

Effects of Mechanical Action on the Electron Paramagnetic Resonance of Wool and Silk

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Synopsis

Wool fibers and cloth damaged by mechanical action such as abrading and grinding show strong EPR signals. Silk fibers after grinding also show strong EPR signals. Both wool and silk ground in a dry nitrogen atmosphere exhibit a doublet spectrum attributed to a $\cdot\text{CH}$ radical on the polypeptide chain. In wool, a complex curve centered at $g = 2.025$ is observed. It is attributed to a $-\text{CH}_2\text{S}\cdot$ radical resulting from the rupture of the disulfide bond in the cystine residue. These radicals are stable for many months in a nitrogen atmosphere or vacuum. In dry oxygen the spectrum of the $\cdot\text{CH}$ radical is transformed within a few hours from a double into a single, narrow curve, whereas the spectrum characteristic of the cystine radical disappears more slowly. In the presence of water vapor both radical species are rapidly destroyed and their spectra disappear. The mechanisms for the production of free radicals by mechanical action are believed to be direct chain rupture or localized pyrolysis, coupled with migration to specific radical traps in the protein.

Introduction

In this paper we describe the electron paramagnetic resonance (EPR) of free radicals produced by mechanical action on wool and silk. A naturally occurring EPR in wool and silk is discussed in an earlier paper.¹ Other papers in preparation will discuss free radicals produced in wool by ultraviolet irradiation, including the effects of moisture, oxygen, temperature, and chemical modification on the EPR spectra and the relationships between the induced radicals and the color of ultraviolet-irradiated wool.

Free radicals are commonly produced in materials by ultraviolet, x-ray, γ -ray, or other irradiation and also by pyrolysis. Early in 1960, H. P. Lundgren (in conversation) suggested that mechanical damage might give rise to detectable free radicals. Following this suggestion, we ground wool fibers in a Wiley mill and obtained a strong EPR spectrum. Subsequently we found that the much less severe mechanical action encountered in normal wool processing also produced detectable EPR spectra. To determine if electron resonance was produced in other natural protein fibers by mechanical action, ground silk was also investigated.

Since the start of this work, paramagnetic resonance has been reported from defects introduced near the surface of inorganic solids by mechanical damage,² and free radicals have been detected by EPR in polymers degraded mechanically by milling at temperatures below the vitrification point,³ in some keratinaceous proteins degraded by filing with a rough file,⁴ and in wood as a result of grinding and pulping.⁵

Experimental Apparatus and Techniques

The EPR spectrometer, operating at *X*-band, was of our own construction. With this spectrometer, a Varian 6-in. electromagnet and 100 kcycle sec. multipurpose cavity are employed. The first derivative of the absorption curve is recorded versus the applied magnetic field, H_0 . The g values have been measured with respect to diphenyl picryl hydrazyl ($g = 2.0036$) and are accurate to within $\pm 0.1\%$.

Fibers were ground in a Wiley mill to pass a 60-mesh screen. The fiber fragments had an average length of about 300 μ . To grind the fibers in an inert atmosphere, the mill was placed in a dry box pressurized with a constant stream of dry nitrogen gas. After grinding, the samples were packed in nitrogen-filled quartz tubes, 3.0 mm. i.d., which were then evacuated and sealed.

The C.S.I. Stoll Flex tester⁶ was used to abrade samples of wool cloth. All abrading was done at room temperature and humidity ($\sim 25^\circ\text{C}$. and $\sim 40\%$ R.H.). Samples of wool fibers at various stages of worsted processing were obtained from our wool processing laboratory. Spectra were obtained on these samples within 15 min. after the processing step had been completed.

Experimental Results

Ordinary wool fibers or cloth exhibit a characteristic, stable, relatively weak EPR spectrum which is not detectable in the presence of the spectrum produced by grinding. Grinding, however, does not destroy this natural resonance. If ground wool is wetted and then dried, the induced free radical spectrum is destroyed, but the naturally occurring resonance remains. Typical EPR spectra for wool before and after grinding in a dry nitrogen atmosphere are shown in Figures 1*a* and 1*b*. The spectrum for ground wool consists of a doublet, centered at $g = 2.003$ and separated by about 18 gauss, superimposed on a line of complex shape centered at a g value of 2.025. For comparison, spectra of ultraviolet and x-ray irradiated wool are shown in Figures 2*a* and 2*b*. The spectrum for ground wool is very similar to the spectrum for x-ray irradiated wool and remains unchanged for many months when the sample is stored in vacuum or dry nitrogen.

