The Source of NMR-Detected Motional Anisotropy of Water in Blood Vessel Walls

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ABSTRACT ^{2}H Double quantum-filtered (DQF) NMR spectroscopy of deuterated water is sensitive to the presence of order in biological systems. This is because the only nuclei that are detected are those with residual quadrupolar interactions due to their anisotropic motion. In the present study, samples of aorta, coronary and carotid arteries, and vena cava were studied in parallel by ^{2}H DQF NMR and by light microscopy. The average quadrupolar splitting, calculated from the NMR data, varies considerably among the different blood vessels, with high reproducibility for each type of vessel. Polarization microscopy examinations using collagen-specific staining with picrosirius red, have shown a variety of color profiles for the different blood vessels. These reflect different physical modes of aggregation (packing and thickness) of collagen fibers. A correlation was found between the NMR parameters and the color profiles of the picrosirius red-stained sections. Treating the blood vessels with 90% formic acid resulted in the elimination of the ^{2}H DQF NMR signal. Histological analysis demonstrated a complete degradation of collagen and muscle, whereas the elastin filaments were preserved. Evidence is given that the ^{2}H DQF NMR signal is dominated by the contribution of water molecules interacting with the collagen fibers.

INTRODUCTION

^{2}H Double quantum-filtered (DQF) NMR spectroscopy is a sensitive technique that can reveal otherwise undetectable anisotropic motion of water molecules in biological systems (Sharf et al., 1995, 1997a; Shinar et al., 1995). Because deuterium is a $I = 1$ nucleus, ^{2}H DQF NMR spectra are detected only in microscopically ordered systems in which the quadrupolar interaction is not averaged to zero because of the anisotropic motion of the water molecules. Thus detection of a ^{2}H DQF NMR signal is indicative of the presence of local order in the studied system. The spectral lineshapes obtained are determined by the residual quadrupolar interaction, relaxation time, and their spatial distribution and heterogeneity (Sharf et al., 1995; Eliav and Navon, 1994).

By the method described above, anisotropic motion of water molecules has been detected in a variety of biological systems such as brain tissues (Assaf et al., 1997), and skin and connective tissues, such as cartilage and tendon (Shinar et al., 1995). The ^{2}H DQF NMR lineshapes and linewidths vary considerably among cartilage from various organs with different degrees of local and macroscopic order. Lineshape analysis was performed for these tissues, yielding values of residual quadrupolar interaction that differ by almost two orders of magnitude (Shinar et al., 1995).

Recently we have reported the observation of local order in various large blood vessels, including the aorta, coronary artery, carotid artery, and vena cava (Sharf et al., 1997a). In contrast to connective tissues that are mainly composed of collagen and proteoglycans, elastin is a significant component of the extracellular matrix of the blood vessels (Darnell et al., 1990; Ham, 1974). Whereas proteoglycans form hydrated gels, both collagen and elastin are fibrillar macromolecules with a highly ordered structure (Darnell et al., 1990). We have already proved that when water interacts with collagen fibers, its motion becomes anisotropic: ^{2}H DQF NMR spectra have been taken of moistened collagen powder (Sharf et al., 1995), but it was not possible to observe spectra for gelatin, which is denatured collagen (Shinar et al., 1994). When cartilage powder was incubated with collagenase, the anisotropy effect was eliminated, most probably because of the degradation of collagen fibers (Knubovets et al., unpublished results). The observation of a ^{2}H DQF NMR signal of water in the blood vessel wall may be due to the interaction of water molecules with elastin filaments. Blood vessel walls also contain muscle tissue, which is an additional ordered component and could contribute to the observed motional anisotropy of water. However, we have already shown that in ^{2}H DQF NMR spectra of blood vessel walls, the contribution of water molecules interacting with muscle tissue is small compared to that of water in contact with collagen fibers (Knubovets et al., manuscript in preparation).

Microscopy is one of the preferred methods for investigating tissue structure. It enables detection of collagen, elastin, muscle, and different glycoproteins by various histochemical techniques. Picrosirius red staining of deparaffinized tissue sections, followed by examination with polarization microscopy, was proposed for the selective display of collagen (Constantine and Mowry, 1968) and has proved to be highly specific for collagen identification in heterogeneous tissues (Junquiera et al., 1979). Moreover, the procedure enables one to obtain some measure of the physical aggregation, thickness (Junquiera et al., 1982), and

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packing (Dayan et al., 1989) of the collagen fibers in the studied tissue.

