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Synergistic effect of age and corticosteroid treatment on connective tissue metabolism

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McConkey *et al.* (1963), Shuster and Bottoms (1963), Rychewaert *et al.* (1967), and Roberts *et al.* (1975) have reported decreasing skin thickness with increasing age. Meema and Reid (1969) suggested that this is due to a reduction in the collagen content of the tissue, but both Harris and Sjoerdsma (1966) and Shuster and Bottoms (1963) were unable to report any consistent age-dependent reduction. Reed and Hall (1974) confirmed this for 'normal' osteoporotic subjects, but in a study which included a group of patients with osteoporosis who had biconcave vertebral bodies with microfractures of the articular processes they observed a striking reduction in total collagen content of the skin with increasing age (Hall *et al.*, 1974a,b). Sheppard and Meema (1967) observed similar reductions in skin thickness associated with excessive osteoporosis accompanying long-term therapy with adrenal or synthetic corticosteroids. Hall *et al.* (1974b) showed that the catabolic effect of the synthetic steroid prednisolone on the collagen of the dermis is dependent on the age of the subject.

This paper carries these studies further. It takes into account not only the interplay of corticosteroid therapy and age on the collagen present in the skin but also correlates these changes with the bone rarefaction which accompanies them in women of postmenopausal age and the duration of the rheumatoid condition for which the corticosteroid was first prescribed.

Methods

Skin samples were obtained with a 6-mm diameter rotary biopsy punch (Mill-Bilt Equipment Co. Inc., Toledo, Ohio, U.S.A.) driven at 20 000 rpm by a power-operated hand drill (Heathcraft, England). Samples were removed under xylocaine anaesthesia from the mid-line of the thigh of 40 women aged 21–80 with no skin or bone lesions and from 22 women of similar age (22–72) with rheumatoid arthritis of varying duration, all but five of whom had received corticosteroid therapy for periods of between 1 and 12 years (that is, up to one-sixth of the life span). The remaining five had been on corticosteroid therapy for periods of less than one year.

The samples, which were taken from the full thickness of epidermis and dermis, were trimmed free of the latter and of all subcutaneous fat and were hydrolysed at 110°C for 18 hours in sealed tubes containing 4 ml 6N HC1. The hydroxyproline content of these hydrolysates was determined by the automated procedure of Grant (1964) and was converted to a value indicative of the amount of collagen present by multiplication by a factor of 7·46 (Neuman and Logan, 1950). Division by 9π gave a figure which is representative of the total amount of collagen in a column of full skin thickness beneath 1 mm^2 of epidermis. All the results are expressed in these terms so as to give an indication of changes in the total amount of collagen rather than the collagen concentration which would result from the expression of the collagen content as a fraction of the mass of the biopsy sample.

The inner (d) and the outer (D) diameters of the cortical region of the second metacarpal bone were measured using vernier calipers on radiographs of the hand. From these dimensions the metacarpal index (MI) was calculated by the formula

$$\frac{(D^2-d^2)}{D^2} \times 100$$

(Nordin, 1971), which records the cortical area as a percentage of the total cross sectional area.

Results

Details of age, number of years past the menopause, the duration of the rheumatoid arthritis, the metacarpal index, and the total skin collagen for the 22 patients receiving corticosteroid therapy are shown in the Table. No details of dosage are included since it had previously been shown (Hall *et al.*, 1974b) that the loss of collagen from the skin is independent of dose. Total dosage over periods of between 4 and 144 months ranged from 1700 to 36 000 mg of prednisolone. The mean age (± SD) at menopause of the 18 women who had passed this stage of life was 47·5 ± 5·5 years.

METACARPAL INDICES OF NORMAL AND OSTEOPOROTIC SUBJECTS

Metacarpal indices were calculated from *x*-ray pictures of the hands of 40 normal and 22 corticosteroid-treated subjects. The indices are displayed

Table *Details of 22 patients receiving corticosteroid treatment for rheumatoid arthritis*

