C. produced a slight increase in "elastin", which was allowed for when the effect of incubation with chemical reagents was assessed. Only the latter gave a true transformation picture to "elastin". The tabulated results are based on the careful scrutiny of a minimum of 120,000 microscopic fields, and a total of 21,576 elastin structures were counted.

Biochemical studies confirmed the electron microscopic findings and the shrinkage temperature also reflected the different reactivity of these three groups of collagen.

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Transformation du collagène en "élastine" dans les collagènes dermiques avec sensibilité variable à la collagenase

RÉSUMÉ

On procéda à de plus amples recherches sur six

substrata collagenase-résistants des dermes humains et sur les deux qui présentaient une digestion excessive par la collagenase, précédemment décrits par Keech (1955). On les compara à cinq substrata présentant une sensibilité moyenne à la collagenase.

Sous le microscope électronique, une augmentation significative des structures ressemblant à l'élastine se produisait par un tampon de phthalate, de périodate et un tampon alcalin, l'intensité de la réaction dépendant de l'âge du sujet. Les résultats étaient parallèles à la sensibilité du substratum à la collagenase, le groupe résistant n'étant pas attaqué par l'alcali et périodate, et seulement deux des six étant légèrement attaqués par le phthalate. Dans les cas restants, le tampon de phthalate seul s'est révélé un réactif plus puissant, pour la transformation apparente du collagène en structures semblables à l'élastine, que le périodate ou l'alcali. Le traitement par l'eau à 37° C. produisait une légère augmentation d'"élastine"; on en tenait compte en évaluant l'effet de l'incubation avec des réactifs chimiques. Seule cette dernière méthode donnait un vrai tableau de la transformation en "élastine". Les résultats catalogués sont basés sur l'examen soigné d'un minimum de 120.000 champs microscopiques et un total de 21.576 structures d'élastine a été compté.

Des études biochimiques ont confirmé les observations au microscope électronique et la température de contraction a reflété aussi la réactivité différente de ces trois groupes de collagène.

Transformación del colágeno en "elastina" en los colágenos cutáneos con sensibilidad variable a la collagenasa

SUMARIO

Se efectuaron investigaciones ulteriores sobre seis de los substratos de la piel humana resistentes a la collagenasa y sobre los dos que habían presentado una digestión excesiva por la collagenasa, anteriormente descritos por Keech (1955). Se los comparó a cinco substratos presentando una sensibilidad media a la collagenasa.

Bajo el microscopio electrónico, un aumento significativo de estructuras parecidas a elastina se produjo por un tapón de ftalato, de periodato y por un tapón alcalino, la intensidad de la reacción dependiendo de la edad del sujeto. Los resultados fueron paralelos a la sensibilidad del substrato a la collagenasa, el grupo resistente no siendo atacado por el alcali o el periodato, y sólo dos de los seis siendo ligeramente atacados por el ftalato. En los demás casos, el tapón de ftalato solo se reveló un reactivo más fuerte, para la transformación aparente del colágeno en estructuras parecidas a la elastina, que el periodato o el alcali. El tratamiento por el agua a 37° C. producía un ligero aumento de "elastina"; esto fué tomado en cuenta en la valoración del efecto de la incubación con reactivos químicos. Tan sólo este método daba el cuadro auténtico de la transformación en "elastina". Los resultados enumerados se basan en la investigación cuidadosa de un mínimo de 120.000 campos microscópicos y un total de 21.576 estructuras de elastina fué contado.

Estudios bioquímicos confirmaron las observaciones al microscopio electrónico y la temperatura de contracción también reflejó la reactividad diferente de estos tres grupos de colágeno.