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^{1}H Spin–Spin Relaxation in Normal and Cataractous Human, Normal Fish and Bird Eye Lenses

P. RÁCZ[a], C. HARGITAI[b], B. ALFÖLDY[b], P. BÁNKI[b] AND K. TOMPA[b]

[a] *Department of Ophthalmology, Markusovsky Hospital, H-9701 Szombathely, P.O.B 143, Hungary and* [b] *Research Institute for Solid State Physics and Optics, Hungarian Academy of Sciences, H-1525 Budapest, P.O.B. 49, Hungary*

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A systematic study on nuclear spin–spin relaxation of water protons in human, fish and bird eye lenses/lens nuclei is reported. The purpose of this study is to clarify the real nature of the relaxation processes not describable as a single exponential decay. The characterization of the spin–spin relaxation by a single exponential is commonly used both in literature and in MRI diagnostics. However, in our opinion, this single exponential decay hypothesis is an oversimplification that can lead to the loss of essential information. Our measurements were performed by Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences on human, carp, chicken and turkey eye lenses/lens nuclei. Several hundreds of CPMG echo amplitude were detected and the time-dependence of their decay was determined by careful fitting procedures. Our results clearly rule out the single exponential decay hypothesis for eye lenses: at least two or three decaying components are observed. These phenomena need further investigations: it should be decided which of the relaxation parameters, T_{2i}-s and A_i-s gives the most characteristic physiological or pathological information. It is claimed that the amplitude ratio of the bound and the free water fraction carries the most characteristic information. During the cataract formation the weight of free water is raised by more than 25%.

Key words: eye lenses; cataract; proton spin–spin relaxation; multi-exponential decay; syneresis.

1. Introduction

In our earlier investigations on the state of water in human normal and cataractous lenses and in normal bird and fish eye lenses, we measured different NMR parameters: the free induction decay signal, FID, the longitudinal relaxation time (T_1) and the transversal time (T_2) (Rácz, Tompa and Pócsik, 1979a, b; Rácz et al., 1983).

As it is well known, T_1 relaxation time characterizes the interaction between the resonant nuclei, (protons in our case) and the lattice, the spin–spin relaxation time, T_2, is characteristic for reaching the internal equilibrium within the spin system. The spin–spin relaxation process is much faster than the spin–lattice one, moreover, it can not be described as a single exponential decay, and there are at least two or more processes with different T_2 according to our measurement on eye lenses.

The spin–spin relaxation processes can reveal very essential information on water molecules in living tissues. There are some data in the literature concerning the multi-exponential character of the spin–spin relaxation measured on different living tissues Belton, Jackson and Packer, 1972; Hazlewood et al., 1974; Stankeiwicz et al., 1989; Lerman and Moran, 1989). Belton et al. (1972) studied the state of water on striated muscles (gastrocnemius and sartorius of frog). Their measurements were carried out using a coherent pulsed NMR spectrometer operating at 60 MHz. The transverse nuclear spin relaxation observed by Carr–Purcell–Meiboom–Gill (CPMG) sequence at room temperature was found to be multi-exponential. The three distinct relaxation components were thought to correspond to physically distinct groups of water molecules. Hazlewood et al. (1974) investigated the biological water in the gastrocnemius muscle of white rats by NMR. The spectrometer was operating at frequencies of 25 and 50 MHz. The samples were at room temperature during the measurement. The results clearly indicated three different spin–spin relaxation times; they were identified with different parts of 'slowly exchanging' tissue water. According to the authors (Hazlewood et al., 1974), ''the observation of three water proton fractions requires that exchange between these three fractions be considered''. Stankeiwicz et al. (1989), having performed proton NMR measurements on different normal animal eye lenses (guinea pig, rabbit, rat, frog, trout and chicken) at 80·285 MHz at 37°C, found two T_2 components. The short T_2 fraction was found to be proportional to the protein concentration of the lenses. This fraction was therefore regarded to refer to the waterbound to the lens protein, similarly the long T_2 fraction to the free lenticular water. Two spin–spin relaxation times from the NMR study of Lerman and Moran (1989) demonstrated the existence of two water phases in the normal human and rabbit eye lenses as age-relating changes. Our earlier NMR measurements (Rácz et al., 1979a, b) have also shown a multi-exponential behavior of the spin–spin relaxation. At the same time we could separate the

