iting factor in the synthesis of dopamine and norepinephrine. Rats are born with only 15 percent and 30 percent of the normal adult amounts of brain dopamine and brain norepinephrine, respectively (*22*). A deficiency in the tyrosine available for the biosynthesis of catecholamines might alter the mechanisms that are responsible for the complex regulation of these neurotransmitters. It is possible that some of the physiological and behavioral sequellae of early protein-calorie malnutrition result from the changes in brain catecholamine metabolism described in this report.

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24. Supported in part by PHS research grant (AM-14228) and training grant (GM 01337-05). We thank Frances Larin and Chuan Chou for technical assistance.

19 October 1970

Scleroderma and the Subcutaneous Tissue

Abstract. *Our study revealed that as observed with both light and electron microscopy the most specific abnormality in scleroderma skin is the replacement of the subcutaneous tissue by markedly abnormal connective tissue.*

Systemic scleroderma is a disease of connective tissue which may affect the skin, lungs, kidneys, gastrointestinal tract, and heart. The disease may be fatal. The skin, the most common site of involvement, shows marked induration, ischemia, and sometimes gangrene. Despite these striking clinical manifestations, numerous histochemical, electron microscopic, and biochemical studies have failed to demonstrate the nature of the alteration in the connective tissue of the skin (*1*). On regular biopsies, the following features have been emphasized: (i) thickening of the dermis by fibrosis, (ii) homogeneous appearance of the connective tissue, and (iii) vascular changes. However, in many instances the dermal changes are minor and are not diagnostically significant. We now report evidence that the most striking pathology in scleroderma skin consists of the replacement of the subcutaneous fat by markedly abnormal connective tissue.

Ten patients (seven females and three males, 21 to 69 years old) who had had systemic scleroderma for 2 to 14 years were selected for this study. All patients had acrosclerosis and Raynaud's phenomenon, six had pulmonary fibrosis with impairment in pulmonary diffusion, and seven had dysphagia. Kidney involvement, as determined by biopsy, was noted in five cases. Skin biopsies were only performed on markedly indurated areas (dorsum of hand and forearm in eight patients; dorsum of finger in two). Specimens of normal skin for controls were obtained from similar areas. In order to include the entire subcutaneous tissue, the biopsies were taken down to the depth of the muscle fascia. Specimens for histochemistry were fixed in 10 percent buffered formalin and stained with the following stains: hematoxylin and eosin, periodic acid–Schiff reagent (PAS), Gomori's trichrome, aldehyde fuchsin, Verhoef's elastic tissue stain, and Alcian blue, *p*H 2.5. Seven cases were also studied by electron microscopy. Each biopsy was sectioned with a scalpel at three different levels, from the top to the bottom, so that four specimens, about 1 mm^3 each, could be studied independently. The specimens were fixed in 3 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer for 3 hours, washed several times with 12 percent sucrose in cacodylate buffer, treated with 2 percent osmium tetroxide, dried in graded alcohols, embedded in Spurr low-viscosity resin (*2*), and stained with uranyl acetate and lead citrate or phosphotungstic acid. The blocks were sec-

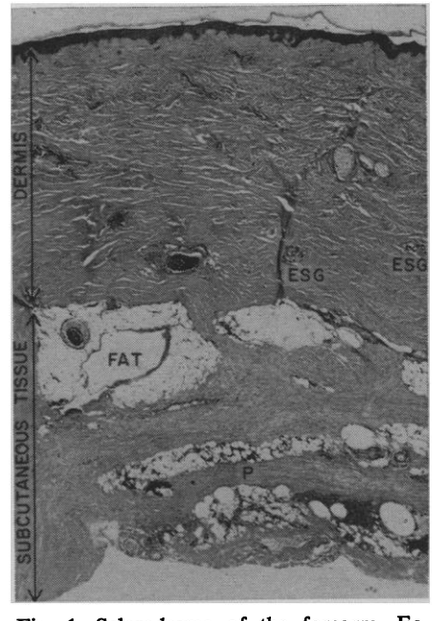

Fig. 1. Scleroderma of the forearm. Eccrine sweat glands (*ESG*) are located in the lower third of the dermis. Note the difference in connective tissue structure between the dermis and subcutaneous tissue area. Focal areas of panniculitis at the lower level (*P*) (hematoxylin and eosin, × 32).