The present study is aimed at investigating the anisotropic motion of water molecules in various blood vessel walls and correlating it with the structural information gained by histological analysis. In addition, the relative contributions of collagen and elastin to the observed NMR spectra are presented.

THEORETICAL BACKGROUND

The single-pulse ^{2}H NMR spectrum of biological tissues immersed in D_2O is dominated by the strong water signal from molecules in bulk. This frequently masks the contribution of anisotropically rotating water molecules. The use of double quantum (DQ) filtering methods enables one to overcome this problem. For deuterium ($I = 1$) DQ coherences can be obtained only in the presence of a nonvanishing residual quadrupolar interaction. However, the isotropic motion of water molecules in bulk results in complete averaging of the quadrupolar interaction. Thus only those water molecules moving anisotropically are detected by the ^{2}H DQF experiment.

Because the blood vessel wall is a heterogeneous system, it is convenient to describe it in terms of independent compartments. The dimension of a compartment is defined by the rate of the water exchange between one compartment and another that is slow relative to the ^{2}H NMR relaxation rates and the residual quadrupolar interaction. Within a compartment denoted as anisotropic, the interaction of water with the structural elements of the tissue induces its anisotropic motion. This results in incomplete averaging of the quadrupolar interaction. Fast exchange among the different anisotropically reorienting water molecules, as well as with the free water within the compartment, leads to a residual quadrupole interaction. This is on the order of 0.01–1.0 kHz, which is less than 0.5% of the ^{2}H static quadrupolar interaction in solid hydrates of 240 kHz (Ketudat and Pound, 1957). This residual quadrupole interaction lifts the degeneracy of the Zeeman energy levels, giving rise to quadrupolar splitting of the ^{2}H NMR signal.

^{2}H DQF NMR spectra were acquired using the conventional pulse sequence (Bodenhausen et al., 1980):

$$90^\circ\text{—}\overset{\tau/2}{}180^\circ\text{—}\overset{\tau/2}{}90^\circ\text{—}\overset{\delta}{}90^\circ t(\text{Acq}) \qquad (1)$$

where τ is the creation time and δ is the DQ evolution time, which is kept short so that relaxation during this period can be neglected. Selection of the DQ coherence pathways is performed using appropriate phase cycling (Bodenhausen et al., 1984).

The deuterium DQF NMR spectrum of blood vessels immersed in D_2O represents the sum of spectra from many anisotropic compartments. Each compartment can be effectively characterized by a local residual quadrupolar interaction, ω_Q, an orientation of the local symmetry axis relative to the external field, θ, and a transverse relaxation rate, R_2. The contribution of the ith compartment to the ^{2}H DQF NMR FID signal can be approximated by (Sharf et al., 1995)

$$\text{FID}^i(\tau, t) = M_0^i \sin(\omega_{Q,\theta}^i \tau)\sin(\omega_{Q,\theta}^i t)\exp[-(\tau + t)R_2^i] \qquad (2)$$

where $\omega_{Q,\theta}^i = \omega_Q^i \tfrac{1}{2}(3\cos^2\theta^i - 1)$ is the local residual quadrupole interaction in the laboratory frame of reference, and M_0^i is the equilibrium magnetization of the spins in the ith compartment. This approximation is valid for local residual ^{2}H quadrupolar interactions larger than the transverse relaxation time, i.e., $\omega_{Q,\theta}^i > R_2^i$. This condition is satisfied for all of the samples of blood vessel walls that we have encountered so far (Sharf et al., 1997a). In the derivation of Eq. 2 it was assumed that the asymmetry parameter was zero.

For a heterogeneous system it is convenient to define a probability function $P_i(\omega_{Q,\theta}^i)$ for finding a nucleus in the ith compartment (Sharf et al., 1995). Thus the DQF spectrum, $S_{DQF}(\tau, \nu)$, is a superposition of contributions, $S^i(\tau, \nu)$, from all of the anisotropic compartments. $S^i(\tau, \nu)$ is the Fourier transform of $\text{FID}^i(\tau, \nu)$:

$$S_{DQF}(\tau, \nu) = M_0 \sum_i P_i(\omega_{Q,\theta}^i) S^i(\tau, \nu) \qquad (3)$$

where M_0 stands for the total magnetization of spins in anisotropic compartments. At short creation times, τ, that satisfy the condition

$$\max(|\omega_{q,\theta}\tau|) \ll \pi/2 \qquad (4)$$

the average quadrupolar splitting, can be approximated by $\langle \Delta\nu_q \rangle$ given by Sharf et al., 1997b)

$$\langle \Delta\nu_q \rangle = \frac{-2/\pi(\int_0^\infty \text{Im}[S_{DQF}(\tau, \nu)]d\nu - \int_{-\infty}^0 \text{Im}[S_{DQF}(\tau, \nu)]d\nu)}{|S_{DQF}(\tau, 0)|}. \qquad (5)$$

