SIGNIFICANCE OF THE OCCURRENCE OF TRANSPARENT SKIN
A STUDY OF HISTOLOGICAL CHARACTERISTICS AND BIOSYNTHESIS OF DERMAL COLLAGEN

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Transparent skin has been observed in a proportion of elderly people and its association with senile osteoporosis has already been noted (McConkey, Fraser, Bligh, and Whiteley, 1963).

In patients with rheumatoid disease, whether treated with steroids or not, it was found to be commoner than in elderly people unaffected by rheumatoid disease (McConkey, Fraser, and Bligh, 1965).

Clinical studies led to the hypothesis that transparent skin is the consequence of a change in the connective tissues of the skin and that a similar change might be taking place in the connective tissues of the bones.

This is an account of the histological changes characteristic of transparent skin and of some experiments undertaken to investigate its pathogenesis.

Methods
Selection of Patients
49 patients were studied in all, including one case of scleroderma studied for comparative purposes (see below). Whether for histology, or for estimation of skin collagen or of water content, the procedure adopted was to select a number of patients with unequivocally abnormal skin and to compare the results obtained in these with those obtained in matched controls. The thickness of a fold of skin on the dorsum of the hand was measured with Harpenden calipers. For each patient with transparent skin (as previously defined, McConkey and others, 1963), a control patient was selected who corresponded in age, sex, and skin thickness but whose skin was opaque.

No patient was receiving treatment with corticosteroids or had other evidence of skin disease. Skin biopsies were taken from the dorsum of the hand over the 3rd-4th metacarpal.

Histology
Twenty biopsies, ten from transparent skin and ten from opaque skin, were studied. The biopsies were taken in pairs by one of us and prepared and stained in batches. The sections were then examined by another of us who was unaware of how each patient had been categorized clinically.

Staining Methods
The stains used were haematoxylin and eosin; van Gieson’s stain; the picropolychrome method of Herovici (1963); toluidine blue or Azure A (method of Hughesdon, 1949); the periodic acid-Schiff technique (McManus, 1946; Hotchkiss, 1948); the colloidal iron method of Hale (1946), and Weigert’s elastic-tissue stain. In some instances, sections were stained, before and after treatment with bovine testicular hyaluronidase, with Alcian blue in varying salt solutions as described by Scott, Dorling, and Quintarelli (1964).

Autoradiographs of sections of skin after incubation with 14C-L-proline were carried out by the stripping film technique of Pelc (1947).

Water Content
Three pairs of patients were selected as described above and biopsies of skin taken. The biopsies were placed immediately in small air-tight containers of known weight and were weighed. The water content was then determined by drying the tissue to constant weight over phosphorous pentoxide at 20°C.

Collagen Estimation and Measurement of Turnover
Eleven patients were selected and biopsies taken as described above. The biopsy specimens were divided into three or four pieces and incubated with (14C) proline as described by Carney, Lawrence, and Ricketts (1965) for estimation of the incorporation of the isotope, except that, in the present instance, after incubation, the sterility of the cultures was checked and the skin samples incubated...
for 1 hour on 1 per cent. β-phenoxy-ethylmethyldecylammoniumbromide ("Bradosol"; Ciba), in order to facilitate separation into samples of dermis and epidermis.

The separated portions were then treated as previously described (Carney and others, 1965) to obtain gelatin extracts, for the estimation of hydroxyproline and for counting of radioactivity.

 Autoradiographs on some samples of skin after incubation with the isotope and washing were carried out as described above.

**Intradermal Injections**

Twelve patients with transparent skin had intradermal injections of 0·1 ml. of their own blood into the skin of the dorsum of the hand and on the forearm.

**Results**

**Histological Appearance of Transparent Skin**

In some of the patients with transparent skin the epidermis was thinner and less keratinized than that in patients with opaque skin. But this was not a constant feature. On the other hand, marked and constant differences were observed in the dermis as between transparent and opaque skin. These differences could be appreciated even in haematoxylin and eosin stained sections, were more marked with van Gieson's stain, and were even more obvious in sections stained by the picropolychrome method of Herovici (1963).

The essential differences lay in the texture of the dermis and in the staining-properties of the collagen fibres. In transparent skin the collagen fibres were loosely arranged as fine individual fibrils with an apparent increase of interstitial "ground substance" between the fibrils. The fibrils stained a faint pink with eosin, an orange-yellow shade with van Gieson's stain, and a clear blue colour with the methyl blue component of the picropolychrome method.

In contrast, in opaque skin the collagen occurred as coarse bundles, much more closely packed. The fibrils stained more intensely pink with eosin, bright red with the picrorufusin of the van Gieson stain, and deep red with picropolychrome method. These differences are illustrated in Figs 1 to 6 (see col. plate).