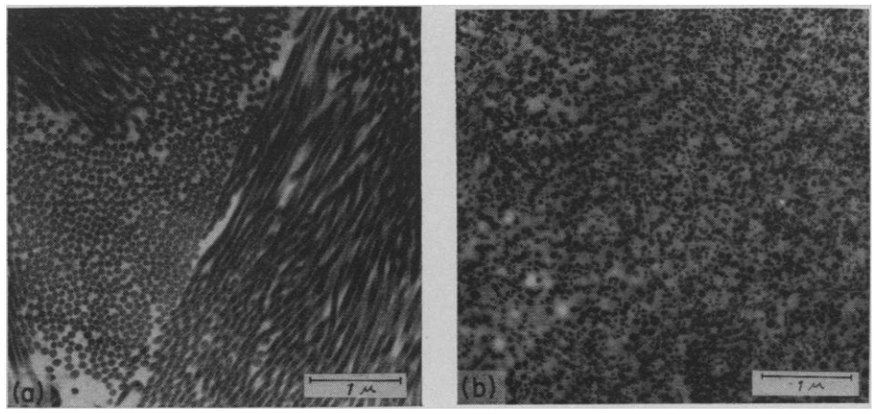

Fig. 2. (a) Electron micrograph of the upper dermis. Note the uniform diameter of fibrils, which are organized in bundles. The ground substance appears as clear, electron-transparent areas (× 27,000). (b) Lower level revealing great variability and reduction in fibril diameter. The ground substance shows an increase in electron opacity (× 27,000).

tioned in a Reichert ultramicrotome and studied with a Jem-7 electron microscope.

Epidermal changes were not remarkable except for some atrophy and effacement of the rete ridges. The pilosebaceous apparatus was usually atrophic or absent. Eccrine sweat glands were frequently preserved although they were devoid of their surrounding adipose tissue. These glands were located in the upper third or middle of the sections. Normally, eccrine sweat glands are found in the lower third of the dermis or at the junction of the dermis and the subcutaneous tissue. The actual dermis, comprising the area between the epidermis and the region just below the eccrine sweat glands, showed normal thickness. The collagen was arranged in thick bundles which appeared compressed by the reduction in interfibrillary spaces (Fig. 1). This collagen stained an intense blue with the trichrome stain. The number of cells, mostly fibroblasts, was normal or reduced. The elastic tissue was normal. The area corresponding to the subcutaneous tissue, which comprised half to two-thirds of the thickness of the sections, revealed marked replacement of fat by abnormal connective tissue. The zone of demarcation between the dermis and the subcutaneous tissue area was easily discernible with all the stains used in this study (Fig. 1). The connective tissue in the subcutaneous area was homogeneous although at high power it revealed fine, wavy collagen fibers, which stained pale blue with the trichrome. These areas were frequently stained positively with PAS and Alcian blue. In most specimens, there were isolated islands of fat tissue, sometimes invaded by fibroblasts and lymphocytes. Oval, clear spaces, with or without flat peripheral nuclei suggesting remnants of fat cells, were noted. The elastic tissue was markedly reduced and consisted of short, thin, wavy fibrils. The number of fibroblasts was larger than that seen in the actual dermis. However, in long-standing cases very few cells were noted throughout the sections.