When either ground wool or x-ray-irradiated wool is exposed to oxygen, the doublet changes in a few hours to a singlet with a width of about 10 gauss which is also centered at a g value of 2.003 and is superimposed on the complex signal (Fig. 1*c*). After a few days of exposure to oxygen the



Fig. 1. Room temperature EPR spectra of wool fibers (the vertical arrow indicates $g = 2.003$): (a) naturally occurring EPR spectrum for wool fibers; (b) spectrum for wool fibers after grinding in a dry nitrogen atmosphere (sample packed in quartz tube, evacuated and sealed), this spectrum is a superposition of a $\cdot\text{CH}$ doublet and the complex curve of $-\text{CH}_2\text{S}\cdot$ radical; (c) sample (b) after exposure to dry oxygen for a few hours (the doublet has changed to a singlet while the $-\text{CH}_2\text{S}\cdot$ spectrum remains unchanged); (d) same as (c) after a few days' exposure to dry oxygen (the $-\text{CH}_2\text{S}\cdot$ spectrum has vanished, leaving only the singlet).

complex signal disappears, leaving only the singlet curve (Fig. 1d). When wool is x-ray-irradiated or ground in air, the singlet is obtained directly along with the complex spectrum, but without any evidence of a doublet.

The EPR spectrum of silk ground in dry nitrogen consists of a simple doublet centered at $g = 2.003$ with a spacing of about 18 gauss (Fig. 3a). Silk x-ray-irradiated in vacuum shows a similar spectrum, except that the

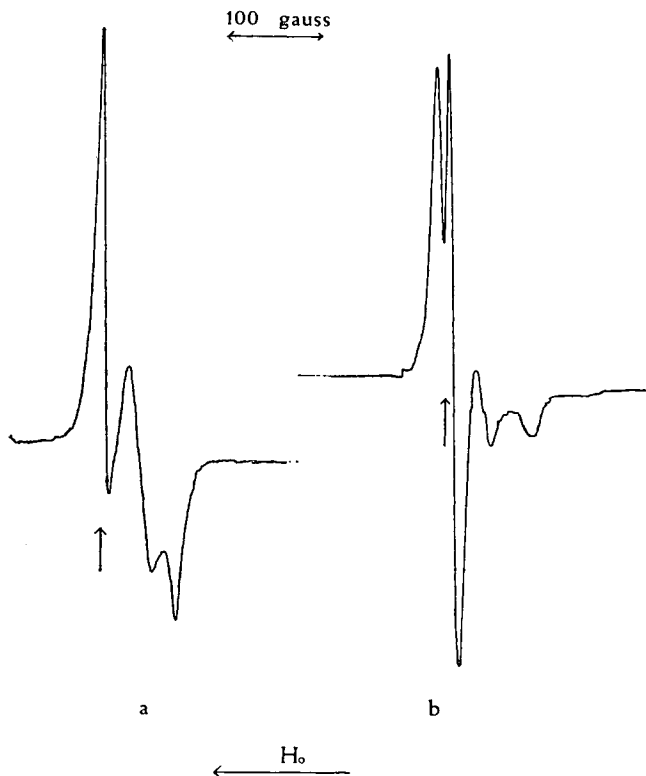


Fig. 2. Typical room temperature EPR spectra for wool cloth: (a) after ultraviolet irradiation at 2537 Å. for 17 hr. in vacuum; and (b) after x-ray-irradiation for 17 hr. in vacuum. To avoid spurious signals from the x-ray-irradiated glass, the sample was shaken down to the end of the sample tube that was not irradiated.

spacing of the doublet is only about 13 gauss (Fig. 3b). Both the x-ray-irradiated and the ground silk spectra differ from that of ultraviolet-irradiated silk (Fig. 3d). The doublet spectrum of silk stored in vacuum or dry nitrogen is stable for many months.

When irradiated or ground silk is exposed to room air, the doublet changes to a singlet centered at the same g value (Fig. 3c). Grinding or x-ray irradiation in room air results in the appearance of only the single curve.

In the presence of water vapor, the induced EPR spectra in wool and silk disappear rapidly and irreversibly.

Wool which is abraded in the presence of air shows an increase in the peak-to-peak amplitude of the naturally occurring free radical resonance as the number of abrading cycles increases. A typical change in the intensity of the EPR signal with abrasion is shown in Figure 4. In this example the cloth disintegrated at 1000 cycles.