Although the changes described above as characteristic of transparent skin sometimes involved the whole thickness of the dermis, they were commonly most marked in the upper third of the dermis (Fig. 7). The alteration in size, distribution, and staining-properties of the collagen in transparent skin was not accompanied by any marked alteration in elastic tissue (i.e. in sections stained by Weigert's method there was no evidence of so-called "senile elastosis",

Fig. 8). Similarly, in sections stained with toluidine blue, mast cells were present in normal proportions and there was no metachromatic material to be seen in the interstitial spaces between the fibres.

Sections of transparent skin fixed in alcohol and stained by the periodic acid-Schiff (PAS) technique showed the presence of PAS-positive material in spaces between the fibres. This material also stained positively with the colloidal iron technique (cf. Figs 9 and 10) and was diminished, but not entirely removed, by previous treatment with hyaluronidase.

In alcohol-fixed tissues the material was not metachromatic when stained with toluidine blue or Azure A.

These results suggested the presence in the interstitial spaces of a tissue mucopolysaccharide of relatively low charge (i.e. non-sulphated). A method of categorizing tissue polysaccharides was recently described by Scott and others (1964). In essence, this entails the determination of the molarity at which certain divalent cations inhibit the uptake, by a given tissue polysaccharide, of the dye Alcian blue at pH 5·7. On applying this technique, it was found that staining of the polysaccharide material in question in this instance, by Alcian blue, was inhibited at pH 5·7 by a concentration of 0·25 M MgCl₂. According to the originators of this technique, such behaviour is characteristic of mucins and carboxyl-containing glycosaminoglycans but not of sulphated tissue polysaccharides.

**Water Content**

On comparing the loss in weight which occurred when matched skin biopsies were dried to constant weight over phosphorus pentoxide, the results shown in the Table were obtained. It will be seen that the loss of weight of biopsies of opaque skin varied between 75 and 80 per cent. of the fresh weight, whereas the loss of weight on drying transparent skin was markedly higher, being between 94 and 98 per cent. of the fresh weight, indicating a higher initial water content.

<table>
<thead>
<tr>
<th>Skin</th>
<th>Opaque</th>
<th>Transparent</th>
</tr>
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<tbody>
<tr>
<td>Specimen No.</td>
<td>Water Content</td>
<td>Specimen No.</td>
</tr>
<tr>
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</tr>
<tr>
<td>Content</td>
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<td>83·0</td>
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<tr>
<td>H₂O per cent. Wet Weight</td>
<td>3</td>
<td>79·9</td>
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<tr>
<td>Mean</td>
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<td>81·0</td>
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</tbody>
</table>
**Fig. 1.**—Normal skin (Van Gieson’s stain) showing dermal collagen arranged in coarse bundles.  \( \times 100 \).

**Fig. 2.**—Transparent skin (Van Gieson’s stain). Dermal collagen staining poorly and arranged as fine fibrils (compared with Fig. 1). \( \times 100 \).

**Fig. 3.**—Normal skin (Herovici’s stain). Coarse dermal collagen staining bright red with picrofuchsin component of stain. \( \times 100 \).

**Fig. 4.**—Transparent skin (Herovici’s stain). Fine dermal collagen staining blue with methyl blue component of stain. \( \times 100 \).

**Fig. 5.**—High-power view of normal skin (Herovici’s stain), showing coarse bundles of dermal collagen. \( \times 450 \).

**Fig. 6.**—Transparent skin (Herovici’s stain), showing loose texture and fine fibrils of dermal collagen. \( \times 450 \).

**Fig. 7.**—Transparent skin (Herovici’s stain), showing changes more marked in upper third of dermis (see text). \( \times 100 \).

**Fig. 8.**—Transparent skin (Weigert’s elastic stain). Note absence of alteration in distribution of elastic tissue. \( \times 100 \).

**Fig. 9.**—Transparent skin (colloidal iron stain) before treatment with testicular hyaluronidase. \( \times 100 \).

**Fig. 10.**—Transparent skin (colloidal iron stain). Adjacent section to that shown in Fig. 9, stained after treatment with testicular hyaluronidase. \( \times 100 \).

*Facing p. 220.*
Collagen Content and (14C)-Proline Uptake

Previous work by Prockop (for references see Carney and others, 1965) has shown that (14C) proline is incorporated into collagen, a proportion of the proline being simultaneously converted to hydroxyproline. Consequently, the radioactivity of the collagen is a measure of its biosynthesis. When autoradiographs were made of intact skin biopsies, radioactivity was found to be present in both epidermis and dermis. Separation of dermis before analysis enabled a pure sample of dermal collagen to be obtained as gelatine for hydrolysis and subsequent radio-activity measurements.