Here $\text{Im}[S_{DQF}(\tau, \nu)]$ is the imaginary part of the DQF spectrum, the zero frequency is at the center of the spectrum, and $|S_{DQF}(\tau, 0)|$ is the intensity of the magnitude calculated spectrum at zero frequency.

MATERIALS AND METHODS

Blood vessels

Samples of the aorta, coronary artery, carotid artery, and vena cava were removed from freshly slaughtered cows. Samples of the aorta were taken from the aortic arch and those of the vena cava were taken from the area of the inferior vena cava. Each sample was divided into two portions. One was immersed in phosphate-buffered saline, prepared in D_2O, and kept at 4°C, and the other was left in 90% formic acid at 45°C for 24 h to remove collagen fibers (Vinee et al., 1993) as well as all of the other components of the blood vessel wall, except elastin filaments (Jackson and Cleary, 1968).

After treatment, samples were thoroughly rinsed in distilled water, wiped, and immersed in the phosphate-buffered saline for at least 24 h at

4°C. All experiments were carried out four times on different samples taken from different cows.

NMR measurements

^{2}H DQF NMR spectra were acquired using the conventional pulse sequence (Eq. 1) with a creation time, τ, between 100 μs and 60 ms, and a short (δ = 30 μs) evolution time. The spectra were recorded at an ambient room temperature on the Bruker AMX360 or ARX500 high-field NMR spectrometer at a ^{2}H frequency of 55.5 and 76.8 MHz, respectively.

Histological studies

All of the samples were fixed in buffered formaldehyde upon completion of the NMR experiments. After dehydration and paraffin embedding, 5-μm-thick sections were cut and stained with Verhoeff's iodine stain for elastin or with a modified picrosirius red procedure (Dayan et al., 1989) specific for collagen fibers. The latter sections were examined by polarization microscopy.

RESULTS

All intact blood vessels studied in this work showed a ^{2}H DQF NMR spectra (Fig. 1, *A–D*), indicating anisotropic motion of the water molecules as a result of their interaction with ordered structures in the tissue. The different vessels exhibit a diversity of spectral lineshapes, linewidths, and modulation of peak intensity by the creation time, τ. The aorta sample gives the narrowest peak, which evolves at relatively long τ values, and whose width changes moderately over the whole range of creation times. The spectra of the coronary and carotid arteries and of the vena cava exhibit both a broad and a narrow component. The broad component reaches its maximum intensity at short creation times and then decays relatively quickly, showing oscillatory behavior. This pattern is expected for a broad distribution of residual quadrupolar interaction, the spectral width of which is larger than the relaxation rates (Sharf et al., 1995). The effect is more pronounced for the vena cava and the carotid artery than for the coronary artery. The narrow component reaches its maximum intensity at longer creation times when the broad component has already significantly decayed. As the blood vessel wall is a heterogeneous system composed of compartments differing in their residual quadrupolar interactions as well as in their transverse relaxation rates, the modulation of the peak intensity and spectral width by the creation time, τ, reflects a discrete evolution of contributions from two or more distinct subpopulations.

A quantitative comparison between the DQF spectral lineshapes from different tissues is given in Fig. 2, where each data point is calculated according to Eq. 5. As was stated in the Theoretical Background, for short creation times that satisfy the condition $\max(|\omega_{q,\theta}\tau|) \ll \pi/2$ (see Eq. 4), the value of $\langle\Delta\nu_q\rangle$ can serve as an estimation for the average quadrupolar splitting. $\langle\Delta\nu_q\rangle$ reaches a constant value at short creation times, indicating that the condition of Eq. 4 is fulfilled for all samples. The relatively small standard errors demonstrate the high reproducibility among

