Dupuytren's Contracture

Some Associated Biophysical Abnormalities

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Dupuytren's contracture is a pathological fibrosis involving thickening and contracture of the palmar fascia. It occurs in one to 2 per cent of the adult population.[3] Many studies have pointed out statistical correlations[9] and associations with other diseases.[9, 15] Several etiologies have been suggested,[4, 21] the most prominent being that of trauma proposed by Dupuytren and subsequently by Skoog[15, 16] and Larson[11] who noted the presence of hemosiderin. Biochemical studies have shown differences between normal palmar fascia and Dupuytren's tissue.[10, 17, 18] Histologic patterns have been demonstrated,[4, 8, 11, 15] and ultrastructure studies revealed no abnormality of collagen fibrillar diameter.[5]

The material removed at surgery from hands with Dupuytren's contracture is principally the structural protein collagen. We have been engaged in the study of normal collagen utilizing the biophysical technics of electron paramagnetic resonance (EPR)§ and trace element analysis by emission spectroscopy. Normal parameters have been established by these two technics,[6, 13] and it seemed appropriate to utilize them as a basis for the study of collagen associated with a pathological process. Accordingly, we have studied Dupuytren's material to determine whether it exhibits any biophysical abnormalities which could be related to the etiology or subsequent course of the disease.

MATERIALS AND METHODS

Seven specimens were removed at surgery from hands exhibiting thickening of the plamar fascia. Five had classical Dupuytren's contracture with thickening and fibrosis of the palmar fascia and definite joint contracture either of the metacarpophalangeal or proximal interphalangeal joints or both. Specimens from 2 hands with thickening of the palmar fascia but without any joint contracture were from a patient with Raynaud's phenomenon and pseudo-Dupuytren's contracture. By "pseudo-Dupuytren's" we mean the condition of thickening and nodularity of the palmar skin and fascia but without joint contracture. This association of palmar thickening and Raynaud's disease is not uncommon and has

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§ Electron paramagnetic resonance, also known as "electron spin resonance," measures the change in spin energy of individual *unpaired* electrons when subjected to external microwave energy in a varying magnetic field. Common systems with an unpaired electron such as free radicals in biological systems, paramagnetic ions of the iron group (Table 1), ferromagnetic materials, freed radicals resulting from radiation damage and impurities in semiconductors can be measured very precisely, giving important structural information.

been noted recently.[9] Normal palmar fascia, removed at surgery for carpal-tunnel release, was studied. All specimens were from men in the fourth through sixth decades of life.

Serial sections for light microscopy using hematoxylin and eosin, and Prussian blue for Fe^{3+} iron[12] were prepared. Electron microscopic examination was performed on 3 Dupuytren's specimens. Samples for EPR analysis were air-dried and then cut into single pieces each approximately 40 mg. Individual samples were placed in quartz tubes and examined in a Varian EPR spectrometer operating at X-band. Spectra were recorded at room temperature and at − 159 C. Two autopsy specimens of liver and 2 of spleen with a clinical diagnosis of hemosiderosis were also studied to determine the hemosiderin signal, along with normal human liver and spleen from autopsy specimens. In addition, arc emission spectroscopy studies were done on selected samples utilizing previously described methods.[1, 6]

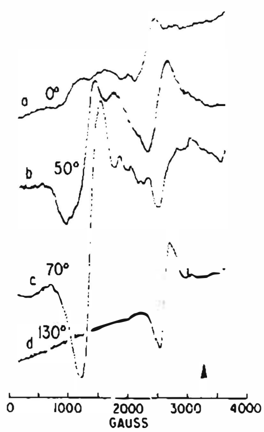

FIG. 1. Spectra from a Dupuytren's sample as a function of its angular position in the magnetic. field. No periodic g-value variations were observed. The illustration is constructed to show the extent of signal variation encountered. A narrow isotropic resonance at g = 2.0 (arrow) similar to that from normal tendon collagen is seen in all material studied with an amplitude proportional to sample size. This resonance is not considered here. When we say a sample exhibited no resonance, we mean no additional resonance.

RESULTS

Light microscopy of all 7 specimens showed typical areas of fibroblastic proliferation with swirling, elongated nuclei adjacent to areas of hyalinization and relative acellularity with some areas of vascular proliferation. Electron micrographs showed no overall structural orientation.