The total collagen content and 14C content of the biopsies were related to the fresh weights. The results obtained are shown in Fig. 11. For opaque skin the total collagen content ranged from 3·27 to 6·75 per cent. (mean 4·42 per cent., standard error ±0·27 per cent.; fourteen observations.) These values were significantly higher (P<0·001) than those for transparent skin samples where the range was 0·56 to 3·40 per cent. (mean 2·16 per cent., standard error ±0·28 per cent.; sixteen observations).

The specific radioactivity of dermal collagen also differed between transparent and opaque skin. The means of observations were: opaque skin, 6,261 c.p.m./mg. collagen (standard error ±1,697; nine observations); transparent skin 30,436 c.p.m./mg. collagen (standard error ±10,517; fifteen observations). The significance of this difference (0·02<P>0·05) must be accepted with some reservation. As may be seen from Fig. 11, the mean value for the transparent skin is influenced by some samples with low collagen content (as estimated chemically) having high radioactivity.

Nevertheless, it may be concluded that, although the collagen content of transparent skin is only about half that of normal skin, the biosynthesis of collagen in transparent skin is at least as active, if not more active, than in opaque skin.

This was in marked contrast with the results obtained with a biopsy of skin from a case of scleroderma studied in similar fashion. Histologically, the dermal collagen in this case was extremely dense and closely packed. The total collagen content was high (6·4 per cent.) but the biosynthesis was low (1,384 c.p.m./mg. collagen).

Response of Skin to Intradermal Injection

When 0·1 ml. blood was injected intradermally into areas of transparent skin, in ten out of twelve patients the result was the formation of a normal bleb. In the remaining two patients, the blood spread rapidly in a manner characteristic of patients with senile purpura.

It should be noted that this technique appears to measure essentially the resistance of the dermis to acutely increased pressure rather than the altered diffusion which occurs with the instillation of hyaluronidase (cf. Holborow and Keech, 1951).

Discussion

The suggestion, put forward on the basis of clinical evidence, that transparency of the skin is not due merely to thinning of the skin (McConkey and others, 1963, 1965) is confirmed by the present study. In pairs of biopsies, when skin thickness was matched, constant qualitative and quantitative differences have been found in the dermal collagen as between opaque and transparent skin.

The histological picture of transparent skin was that of an alteration of texture of the dermis in which the collagen was present as thin fibres, widely separated from one another and showing a marked affinity for methyl blue but a poor staining-reaction with acid fuchsin—as contrasted with the collagen of the dermis of opaque skin which was arranged in
ANNALS OF THE RHEUMATIC DISEASES

compact coarse strands or thick bundles showing a strong affinity for acid fuchsin.

The wide separation of the relatively thin collagen fibres in transparent skin appeared to be due to marked hydration of the interstitial ground substance, as supported by the finding of a high water content of skin biopsies showing transparency. These interstitial spaces, in sections appropriately fixed and stained, contained material with the characteristics of a weakly-charged tissue polysaccharide. Although these histochemical reactions were consistent with those of hyaluronic acid, suggesting that this was the predominant component, the material was incompletely removed by previous treatment with hyaluronidase.

Preliminary investigation, by extraction of the skin polysaccharides and their characterization by chromatographic techniques (Long, 1965) has suggested the presence, in extracts of transparent skin, of small amounts of dermatan sulphate, chondroitin-4-sulphate, and heparin in the dermis, in addition to hyaluronic acid (i.e. all the components present in normal skin). The yields of material from skin biopsies were insufficient to allow quantitative comparisons of the relative proportions of these compounds in opaque and transparent skin. In view of the possible importance of this in relation to the pathogenesis of transparent skin (see below) these investigations are being continued and the results will be reported in due course.

The work with isotopically-labelled proline confirmed the depletion of total collagen in transparent skin but suggested that the collagen present was metabolically as active, or even more active, than that present in opaque skin. It is known that the collagen of skin exists in two functional forms, of differing metabolic activity (Harkness, Marko, Muir, and Neuberger, 1954): a more labile and rapidly turned-over fraction, situated predominantly in the superficial position of the dermis, which has a rapid turnover rate; as compared with the collagen of the deeper layers of the dermis, which has a very slow turnover rate.