The electron microscopic findings correlated well with those obtained with light microscopy. The dermis revealed mature collagen fibrils organized in bundles and surrounded by a clear ground substance. The predominant diameter of the fibrils ranged between 700 and 800 Å. At high magnification (× 450,000) these fibrils revealed the characteristic 600-Å periodicity with eight electron-opaque intraperiod bands. All the above findings are compatible with those for normal collagen and ground substance. The middle sections showed a progressive reduction in the diameter of the collagen fibrils. There was a marked increase in ground substance which appear as electron-opaque granular or structureless material. The lower sections showed striking alterations. At this level most fibrils were immature and arranged in random fashion with the predominant diameter ranging from 200 to 400 Å. A few fibrils had diameters below 100 Å (Fig. 2). Of 200 fibrils measured, only two were normal in thickness. Some fibrils had tapered ends. Microfibrils with a diameter of less than 20 Å were noted in several areas. These represent collagen in a very early stage of differentiation. There was a massive increase in ground substance, represented by granular or structureless material containing few fine fibrils, many of which were bent or curved (Fig. 3). At high magnification these collagen fibrils revealed normal periodicity and intraperiod structure. Normal control specimens revealed at all levels collagen fibrils of uniform diameter (700 to 800 Å) arranged in bundles.

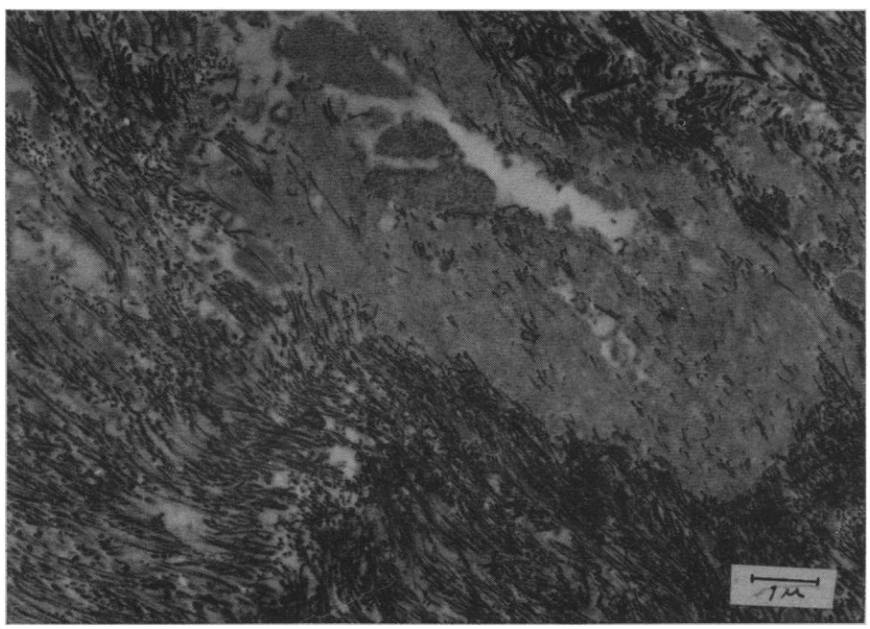

Fig. 3. Lower level showing a massive increase in ground substance. The collagen fibrils are thin and disorganized (× 13,000).

Braun-Falco and Rupec (*3*) noted in the dermis of systemic scleroderma isolated collagen fibrils of reduced thickness (ranging from 500 to 800 Å) intermingled with normal collagen fibrils. Hayes and Rodnan (*4*) also described in the dermis variation in collagen fibrils (200 to 700 Å) and the presence of double stranded beaded filaments of 640-Å periodicity. The above authors concluded that their findings suggested an increase in the rate of collagen synthesis in scleroderma dermis.

Our study suggests that the most specific abnormality in systemic scleroderma is the replacement of the subcutaneous tissue by abnormal connective tissue, both within the fibrils and the ground substance. It is unlikely that the newly synthesized connective tissue arises from the dermis, since in this layer there were very few fine collagen fibrils, no increase in ground substance, and normal or reduced numbers of fibroblasts. Three types of cells were noted in the subcutaneous area: fibroblasts, fat cells, and lymphocytes. It is likely that the fibroblasts are responsible for the synthesis of the newly synthesized connective tissue. The persistence of immature collagen fibrils in long-standing cases suggests an impairment or delay in the mechanism of fibril aggregation probably due to interference by the increased ground substance. We believe that chemical analysis of the abnormal connective tissue in scleroderma may not only improve our understanding of this disease but may also yield useful information on the basic mechanism of collagen fibrillogenesis and its relationship to the ground substance.