Case no.	*Age*	*Years past menopause*	*Duration of arthritis (years)*	*Metacarpal index*	*Total skin collagen (μg/mm²)*
1	23	—	8	73	270
2	35	—	20	46	122
3	39	—	6	75	253
4	46	7	7	81	175
5	50	—	22	81	270
6	52	4	4	78	199
7	56	18	10	73	184
8	56	4	16	45	184
9	56	13	13	42	161
10	56	16	36	46	165
11	56	11	11	57	170
12	58	8	10	54	157
13	59	10	20	55	160
14	60	10	11	83	193
15	62	22	20	34	184
16	63	8	6	56	136
17	65	8	8	59	192
18	65	15	6	63	260
19	66	16	11	54	146
20	66	15	7	54	244
21	71	21	9	50	147
22	72	22	22	56	136

as a function of age in Fig. 1. By comparison with the regression line for the 40 normal subjects it can be seen that the values for MI of 78% of the subjects receiving therapy were significantly below those of normals of comparable age (that is, outside the 5% significance limit). The value for MI of the normal subjects falls by 4·4% per decade. The scatter of results and the small number of subjects receiving corticosteroid therapy precludes the calculation of a meaningful regression line, but the trend seems to indicate a slightly less rapid regression of MI with age.

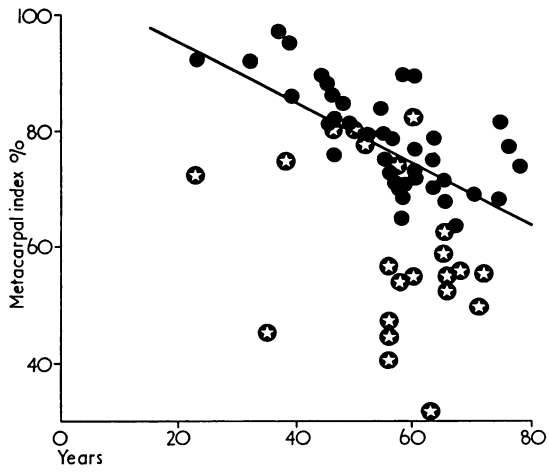

FIG. 1 *Age dependence of metacarpal indices for normal (●) and corticosterold-treated rheumatoid subjects (✪). Regression line for normal group* $y = 107 - 0{\cdot}44\ x$

TOTAL SKIN COLLAGEN CHANGES WITH INCREASING AGE

The total collagen in a column of skin beneath 1 mm² surface was determined for all 40 normal and 24 treated subjects. Again the scatter was appreciable but the collagen content of the skin of the normal subjects fell at a rate of 2 μg/mm²/decade. As with the values for MI, an appreciable proportion of those for the skin collagen content of treated subjects (87·5%) lay well below the regression line for the normals at all ages, three subjects only (cases 5, 18, and 20) retained an amount of collagen typical of 20- to 30-year-old subjects until the ages of 50, 65, and 66 respectively. Here a hypothetical regression line for the treated subjects would lie roughly parallel to that of the normal subjects but about 100 μg/mm² lower (Fig. 2).

Discussion

The correlation of MI with values for total skin collagen provides the distribution diagram in Fig. 3. The field may be divided into four quadrants by lines representing the mean value for MI for all subjects (the horizontal line at 70%) and the mean value for collagen content (the vertical line at 216 μg/mm²). The lower left hand quadrant, representing those subjects for whom both bone and skin parameters are below the mean, contains 62% of the values for treated subjects and only 5% of the normals. Only 9% of the treated subjects retain both high values for MI and for skin collagen, and these are the 'normal' subjects aged 50, 65, and 66 referred to above.