Histological examination suggested that the collagen changes in the dermis of transparent skin affected predominantly the superficial layers of the dermis, in some cases being confined to this region, and only extending throughout the thickness of the dermis where the skin changes were advanced. The altered staining properties of the fibres in the affected area were also consistent with this collagen being different from normal. The picro-polychloro blue stain, as introduced by Herovici (1963), was stated to differentiate between young collagen (pro-collagen) by its affinity for methyl blue; and mature collagen by an affinity for picro-fuchsin.

At the other end of the scale, the dermis from the case of scleroderma which showed exceptionally dense and compact bundles of collagen with a marked affinity for picrofuchsin, although found to have an increased total collagen content, exhibited markedly lower biosynthetic activity of this collagen as compared with normal skin.

At first sight, it seemed inherently unlikely that the elderly patients with transparent skin who formed the subjects of this study could be inferred to be distinguished by a capacity to make new collagen at an unusual rate in the skin. But, on the other hand, it seemed possible that this picture might also result from an interference with the normal maturation of collagen, as proposed in the following hypothesis which is based on the work of Jackson (1956), Meyer (1957), Meyer and Hoffman (1960), Gross (1959), Wood (1960a, b), Wood and Keech (1960), and others.

Collagen is formed initially in a soluble form and it is this fraction of the collagen in a tissue which is the most active metabolically. Maturation of collagen involves its transformation, by the aggregation of sub-units to the insoluble form. Immature insoluble collagen exists as fine fibres which by further aggregation become coarse bundles which are the stable, metabolically-inactive form. At all stages of the maturation process (i.e. orientation and steric arrangement to allow cross-linking and aggregation) it is likely that the presence of mucopolysaccharides of normal constitution in the ground substance is required.

Thus, our findings could represent an arrest in the maturation of collagen arising as a specific defect giving rise to a smaller proportion of mature and metabolically-inert collagen and a reduced total collagen content because of loss from the skin as soluble collagen.

Alternatively, the failure of maturation of collagen might be secondary to changes in the constitution of the ground substance. Such changes might be in turnover rate, in the relative proportions, or in the degree of polymerization, of the mucopolysaccharides of the ground-substance.

It is known that the total concentration (Clausen, 1962) and turnover rate of the mucopolysaccharides of skin diminish with age. If the rate of turnover of mucopolysaccharides is one of the limiting factors in the normal maturation of collagen, it is possible that marked decrease, in itself, might account for the occurrence of transparent skin in some otherwise healthy elderly people. Such decrease of turnover might affect all the mucopolysaccharides together or
might affect some more than others. It is known from studies of the incorporation of $^{35}$S-sulphate and $^{14}$C-acetate into skin mucopolysaccharides that uptake occurs much more readily into some of these compounds than others, suggesting different rates of synthesis and hence of turnover (Dorfman and Schiller, 1958; Sobel and Marmorston, 1958). Decreased turnover might thus affect not only the total but also the relative proportions of the skin mucopolysaccharides.

It was first suggested by Meyer (1957), on the basis of the failure of normal collagen fibre formation in scorbutic animals or those given large doses of cortisone, that this might in turn be dependent upon failure of production of sulphated mucopolysaccharides in normal amounts and proportions. In relation to cortisone dosage, it should be noted that, although the patients in the present study had not been treated with corticosteroids, in other patients we have observed that transparency of the skin becomes more marked with prolonged steroid therapy (See also Greenwood, 1966).

It was shown by Loewi and Meyer (1958) in pigs that, when embryonic skin (characterized by fine collagen fibres arranged in a loose texture) was compared with adult skin (characterized by a compact arrangement of coarse collagen bundles), there were marked differences in the relative proportions of hyaluronic acid and chondroitin sulphate B (dermatan sulphate). Loewi (1961) was unable to show similar differences in humans, using skin from the back of the hand, with pooled samples from the age group 50–70 years. However, no observations were recorded on the relative incidence of "transparent" and "opaque" skin among the latter group, so that it is possible that differences might have existed but have been obscured by the pooling procedure adopted. This possibility is being re-investigated and the results will be reported later.

With regard to the degree of polymerization of the mucopolysaccharides, there may be an additional factor to consider in rheumatoid arthritis, a disease with a high incidence of transparent skin. It has been shown by Barker, Bayyuk, Brimacombe, Hawkins, and Stacey (1963) that in rheumatoid arthritis the hyaluronic acid of synovial fluid is depolymerized, and there is some evidence that hyaluronic acid elsewhere in the body may be similarly affected, including skin as first shown by Bywaters, Holborow, and Keech (1951) and more recently by Herp, Fabianek, Calick, and Pigman (1966).

The alteration of the hyaluronic acid of the ground substance is not necessarily accompanied by a decrease in resistance to an acute increase in pressure as shown by the formation of a normal bleb on the injection of blood intradermally in our patients.