The mechanical action of wool processing also causes variations in the free radical concentration in wool. Figure 5 shows the peak-to-peak

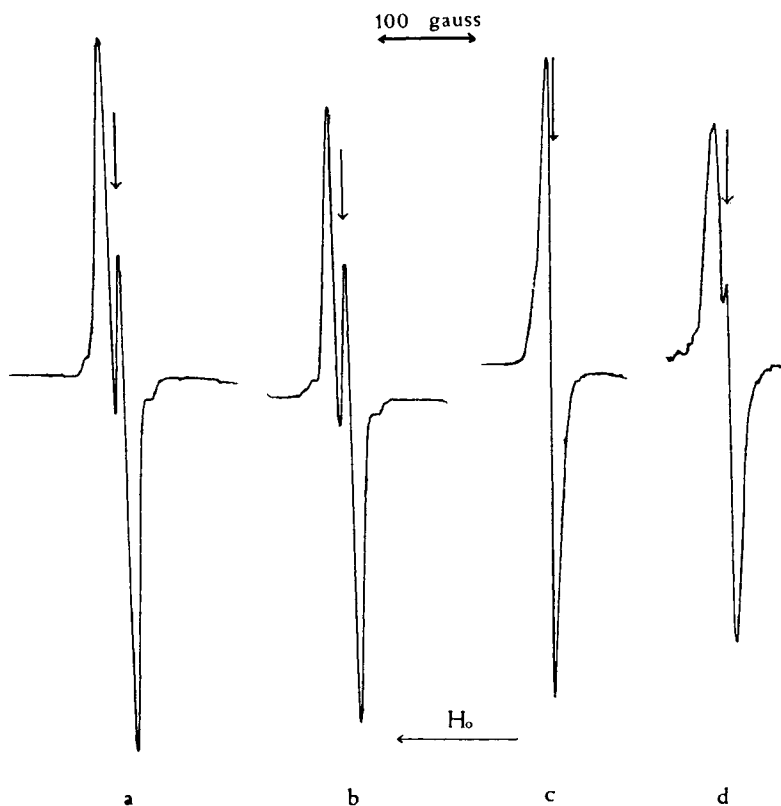


Fig. 3. Room temperature EPR spectra for silk fibers: (a) silk ground in a dry nitrogen atmosphere, then packed into a quartz tube which was evacuated and sealed; (b) silk fibers after x-ray irradiation in vacuum for 17 hr.; (c) typical singlet spectrum after exposure to air of x-ray irradiated or ground silk; (d) typical spectrum of silk fibers after ultraviolet irradiation of 2537 Å. in vacuum for 17 hr.

amplitude (in arbitrary units) of the EPR spectra in wool samples of equal weight after scouring, carding, pin drafting, and combing. (The noils are the short fibers or broken fibers separated during combing.) In this example, the second pin drafting operation produced the most radicals as measured by the amplitude of the EPR signal. These results should be considered only as illustrative of the effect of wool processing, because we have not determined their reproducibility with other lots of wool or different processing conditions.

Discussion

The doublet spectrum observed in ground wool and silk is similar to that found by Gordy in a number of proteins x-ray-irradiated in vacuum and has been explained as arising, most probably, from a $\cdot\text{CH}$ radical formed by the loss of a proton from the glycine residue in the polypeptide chain.⁷

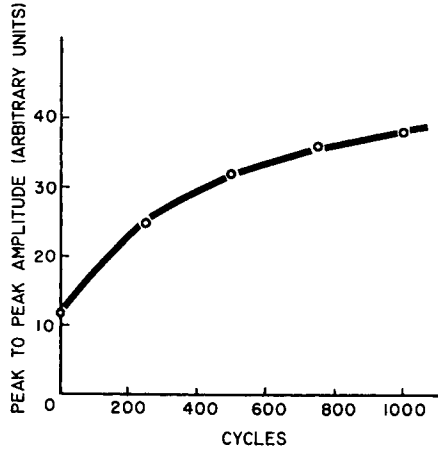


Fig. 4. Peak-to-peak amplitude (arbitrary units) of the singlet EPR spectrum for wool cloth vs. cycles of action of Stoll Flex tester. After about 1000 cycles the cloth disintegrated.