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18 November 1970

Extracellular Recordings from Human Retinal Ganglion Cells

Abstract. *Ganglion cells were studied in the isolated retina, with extracellular recordings. Activity was found similar to that seen in the retinas of other animal species.*

The isolated retinal preparation has been employed for the investigation of biochemical and physiological activity in a variety of species, including mammalia (*1–4*). In previous reports, we have described studies of adaptation in the rat retina (*5–8*). When suitable conditions exist, the human retina may be studied in a similar fashion.

On several occasions, we have been able to learn in advance of the need for enucleation of otherwise normal eyes from patients harboring malignant melanomas of the choroid. Cooperation from both patient and surgeon was obtained so that a black, opaque contact lens could be worn for several hours prior to and during the operation, thereby permitting the retina to remain relatively dark-adapted. Surgical technique was modified so as to avoid interfering with the retinal circulation until the moment of severance of the optic nerve. The globe was then rapidly opened in dim red light, and the retina, in an area remote from the tumor, was gently dissected from its pigment epithelium and from the vitreous.

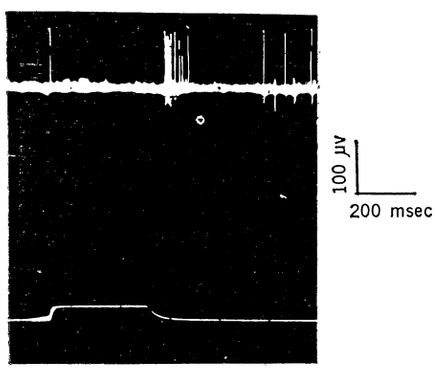

Fig. 1. Extracellular recording from a single human retinal ganglion cell. Upper trace shows maintained activity and burst of responses following cessation of stimulus, shown on lower trace. A few smaller action potentials from a second ganglion cell are also visible.

The procedure for mounting, incubating, and recording from the retina was identical to that used in our previous studies. The light source was a well-regulated, xenon-arc lamp (Bausch and Lomb). Narrow band interference filters (Baird-Atomic) were used for chromatic stimulation, together with a pair of rotating-wedge neutral density filters (Kodak Wratten) for stimulus attenuation. Most often, electroretinographic (ERG) responses, which closely resembled those from the living eye, could be obtained, although occasionally no ERG could be recorded. Ganglion cell action potentials of 100 to 300 μv were frequently encountered; although most units showed marked spontaneous activity, only a few were responsive to light stimuli (Fig. 1). Both ERG and ganglion cell responses usually remained stable for several hours.

When we used small (80 to 100 μm) spots, some units were found with center-surround receptive field organization. Both on and off center types were found.

Two units in one retina were studied with chromatic stimuli, although adapting backgrounds were not employed. The portion of the retina studied was from the temporal midperiphery. The sensitivity data obtained for these units are shown in Fig. 2. The data are plotted in relative log quanta per pulse incident on the retinal tissue versus wavelength in nanometers. Also illustrated is the π_5 curve of Stiles (*9*). Most points for both units lie on or close to this curve at about 540 nm, but above the curve at shorter wavelengths. These data also agree closely with Wald's sensitivity curve for the red receptors, obtained by microspectrophotometry

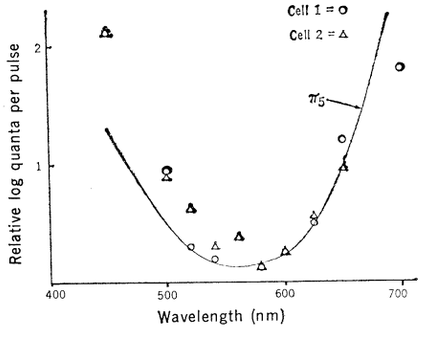

Fig. 2. Spectral sensitivity data for two human retinal ganglion cells. The continuous curve is the π_5 curve of Stiles.