It has been suggested that senile purpura, another abnormality of the skin of elderly people, is the consequence of lack of support by the connective tissues to the small blood vessels of the skin (Tattersall and Seville, 1950; Shuster and Scarborough, 1961). If this were so, it might be expected that the "senile" or "steroid" type of purpura would occur readily in transparent skin where collagen is so clearly diminished and where changes in the ground substance may also be present. However, although there is a partial association between transparent skin and senile purpura (McConkey, Fraser and Bligh, 1962), in patients who have both conditions the purpura does not necessarily occur in areas where the skin is most obviously transparent. Moreover, Shuster and Scarborough (1961) showed that the lesion of senile purpura could be reproduced in susceptible persons by an intradermal injection of their own blood. As already stressed, we have found that intradermal injection of blood in areas of transparent skin in twelve patients led to the formation of normal blebs in ten of them.

The opacity of normal skin was held by Montagna (1956) to be due to the constitution of the epidermis. In our patients with transparent skin, however, changes in the epidermis were not a marked or constant feature. The appearance of transparency can probably be accounted for by the reduction in the amount of collagen with accumulation of homogeneous and highly hydrated material between the widely dispersed fibres; the effect of this change would be to render the tissue as a whole optically more uniform.

**Summary**

(1) Transparency of the skin has been found to be due not simply to thinning but to changes in the dermal collagen. These changes have a characteristic histological pattern in that the collagen fibres are fine and widely-dispersed and show altered staining characteristics from the coarse compactly-arranged collagen bundles of normal (opaque) skin.

(2) Transparent skin is more hydrated than normal skin and shows a diminution of total collagen as compared with controls matched for age and sex. But biosynthesis, as judged by the uptake of $^{14}$C-proline with the collagen, is as active or even more active than in normal skin.

(3) The reaction of transparent skin to the intradermal injection of autologous blood was found, in the majority of cases, to be similar to that found in normal skin.

(4) It is suggested that the changes in the dermal
collagen of transparent skin result from a failure of maturation of the collagen fibres. As yet it is not possible to say whether this arises as a specific defect or whether it is secondary to changes in the glycosaminoglycans (acid mucopolysaccharides) of the ground substance.

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REFERENCES


La portée de l'occurrence de la peau transparente—Etude des caractères histologiques et de la biosynthèse du collagène dermique

Résumé

(1) On a trouvé que la transparence de la peau n’est pas due simplement à l’amincissement mais aux altérations du collagène dermique. Ces altérations présentent un tableau histologique caractéristique dans le sens que les fibres du collagène sont fines et très dispersées et se colorent différemment des faisceaux de collagène grossièrement tassé de la peau normale (opaque).

(2) La peau transparente est plus hydratée que la normale et contient moins de collagène total que celle des témoins d’un âge et d’un sexe comparables. Toutefois la biosynthèse, déterminée par l’absorption de la 14C-proline avec le collagène, est aussi active et même plus active que dans la peau normale.

(3) On a trouvé que dans la plupart des cas la réaction de la peau transparente à l’injection de sang autologue était similaire à celle de la peau normale.

(4) On suggère que les altérations du collagène cutané de la peau transparente sont dues à un défaut dans la maturation des fibres du collagène. On ne sait pas encore s’il s’agit ici d’un défaut spécifique ou secondaire aux altérations des glycosaminoglycans (mucopolysaccharides acides) de la matrice.

El significado de la ocurrencia de la piel transparente. Estudio de los rasgos histológicos y de la biosíntesis del colágeno dérmico

SUMARIO

(1) Se halló que la transparencia de la piel no se debe simplemente al adelgazamiento sino a alteraciones del colágeno cutáneo. Estas alteraciones presentan un cuadro histológico característico: las fibras del colágeno son finas y muy dispersas y ofrecen características de coloración diferentes de las de haces de colágeno grosse-mente empacadas de la piel normal (opaca).

(2) La piel transparente es más hidratada que la normal y contiene menos colágeno total que la de testigos de edad y sexo comparable. Sin embargo la biosíntesis, determinada por la absorción de la 14C-prolina con colágeno, es tan activa o más que en la piel normal.

(3) Se halló que en la mayoría de los casos la reacción de la piel transparente a la inyección de sangre autóloga fue similar a la de la piel normal.

(4) Se sugiere que las alteraciones del colágeno cutáneo de la piel transparente se deben a un defecto de la maduración de las fibras del colágeno. No se sabe todavía si se trata aquí de un defecto específico o secundario a alteraciones de los glicoaminoglicanos (mucopolisacaridos ácidos) de la matriz.
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