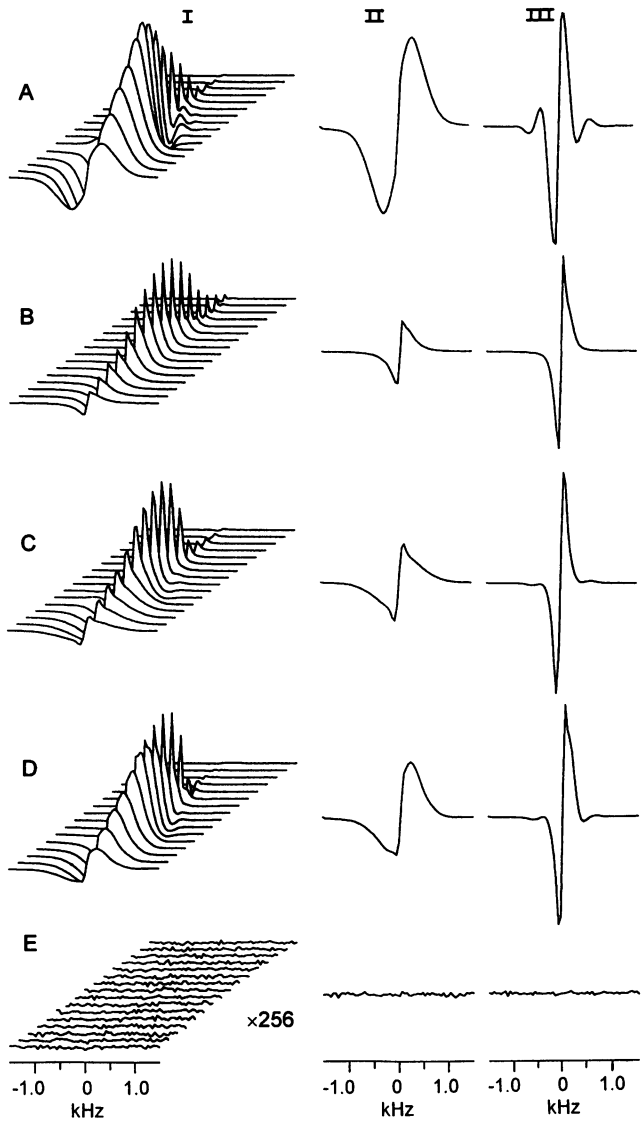

FIGURE 1 ^{2}H DQF NMR spectra of intact bovine vena cava (*A*), aorta (*B*), and coronary (*C*) and carotid (*D*) arteries, and carotid artery after incubation in 90% formic acid for 24 h (*E*). *Column I*, Stack plot of spectra were measured with the following τ values (ms): 0.1, 0.2, 0.3, 0.4, 0.6, 1.0, 1.4, 2.0, 3.0, 4.0, 6.0, 10.0, 20.0, 30.0, 40.0, 60.0. *Columns II* and *III*, The spectra measured with τ = 0.3 ms and 2.0 ms, respectively.

samples of the same tissue taken from different animals. The characteristic values of the average quadrupolar splitting obtained for each blood vessel are summarized in Table 1. The value $\langle\Delta\nu_q\rangle$ from the aorta sample is the smallest, followed by those from the coronary artery, the carotid artery, and the vena cava. These distinctions are in good agreement with the qualitative description of the lineshapes given above and, thus, reinforce our confidence in the method of analysis. At longer creation times ($\tau \geq 3$ ms) the broad component of the DQF spectrum has almost decayed (Fig. 1, *A–D*); therefore the values of $\langle\Delta\nu_q\rangle$ correspond to the narrow component of the spectrum. At τ = 3 ms the values of $\langle\Delta\nu_q\rangle$ for all samples range between 80 and 120 Hz such that the condition in Eq. 4 is satisfied. Therefore,