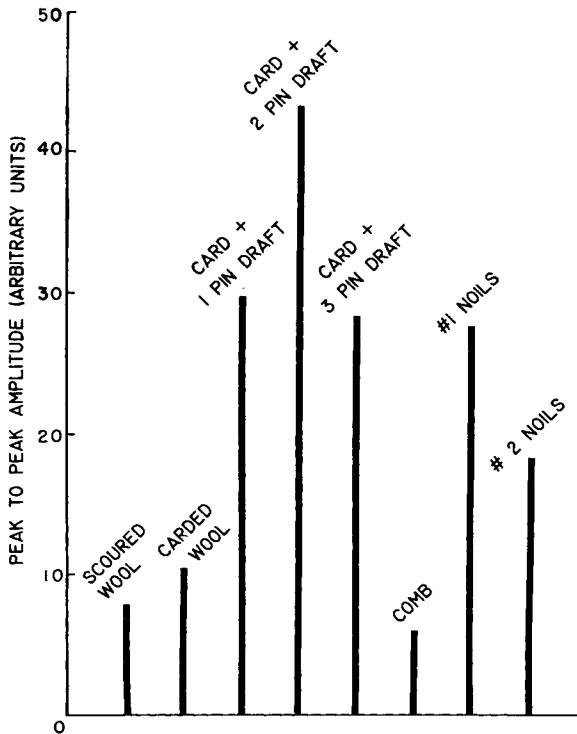


Fig. 5. Peak-to-peak amplitude (arbitrary units) of singlet EPR spectrum for wool fibers after various steps in the processing of wool fibers to cloth.

The difference in the spacing of the doublet in x-ray-irradiated and ground silk can be explained by the anisotropy of this doublet signal. In silk fibers the polypeptide chains are elongated in the direction of the fiber axis. Gordy found that when x-ray-irradiated silk fibers are oriented parallel to the applied field, the doublet splitting (A_{\parallel}) is 26 gauss, and when oriented perpendicular to the field, the splitting (A_{\perp}) is 13 gauss. The x-ray-irradiated silk fibers in this experiment were oriented perpendicular to the field and showed the expected splitting of 13 gauss. The ground silk fibers had a random orientation and a splitting of about 18 gauss, which is close to the expected value of $\frac{2}{3}A_{\perp} + \frac{1}{3}A_{\parallel} = 17$ gauss.

The doublet spectrum for ground wool is identical to the spectrum in ground silk and is also attributed to a $\cdot\text{CH}$ radical. The complex curve, which is superimposed on the doublet spectrum, appears to be similar to the spectrum observed in x-ray-irradiated keratinaceous proteins. The latter has been attributed to a free radical on the cystine residue, because of its similarity to the spectrum of x-ray-irradiated polycrystalline cystine.⁸ The g value of 2.025 is characteristic of radicals containing sulfur,⁹ suggesting that the disulfide linkage in cystine has been broken to form a $-\text{CH}_2\text{S}\cdot$ radical. Further evidence that this complex curve is due to a cystine radical is the absence of this spectrum in silk, which has practically no cystine, and in wool in which the cystine has been destroyed. For example, wool treated with peracetic acid does not show any cystine-type of resonance upon x-ray irradiation or grinding.¹⁰

It is concluded that at least two free radicals are produced in wool ground in the absence of air at room temperature: one is a $\cdot\text{CH}$ radical located on the polypeptide chain, presumably at the glycine residue; the other is a $-\text{CH}_2\text{S}\cdot$ radical on the cystine residue.

When room air is admitted to the wool and silk samples, the doublet spectrum is transformed within a few hours to a singlet. This is an irreversible reaction. The spectrum obtained is the same as that found when silk and wool are ground or x-ray-irradiated in the presence of air. This reaction is attributed to oxygen, because the same effect has been obtained in a pure, dry oxygen atmosphere.

An irreversible oxygen effect has also been observed for some γ -ray-irradiated polymers, such as low-pressure polyethylene and polychlorotrifluoroethylene,¹¹ and for γ -ray-irradiated bone.⁷ In these specimens this reaction results in the formation of a stable peroxide radical of the form $\text{R}-\text{O}-\text{O}\cdot$.^{7,11} The EPR spectrum observed is an asymmetric curve about 30 gauss wide.^{7,11}

A quite different spectrum is observed for wool and silk after exposure to oxygen, indicating that a different oxygen reaction has probably occurred. This spectrum is believed to be due to a radical formed by the loss of a proton from the hydroxyl group of tyrosine in wool and silk. The spectrum is similar to that of ultraviolet-irradiated tyrosine. The stability of this free radical can be explained by resonance of the unpaired electron in the benzene ring.¹

The cystine radical in wool also reacts irreversibly with oxygen. The reaction takes many days to complete and eventually results in the disappearance of the cystine spectrum, leaving only the single resonance curve described above.