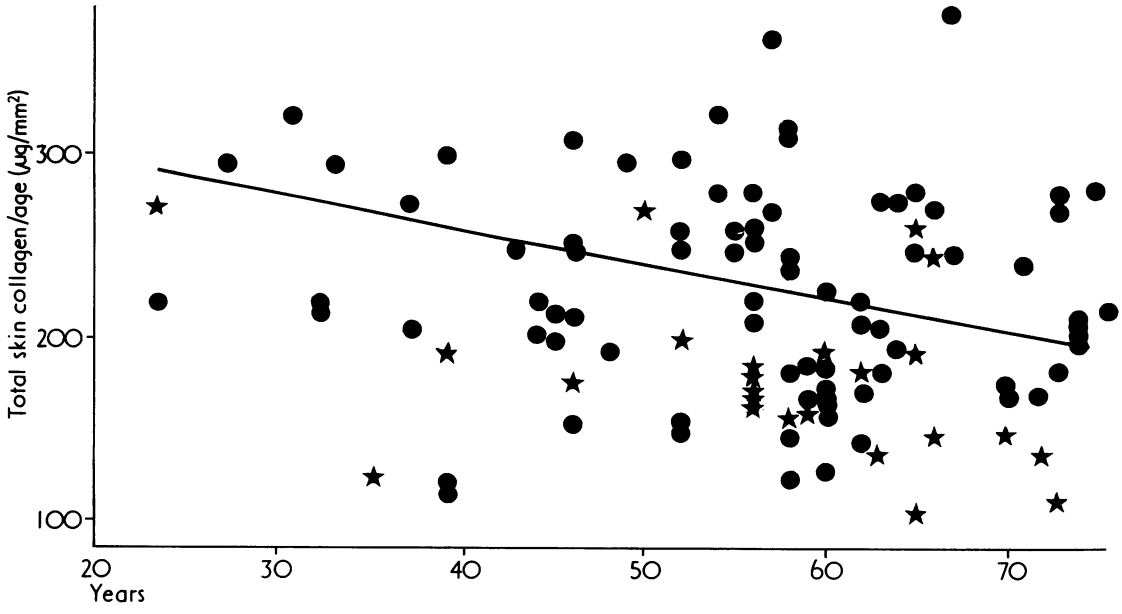

FIG. 2 *Age dependence of collagen content of a column of full skin thickness beneath 1 mm² of skin thickness for a group of normal subjects (●) and corticosteroid-treated rheumatoid arthritic subjects (★)*

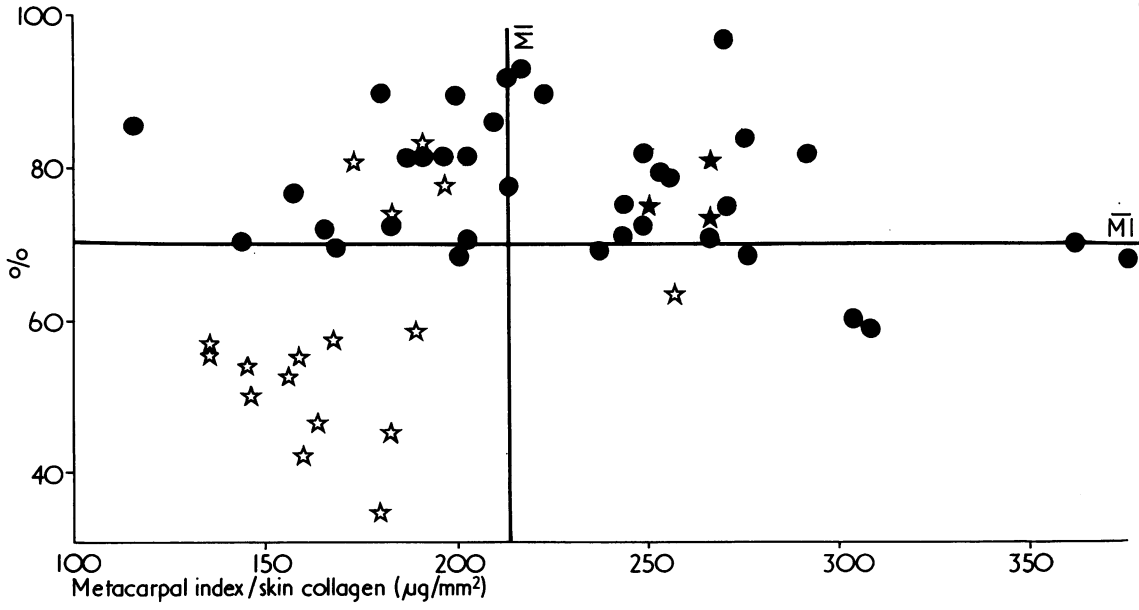

FIG. 3 *Relationship between metacarpal indices and skin collagen content for normal (●) and treated (★) subjects. Horizontal line at 70% represents mean value for MI of all subjects. Vertical line at 216 μg/mm² represents mean value for collagen content*

An assessment of the overall effects of corticosteroid therapy on both bone and skin together can be obtained by multiplying the value for MI by the figure for collagen content. A comparison of values for this composite factor for normal and treated subjects in three age groups is shown in Fig. 4. There are too few individuals in the lowest age groups to justify statistical analysis by normal numerical methods, but the Mann-Whitney ranking methods (Siegel, 1956) shows that the treated rheumatoid arthritic group is not significantly lower ($P > 0{\cdot}2$) than the normal group. The other two groups, however, may be compared using Students *t* test, when differences between the mean values for the normal and pathological groups of 178·6 and 109·4 and 164·5 and 95·3 respectively can be shown to be highly significant (40–60, $P \leqslant 0{\cdot}0005$; 60–80, $P \leqslant 0{\cdot}0005$).