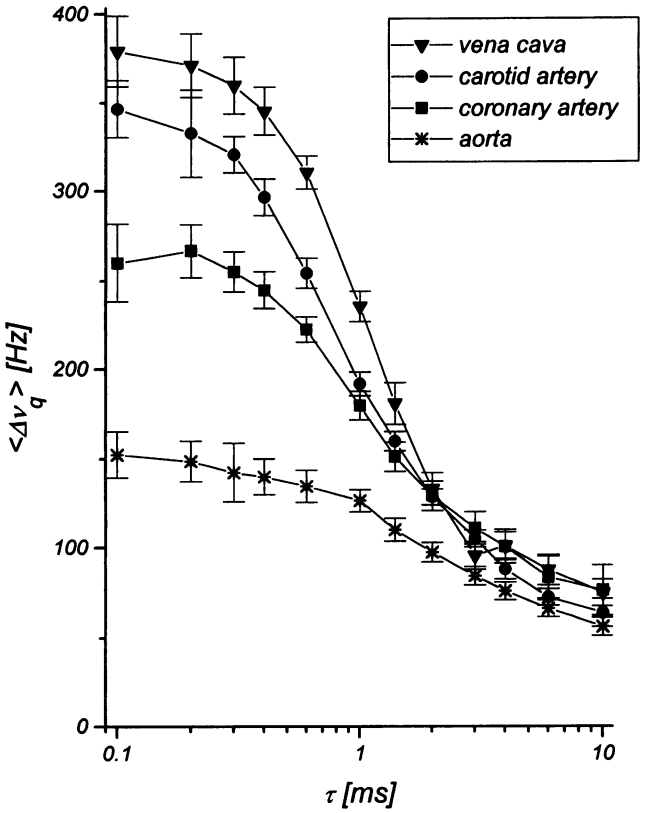

FIGURE 2 The values of the average quadrupolar splitting for vena cava, aorta, and coronary and carotid arteries obtained by the ^{2}H DQF NMR experiments as a function of the creation time, τ. Each point is calculated according to Eq. 5. The standard error is calculated for four samples of each type of blood vessel taken from different cows. The lines are given to lead the eye.

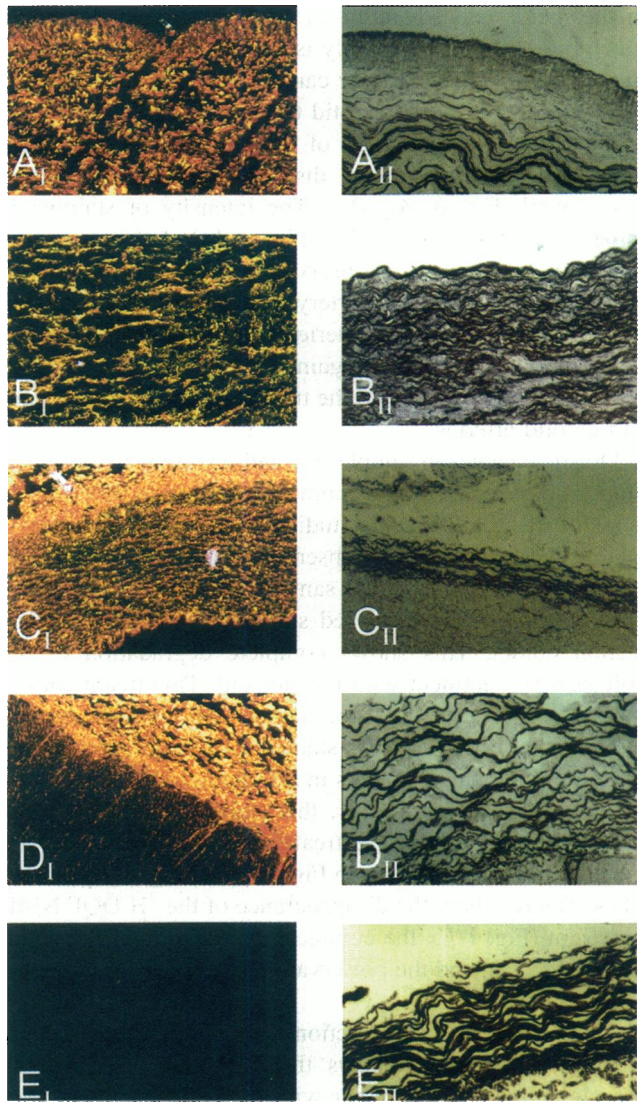

FIGURE 3 (*Left column*) Picrosirius red collagen staining with polarization microscopy of intact bovine vena cava (A_I), aorta (B_I), and coronary (C_I) and carotid (D_I) arteries, and carotid artery after incubation in 90% formic acid for 24 h (E_I). Original magnification, ×100. (*Right column*) Elastin staining (Verhoeff's) of intact bovine vena cava (A_{II}), aorta (B_{II}), and coronary (C_{II}) and carotid (D_{II}) arteries, and carotid artery after incubation in 90% formic acid for 24 h (E_{II}). Original magnification ×100.

they can give a rough estimation of the average quadrupolar splitting of the narrow components of the spectra (see Table 1).

The polarization colors of the picrosirius red-stained sections of the studied blood vessels are shown in Fig. 3, A_I–D_I. These colors are indicative of the state of the collagen fibers. The color profile at the constant section thickness (5 μm in our case) is mainly determined by the fiber thickness and packing (Junquiera et al., 1982; Dayan et al., 1989). The thinner and less tightly packed fibers display green–green-yellow colors, whereas the thicker and more tightly packed show orange-red–red ones.