The stability of the radicals produced in ground wool and silk also depends upon the water content of the fibers. The radicals are quite stable in dry fibers but quickly disappear when the fibers are allowed to absorb moisture. Subsequent redrying does not cause the radicals to reappear, indicating that irreversible radical reactions have occurred. The nature of these reactions is not yet completely understood.

The detection of radicals produced in wool by mechanical action that is less severe than grinding, such as abrasion (Fig. 4) and wool processing (Fig. 5), indicates that damage has occurred. In the case of wool processing, the relatively large signal from the noils indicates that we are measuring mainly the effects of fiber breakage, although some of the signal undoubtedly results from surface damage produced by abrasion in which the scales and epicuticle of the fiber are damaged or destroyed. These experiments demonstrate the sensitivity of EPR to the effects of mechanical action.

There are two possible mechanisms for the production of free radicals by mechanical action: one is chain rupture, which has been proposed for the production of free radicals in synthetic polymers by mechanical degradation;³ the second is localized pyrolysis caused by intense frictional heating during grinding. It is possible by this means to produce local "hot spots" as high as 600°C. which can result in the breakdown of organic molecules.¹²

The radicals produced in synthetic polymers were localized at the cut surfaces and reacted quickly with the surrounding atmosphere.³ The relative stability of the radicals in ground wool and silk in air, on the other hand, suggests that they are not localized on the freshly cut surfaces, but are trapped within the fiber where they cannot react as readily with the atmosphere. Thus, the radicals observed after grinding are probably not the ones formed initially, but are more likely a result of radical migration from the site of damage to specific free radical traps in the protein. Such a mechanism has also been postulated to explain the formation of specific free radicals in x-ray-irradiated proteins.¹³ The formation of identical free radicals in wool and silk fibers by the two widely different methods of x-ray irradiation and grinding thus can be understood in terms of this mechanism of radical migration.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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Résumé

Des fibres de laine et des tissus endommagés par action mécanique telle que l'abrasion ou la mouture montrent d'intenses signaux RPE. Des fibres de soie après mouture montrent également d'intenses signaux RPE. La laine ainsi que la soie moulués sous atmosphère d'azote sec présentent un doublet spectral attribué à un radical $\cdot\text{CH}$ sur la chaîne polypeptidique. Dans la laine on observe une courbe complète centrée en $g = 2.025$. Elle est attribuée à un radical $-\text{CH}_2\text{S}\cdot$, provenant de la rupture d'un pont disulfure au niveau du fragment cystine. Les radicaux sont stables durant plusieurs mois dans une atmosphère d'azote ou sous vide. Dans l'oxygène sec, le spectre du radical $\cdot\text{CH}$ passe endéans quelques heures d'un doublet à une courbe simple et étroite tandis que le spectre caractéristique du radical cystine disparaît plus lentement. En présence de vapeur d'eau chacune des espèces radicalaires est rapidement détruite et leur spectre disparaît. On suppose que le mécanisme de production des radicaux libres par action mécanique est dû à une rupture directe des chaînes ou à une pyrolyse localisée, couplée avec une migration vers des pièges spécifiques dans la protéine.

Zusammenfassung

Durch mechanische Einwirkung wie Abreibung und Zerreiben beschädigte Wollfasern und -gewebe geben starke EPR-Signale. Seidenfasern zeigen nach dem Zerreiben ebenfalls starke EPR-Signale. Sowohl Wolle als auch Seide weisen nach dem Zerreiben in trockener Stickstoffatmosphäre ein Dublettspektrum auf, das einem $\cdot\text{CH}$ -Radikal an der Polypeptidkette zugeschrieben wird. An Wolle wurde eine komplizierte Kurve mit dem Zentrum bei $g = 2,025$ beobachtet, die einem durch Spaltung der Disulfidbindung im Cystinrest entstandenen $-\text{CH}_2\text{S}\cdot$ -Radikal zugeordnet wird. In Stickstoffatmosphäre oder Vakuum sind diese Radikale viele Monate beständig. In trockenem Sauerstoff geht das Spektrum des CH -Radikals innerhalb weniger Stunden von einem Dublett in eine einzige schmale Kurve über, während das für das Cystinradikal charakteristische Spektrum langsamer verschwindet. In Gegenwart von Wasserdampf werden beide Radikalarten rasch zerstört und ihre Spektren verschwinden. Als Mechanismus der Bildung freier Radikale durch mechanische Einwirkung wird direkte Kettenspaltung oder lokale Pyrolyse in Verbindung mit einer Wanderung zu spezifischen Radikalfängern im Protein angenommen.

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