The composite factor falls away at a greater rate when plotted against the number of years since the menopause than when plotted against the full age of the individual, the equation of the regression line against age being $y = 217 - 2{\cdot}07\ x$ whereas that against the postmenopausal period is $y = 139 - 2{\cdot}8\ x$. The slopes of these lines are significantly different ($P \leqslant 0{\cdot}05$) whereas the intercepts are not.

That the composite effect on both bone and skin collagen is dependent not only on the treatment with the corticosteroid but also on the underlying rheumatoid arthritis may be seen from Fig. 5, which records the changing value of the composite function MI × collagen content against the duration of the disease. The relationship is not linear but essentially parabolic, the composite function varying as the square of the period since the onset of the disease.

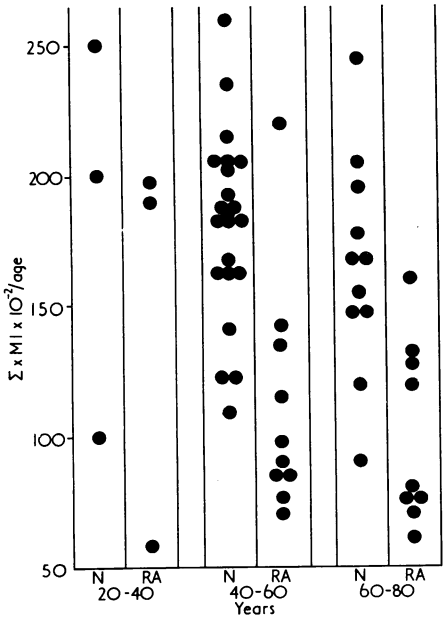

FIG. 4 *Age dependence of the composite function, collagen content (Σ) × metacarpal index (MI). Points in left hand column of each age group represent values for normal individuals, right hand column represents values for corticosteroid-treated group*

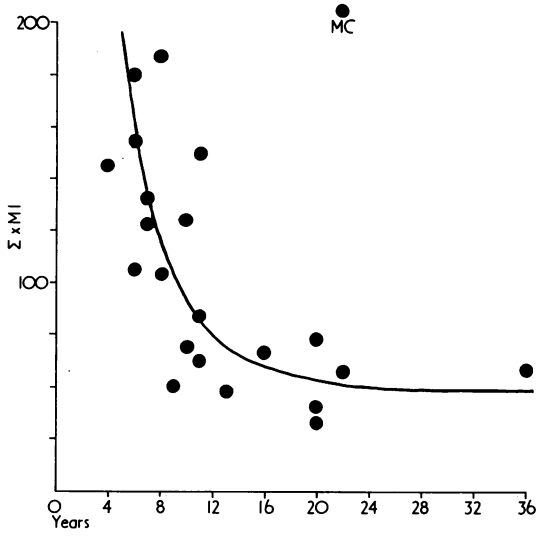

FIG. 5 *Relationship between composite function (Σ × MI) and period since onset of rheumatoid arthritis for group of 22 rheumatoid subjects*

From this it can be deduced that the bone involvement and the skin changes, although individually dependent on the duration of the disease (MI = 75 − 1·2 × duration in years; total skin collagen = 220 − 4·1 × duration in years) are independent of one another. They seem, however, to be complementary, since the scatter of the composite function when plotted against duration (Fig. 5) is appreciably less than that of either individual parameter plotted on its own. The correlation coefficient of the square root of the composite function is in fact 22% higher than the mean value of the two parameters plotted independently.

Two of the 'abnormal' cases fit closely into this linear relationship of MI × Σ collagen/duration of disease, indicating that although most treated subjects show low values for both parameters a certain small proportion, for whom no outstanding characteristics can be demonstrated, provide a composite function which fits closely into the relationship characteristic of the majority by the combination of a relatively high value for one parameter with a low value for the other. The sole remaining outlier

(case 5), although a rheumatoid of 22 years' standing, had not yet started treatment, thus demonstrating the joint dependence of the skin and bone parameters on more than one factor.

It may therefore be concluded that the loss of collagen from the skin of subjects with rheumatoid arthritis and the rarefaction of bone which accompanies corticosteroid therapy are dependent on: (1) the age of the subject (Hall *et al.*, 1974a,b); (2) the loss of hormonal stability resulting from the menopause; (3) the period since the onset of the disease; and (4) treatment with corticosteroid, although not directly dependent on the dosage (Hall *et al.*, 1974b).