TABLE 1 The values of the average quadrupolar splitting calculated* for ^{2}H DQF NMR spectra of various blood vessels

Specimen	$\langle\Delta\nu_q\rangle$ (Hz) Broad + narrow components calculated at τ = 0.1 ms	$\langle\Delta\nu_q\rangle_{narrow}$ (Hz) Narrow component calculated at τ = 3.0 ms
Vena cava	379 ± 20	96 ± 8
Aorta	152 ± 13	85 ± 5
Coronary artery	260 ± 22	111 ± 9
Carotid artery	347 ± 16	105 ± 5

*The average quadrupolar splitting is calculated according to Eq. 5. The condition in Eq. 4 is satisfied for all of the samples. The standard error is calculated for four samples of each specimen taken from different cows.

The vena cava sample clearly shows the red color of the rather thick intima (in the *upper part* of Fig. 3 A_I) and the yellow-orange to red color pattern dominant in the adventitia (in the *middle* and *lower parts* of Fig. 3 A_I). The tunica media is absent from the inferior vena cava (Bloom and Fawcett, 1975). Conversely, in the case of aorta (Fig. 3 B_I), the tunica media is the most developed layer, the tunica adventitia is rather thin, and the greenish colors are mostly displayed in the stained section. In the coronary and carotid arteries (Fig. 3, C_I and D_I), one can easily distinguish between different layers. This is because the color profiles

in the media and adventitia are different, the latter being much more reddish. Not only is the adventitia clearly seen in these two arteries, but one can also see that the adventitia is better ordered in the carotid than in the coronary artery.

Verhoeff stained sections of the samples show different patterns of elastin filament distribution within the blood vessel wall (Fig. 3, A_{II}–D_{II}). The intensity of staining is rather low in the vena cava (Fig. 3 A_{II}). In contrast, the highest color intensity is observed in the aorta (Fig. 3 B_{II}), as expected for this elastic artery with a high level of elastin. The coronary and carotid arteries display rather weak staining (Fig. 3, C_{II} and D_{II}). Again one can easily distinguish between the adventitia and the tunica media in the coronary and carotid arteries.

Drastic changes in sample size and texture were observed after 24 h of incubation at room temperature in 90% formic acid for all blood vessels studied. After treatment, no ^{2}H DQF NMR signals were observed for all of these blood vessels. Examination of the samples by polarized light microscopy after picrosirius red staining exhibited no polarization colors. This shows complete degradation of the collagen by treatment with formic acid. This treatment did not affect elastin in the same manner. Microscopic examination of the same sections stained for elastin revealed the presence of elastin filaments in all samples after incubation with formic acid; moreover, this staining was even more intense. The results of the treatment with formic acid are given for the carotid artery in Fig. 1 *E*, and Fig. 3, E_I and E_{II}. These figures show the disappearance of the ^{2}H DQF NMR spectrum (Fig. 1 *E*), the complete degradation of the collagen (Fig. 3 E_I), and the preservation of the elastin filaments (Fig. 3 E_{II}).

Examination of the sections after Masson-trichrome staining (not shown) shows that degradation of smooth muscle cells occurs together with the collagen fibers after treatment of specimens with 90% formic acid. This result agrees with previous reports (Jackson and Cleary, 1968).

DISCUSSION

In the present study ^{2}H DQF NMR spectra were detected for various large blood vessels, including three major arteries, the aorta (ascending aortic arch) and the coronary and carotid arteries, as well as one of the large veins, the inferior vena cava. All samples were histologically examined to confirm their intactness. The blood vessels exhibited a ^{2}H DQF NMR signal, which indicates the presence of anisotropic motion of water molecules in the tissues. Treatment of the samples of blood vessel walls with formic acid resulted in complete elimination of the ^{2}H DQF NMR signal. Histological examination of the same samples indicates complete degradation of collagen, as well as muscle tissue. The appearance of elastin filaments is different after treatment with formic acid: staining is more intense and the filaments are more tightly packed (Fig. 3). According to the data of Hass (1942), these changes are due to two factors: 1) elastin is no longer hidden by the overlapping collagen fibers and muscle tissue, because these both have been completely degraded, causing more intense staining of the sections; 2) treatment with formic acid leads to spontaneous elongation of elastin networks to an extent that approaches the elongation in response to the application of high mechanical strain. In fact, this elongation imposes higher ordering on the network (which is clearly seen in Fig. 3). Had the water molecules interacting with elastin filaments contributed to the DQF signal, the increased ordering of the elastin network should have resulted in larger values of the residual quadrupolar interaction and thus would have increased their contribution to the DQF NMR signal. It is very difficult to predict the extent to which the DQF spectral lines would vary because of the alteration of the elastin fibers by formic acid treatment. However, the fact that no DQF NMR signal is observed after treatment, despite the increased order of the elastin network, strongly suggests that elastin does not contribute to the observed motional anisotropy of water in the blood vessel walls.