All 5 fascial samples from the Dupuytren's cases demonstrated similar EPR spectra which were markedly different from the singlet at $g = 2.0$ seen in normal collagen.[13] The spectra were composed of complex broad resonances with angularity dependent g values ranging from about $g = 2.0$ to $g = 5.0$. Figure 1 illustrates the extent of this effect; it can be seen that the shape, g-value, and amplitude of the resonance depends strongly on the orientation of the sample in the magnetic field. Figure 2 shows the spectra obtained from samples of each of 4 specimens. Samples from the fifth specimen had similar but less intense spectra. Each sample in Figure 2 exhibited angularly dependent spectra and the "most alike" from each sample were selected for Figure 2 in an effort to determine whether we were dealing with a number of magnetic species, or one magnetic species. We found that the magnetic center was not uniformly distributed; for each specimen, some samples exhibited the resonance, others did not. The percentage in each class varied with the specimen and ranged from 20 to 80 per cent. The 2 specimens of pseudo-Dupuytren's exhibited no resonances, other than the normal singlet

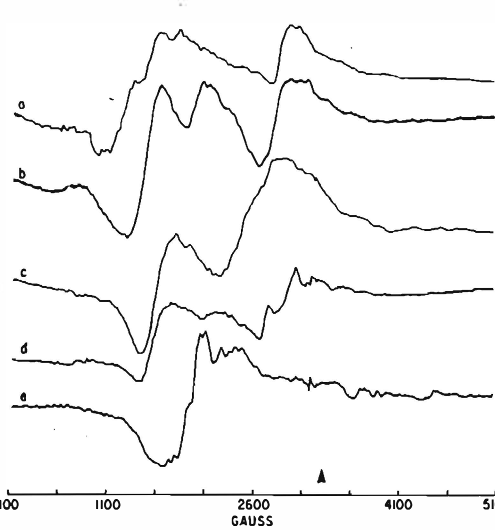

FIG. 2. Spectra of 5 samples from 4 different specimens. c and d are from different samples of the same specimen. The same magnetic species is assumed to be responsible for all spectra.

TABLE 1. Comparison of the Trace Element Content of Samples of Dupuytren's Contracture which Exhibited an EPR Spectrum with Samples of Dupuytren's Contracture which did not Exhibit an EPR Spectrum (Each number represents the results of 4 separate analyses. The results show no significant difference.)

Element	*Concentration (ppm dry weight)*	
	Material Exhibiting an EPR Spectrum	*Material Not Exhibiting an EPR Spectrum*
Iron	34	49
Copper	5.6	7.0
Chromium	1.4	1.0
Titanium	N.D. (10)*	N.D. (10)
Vanadium	N.D. (10)	N.D. (10)
Manganese	N.D. (10)	N.D. (10)
Cobalt	N.D. (10)	N.D. (10)
Nickel	N.D. (10)	N.D. (10)

* N.D. Not detected. Limits of detection (ppm) are noted in parenthesis.
Spectroscopic analyses courtesy of Dr. Joseph A. Spadaro.

at $g = 2.0$, as did the normal palmar fascia.

Samples of Dupuytren's with and without a resonance and samples of pseudo-Dupuytren's were found to be histologically identical when stained with hematoxylin and eosin. Free radicals are a common source of EPR spectra from biological materials; however, unlike the EPR behavior of our samples, they usually produce narrow isotropic resonances around $g = 2.0$. Other possibilities for the origin of the spectra are the ions of the iron group elements which are paramagnetic. Accordingly, we performed arc emission trace element analyses on selected Dupuytren's samples using technics previously described for bone[1] and tendon.[6] The results, given in Table 1, showed no significant differences in trace ion content between samples exhibiting and not exhibiting an EPR spectrum. With the exception of an elevated iron content, the trace element composition is comparable to that of normal human tendon.[6]

Hemosiderin deposits are commonly found in some areas of Dupuytren's material.[8] Since hemosiderin contains a ferric oxide-hydroxide core,[14] the possibility was considered that the spectra were due to the inorganic iron portion of the hemosiderin. Histologic sections were stained for hemosiderin.[12] All of the Dupuytren's samples were found to contain about equal concentrations of hemosiderin, whether the samples showed an EPR signal or not. Figures 4 A and C show EPR spectra from strongly hemosideric spleen and liver. The main resonance, presumed to be hemosiderin, is at $g = 2.0$, has a width of about 500 gauss, and shows no angular dependence. The narrow singlets are the only resonances seen in the normals.

Additional EPR spectra of the Dupuytren's specimens were recorded at — 150 C. If a paramagnetic ion caused the resonance, the Curie Law would dictate a threefold increase in signal amplitude at that temperature.[14] Yet the Dupuytren's samples actually exhibited a small decrease in amplitude, plus an accompanying broadening at that temperature (Fig. 3). The main resonances of hemosideritic spleen and liver behaved similarly (Fig. 4).