Another potential source of motional anisotropy could be the muscle tissue in the blood vessel walls. In particular, the intermediate layer of arteries, the tunica media, is predominantly composed of smooth muscle cells disposed circumferentially. ^{23}Na DQF NMR has revealed anisotropic motion of sodium ions in skeletal muscles (Reddy et al., 1995; Knubovets et al., 1996a), and residual dipolar splitting has been observed for creatine in human muscle (Kreis and Boesch, 1994; Kreis et al., 1997). These results suggest that the interaction of water with muscle tissue might also be the cause for the anisotropy. As we have previously shown, removing the outer layer (adventitia) in the samples of the coronary and carotid arteries resulted in the loss of the broad component of the spectrum (Sharf et al., 1997a). Furthermore, in the case of the aorta, where the muscle tissue is the most pronounced, only a narrow signal is detected. Thus any contribution by the muscle tissue to the broad component of the ^{2}H DQF NMR spectrum of these arteries can be excluded. In a separate study we have shown that treatment with trypsin, which causes complete degradation of muscle tissue and only a minor morphological change in the collagen network, results in a relatively small change in the ^{2}H DQF NMR spectrum (Knubovets et al., manuscript in preparation). Thus, if the interaction of water with muscle tissue contributes to the narrow component of the ^{2}H DQF NMR spectrum of the blood vessel wall, this contribution is small compared to that of water molecules in contact with collagen fibers.

The anisotropic motion of water molecules bound to collagen fibers has already been observed in single-pulse ^{2}H NMR experiments for hydrated collagen from rat-tail tendon (Migchelsen and Berendsen, 1967), and inside various intact connective tissues by using the ^{2}H DQF NMR technique (Sharf et al., 1995; Shinar et al., 1995). These results are also in line with the drastic decrease in the ^{2}H DQF NMR signal intensity when collagen is cleaved by collagenase in a suspension of collagen fibers (Knubovets et al.,

unpublished results). Other studies have revealed anisotropic motion of sodium ions interacting with collagen fibers in connective tissues (Eliav and Navon, 1992; Shinar et al., 1994).

The ^{2}H DQF NMR lineshapes are determined by the distribution of the residual quadrupolar interaction, $\omega^i_{Q,\theta}$, as well as the transverse relaxation times, R^i_2, throughout the specimen. The average quadrupolar splitting, $\langle\Delta\nu_q\rangle$, calculated at short τ values (see Table 1), varies considerably among the different blood vessels with high reproducibility for each type of vessel. On the other hand, the narrow components of the ^{2}H DQF NMR spectra are characterized by practically identical values of the average residual quadrupolar splitting $\langle\Delta\nu_q\rangle_{narrow}$, 100 ± 20 Hz, for all of the blood vessels under study. Thus, because at short creation times $\langle\Delta\nu_q\rangle$ is the weighted average of the contributions of the narrow and broad components, its variation among the various blood vessels must be attributed to the relative weight and the average quadrupolar splitting of the broad component. Whereas $\langle\Delta\nu_q\rangle$ for the aorta sample is relatively small (~150 Hz), those of the coronary and the carotid arteries are much larger (260 Hz and 355 Hz, respectively). The latter two arteries exhibit broad spectra at short τ values, indicating a broad distribution of $\omega^i_{Q,\theta}$. In a previous work (Sharf et al., 1997a) we found that ^{2}H DQF NMR spectra of the coronary and carotid arteries comprise the superposition of a broad and a narrow component. The broad component originates from the water in the outer layer, the tunica adventitia, whereas the narrow component is related to the water in the tunica media. The results obtained for aorta are also in line with this finding. The aorta sample was taken from the ascending aortic arch close to the heart and has practically no outer layer, as proved by our histological examination. This may be the reason for the relatively narrow ^{2}H DQF lineshape of the aorta.

The average quadrupolar splitting obtained for the sample of vena cava ($\langle\Delta\nu_q\rangle$ = 380 Hz) is significantly larger than those obtained for the three arteries. Histological examinations reveal that in a considerable portion of the inferior vena cava, the tunica adventitia makes up most of the venous wall, whereas the tunica media is absent, as can be seen in Fig. 3, A_I and A_{II}. The tunica adventitia consists of loose connective tissue containing elastic filaments and longitudinally oriented collagen fibers, and in contrast to muscular arteries, it also contains prominent longitudinal layers of smooth muscle. In addition, the inner layer of the vena cava, the tunica intima, contains a layer of connective tissue of considerable thickness (exhibited as the homogeneous red layer in Fig. 3 A_I). Thus, unlike the case of the large arteries, in which the tunica intima presents only a small portion of the vessel's wall, the contribution of water in the tunica intima is significant in the inferior vena cava. In fact, by partially removing the innermost layer of the vena cava, we were able to estimate its contribution to the ^{2}H DQF NMR lineshapes. The resulting spectra (not shown) indicate that the water in the intima contributes to the broad component of the spectrum with a linewidth comparable to that of the outer layer. Hence we conclude that the broad component of the ^{2}H DQF NMR spectral lines comes from water in both the tunica intima and the tunica adventitia. We have previously reported that ^{2}H DQF NMR spectral lineshapes of coronary and carotid arteries are highly sensitive to longitudinal strain and are insensitive to circumferential stretching (Sharf et al. 1997a). For these two arteries the effect of strain has been assigned to the outer layer. Moreover, it has been shown that in their strained state the ^{2}H DQF NMR spectrum of the outer layer resembles that of a macroscopically oriented system that has maximum splitting parallel to the external magnetic field, half-maximum splitting perpendicular to the external field, and minimum splitting at the magic angle. A large strain effect has also been found for the vena cava, but has been shown to be independent of the choice of elongation axis (Sharf et al., 1997a). The sensitivity of the ^{2}H DQF NMR spectrum of the vena cava to longitudinal as well as circumferential elongation may be associated with the interlacing bundles of collagen fibers in the venous tunica intima.

According to our model, the diversity of the averaged residual quadrupolar splitting among various samples corresponds to the composition of the blood vessel walls, the order of the collagen fibers, and the fraction of the water molecules interacting with them. Thus this diversity also depends on the water content of the anisotropic compartments. Histological examination by picrosirius red staining of deparaffinized tissue sections, followed by examination with polarization microscopy, demonstrates differences in the polarization colors of collagen fibers among different samples. The color profile is related to the physical aggregation of collagen: green to green-yellow corresponds to thin and/or poorly packed collagen fibers, and orange-red originates from thick, well-packed fibers (Dayan et al., 1989, 1993; Hirshberg et al., 1996). A comparison between the two methods reveals that the green to green-yellow color pattern (as in the aorta, and the tunica media of coronary and carotid arteries) correlates the narrow component of the ^{2}H DQF NMR spectrum, whereas the yellow-orange-red color pattern (as in the adventitia of coronary and carotid arteries, and the adventitia and the tunica intima of vena cava) correlates the broad component of the spectrum. As the polarization colors of the picrosirius red-stained sections are shifted from green to red, the values of the residual quadrupolar splitting increase. This suggests that the higher level of physical aggregation of the collagen fibers results in a higher degree of ordering of the water molecules.

The reasons for the specificity of the observed anisotropy of water molecules for the collagen fibers must still be elucidated. It is possible that the triple helix of the protein is essential for obtaining motional anisotropy of water. We have demonstrated that anisotropic motion of sodium ions bound to the inner side of mammalian red blood cell membranes is dependent on the conformation of the membrane cytoskeleton (Shinar et al., 1993; Knubovets et al., 1996b). The major element of the cytoskeleton is spectrin, a fibrillar protein that has a triple helix as the main motif of its tertiary

structure (Yan et al., 1993). One should also consider the possibility that the water molecules may form bridges between two collagen helices inside a collagen fibril. In this case the presence of fibrils would be essential to detecting motional anisotropy.

This study shows that the order and packing of collagen fibers in the blood vessel wall that are observed by polarization microscopy are also expressed in the motional anisotropy of water molecules and can be measured by ^{2}H DQF NMR of the intact tissue. This latter technique can be used in magnetic resonance imaging (MRI) microscopy to display distinct tissue layers within the blood vessel walls.

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