DISCUSSION

All 5 Dupuytren's specimens yielded broad anisotropic EPR spectra with general similarities in the shape and properties leading us to conclude that they are due to the

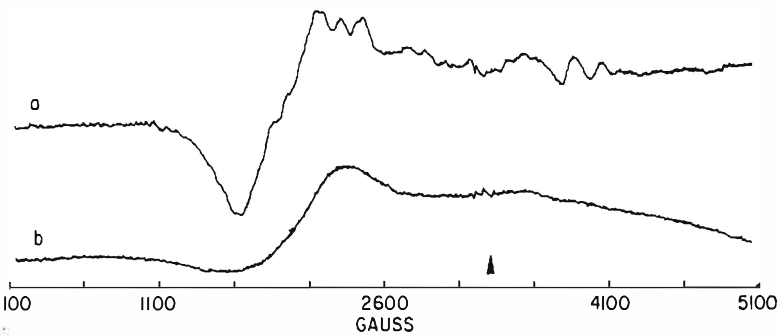

FIG. 3. Spectra from a Dupuytren's sample: (a) at room temperature; (b) at − 159 C.

same magnetic species. The species is nonuniformly distributed throughout the Dupuytren's specimen, and its existence could not be correlated with any histologic feature such as nodularity, collagen organization, etc. (In view of the steps necessary for preparation of histologic slides, however, it cannot be concluded that no such correlation existed.) The strong angular dependence of the signal is indicative of the fact that the magnetic species is organized into some crystalline order. Moreover, the symmetry of the magnetic site is such that the g-value in each of the 3 spatial directions is different; this is inferred from the results seen when samples exhibiting spectra were minced. In an attempt to produce a "powder spectrum," samples exhibiting a spectrum were minced into pieces of less than 0.1 mm. We found, however, that this procedure removed the angular dependent signal, possibly indicating that the signal is related to long-range order in the quasi-crystalline collagen matrix.

The lack of temperature dependence of the signal is considered presumptive evidence that the magnetic species is ferromagnetic rather than paramagnetic. This effect has been reported for both naturally present and artificially added impurities in DNA.[19, 20] Since cobalt and nickel were not detected spectrographically, the ferromagnetic substance is probably an iron compound. Generally organic molecules, such as hemoglobin, which contain iron (Fe^{3+}) are such that the average distance between Fe^{3+} atoms is too large to permit ferromagnetic interaction to occur. For this reason, the EPR spectra from hemoglobin are paramagnetic[7] and cannot account for the observed resonances. Furthermore, the inorganic, crystalline iron compound presumed to be responsible for the observed spectra does not appear to be hemosiderin.

The EPR spectrum of hemosiderin, being isotropic and centered at $g = 2.0$, is quite different from that observed in the Dupuytren's cases. Also, neither the Prussian blue staining nor the spectrographic analysis indicated any difference between samples possessing or lacking a resonance. Since the magnetic species is present in all the specimens of true Dupuytren's contrac-

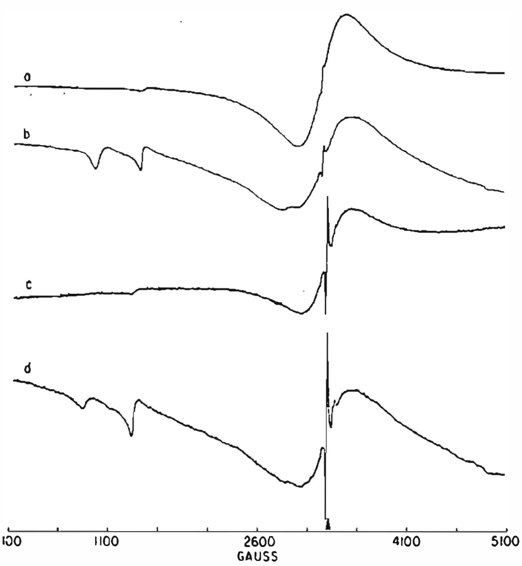

FIG. 4. Spectra from hemosideric spleen (a and b), and liver (c and d); a and c at room temperature, b and d at − 159 C. The low field resonances on b and d are due to paramagnetic Fe^{3+}.

ture, and in neither of the pseudo-Dupuytren's pecimens or normal palmar fascia, it may constitute an etiologic factor. Since the magnetic species appears not to be hemosiderin, we believe the thesis of a traumatic etiology is cast in doubt. In light of our results we suggest alternatively that patients exhibiting Dupuytren's contracture may have some disorder in iron metabolism which is responsible either directly or indirectly for the onset of the pathological state.

SUMMARY

Pathological material from 5 cases of Dupuytren's contracture has been found to exhibit EPR spectra suggesting a non-uniformly dispersed, crystalline, ferromagnetic aggregate. These spectra were not found in 2 specimens of pseudo Dupuytren's and are not present in normal collagen from a variety of sources, including human tendon and normal palmar fascia. Hemosiderin is not the responsible magnetic species. It is postulated that the ferromagnetic material present in the cases of Dupuytren's contracture may indicate a defect in iron metabolism related to the onset of the disease state.

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