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TABLE I

Spin–spin relaxation times and their weights in different eye lenses

Lens code	A1 (%)	A2 (%)	A3 (%)	t1 (msec)	t2 (msec)	t3 (msec)
carp17	38·4	43·3	18·3	6·9	39·8	188·8
carp18	36·2	45·9	18·2	5·27	38·0	159·3
carp27	38·1	42	19·8	5·97	30·1	104·0
carp28	35·4	48·3	16·4	5·85	29·2	102·2
carp37	41·9	39·6	18·4	6·61	36·6	155·1
carp38	43·6	40·3	16·2	7·33	40·6	200·0
carp47	37·4	41·9	20·7	6·45	35·8	153·6
carp48	35·4	48·1	16·5	6·51	36·0	174·0
carp mean	38·3	43·7	18·1	6·4	35·7	154·6
S.E.	1·1	1·2	0·6	1·5	0·2	12·6
chicken0a	4·2	44·1	51·7	1·59	108·8	309·4
chicken0n	5·2	31·4	63·4	1·59	73·8	273·0
chicken1a	6·0	26·3	67·7	0·75	56·1	204·7
chicken1n	9·1	36·0	54·9	0·75	79·6	267·7
chicken2a	4·6	55·4	40·0	1·60	107·7	332·5
chicken2n	5·5	43·1	51·4	1·60	72·2	280·0
chicken3a	4·6	37·1	58·2	1·07	65·5	244·5
chicken3n	5·8	28·6	65·6	1·07	56·2	216·5
chicken mean	5·8	38·6	58·1	1·25	77·5	266·0
S.E.	0·6	3·2	3·5	0·14	7·3	15·4
turkey17an	9·3	67·2	23·5	0·35	40·8	103·6
turkey18an	7·6	61·4	31·0	0·62	42·7	93·5
turkey27an	7·6	64·8	27·6	0·46	36·6	81·5
turkey28an	7·1	73·9	18·9	0·51	43·0	88·4
turkey37an	8·7	54·0	37·4	0·44	36·4	76·0
turkey38an	7·7	40·8	51·5	0·67	36·6	72·3
turkey47an	6·2	47·2	46·6	0·65	39·1	114·8
turkey48an	6·8	42·1	51·1	0·98	43·5	116·7
turkey mean	7·6	56·4	35·9	0·6	39·8	93·4
S.E.	0·4	4·3	4·5	0·1	1·1	6·0
Humcatw71	72·0	28·0		24·0	64·2	
Humcatw72	73·0	27·0		24·1	68·8	
Humcatw73	72·3	27·7		24·0	66·0	
Humcatw74	78·7	21·3		26·4	54·6	
Humcatw75	76·8	23·2		26·1	52·1	
Humcatw76	77·6	22·4		25·3	56·1	
Humcatw77	77·4	22·6		26·0	54·0	
Humcatw81	76	24		24·1	75·5	
Humcatw82	79·5	20·5		26·2	61·8	
Humcatw83	78	22		26·0	60·0	
Humcatw91	71·7	28·3		23·5	71·5	
Humcatw92	71·6	28·4		23·7	70·2	
Humcatw93	71·6	28·4		23·4	70·8	
Humcatw mean	75·1	24·9		24·8	63·5	
S.E.	0·8	0·8		0·3	2·0	
Humnorw11	81·9	18·09		29·8	70·2	
Humnorw12	85·9	14		30·4	85·3	
Humnorw11a	84·2	15·8		30·1	78·8	
Humnorw13	84·2	15·9		29·9	78·9	
Humnorw14	89·4	10·1		31·5	79·6	
Humnorw15	83·9	16		29·9	80·6	
Humnorw16	86·7	13·3		30·7	94·9	
Humnorw16s	86·2	13·8		30·5	90·9	
Humnorw mean	85·3	14·6		30·3	82·4	
S.E.	0·8	0·8		0·2	2·7	
Humnor21	77·3	22·7		20·7	39·3	
Humnor22	81·2	22·9		20·7	39·8	
Humnor23	77·4	22·6		20·5	40·5	
Humnor24	77·4	22·6		20·5	40·5	
Humnor25	81·4	18·6		21·5	38·1	
Humnor26	78·0	22·0		21·2	37·1	
Humnor mean	78·8	21·9		20·8	39·2	
S.E.	0·8	0·7		0·2	0·6	

The CPMG amplitude, $I(t) = \Sigma_i A_i \mathrm{Exp}(-t/t_i)$.

spin–lattice relaxation processes in the eye lenses dehydrated by vacuum and we could determine their relative weights. On this basis, the following questions can be raised: first, how characteristic is the multi-exponential behavior of T_2 to the different eye lenses? and second, could one get information on the structural changes during the cataract formation from NMR measurement?

We now find an appropriate opportunity and possibility to deepen our knowledge concerning the T_2 relaxation times in new experiments by new measurements on human normal lenses and cataractous lenses/lens nuclei and normal bird and fish eye lenses with a developed data acquisition and evaluation. The Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence promises the advantage: the measuring results may be independent of instrumental weakness (inhomogeneities of the magnetic field) and the setting inaccuracy (Bull, 1974; Hughes and Lindblom, 1977). While the measuring points followed each other by 100 msec in the experiments of Hazlewood et al. (1974), our results reported here are based on 250–1500 CPMG echo amplitudes following each other by 1 msec steps. Moreover, in most of the cases investigated, the short time behavior was also measured in 0·2 msec steps below 18 msec.

2. Materials and Methods

Normal (3) and cataractous (25) human lens nuclei, normal turkey (8), chicken (4) and carp (8) lenses were investigated. The cataractous lens nuclei were removed by the regular extra-capsular cataract extraction (ECCE) from the eyes of patients aging 60–75 years. Normal eye lenses were obtained from cadaver eyes within 6 hr after death. Turkey, chicken and carp eye lenses were removed from the eye after having killed the animals.

Before operating, all cataractous lenses were classified by a careful slit-lamp examination in full mydriasis. After removal, the lens nuclei and the normal turkey, chicken and carp eye lenses were immediately placed into and kept in sealed sample holders in a 0°C water-ice mixture for less than 24 hr before the NMR measurement.

The NMR measurements were carried out on a BRUKER SXP-4-100 pulsed NMR spectrometer at 86·7 MHz at room temperature. The measurements were carried out in a high-resolution magnet. The volume of the RF coils was much larger than that of the investigated lenses so that the exciting field can be homogeneous. CPMG pulse sequence was applied with $\tau = 1$ msec (or $\tau = 0{\cdot}2$ msec in short time measurements). Two hundred to 1600 echoes were detected. The CPMG echo amplitudes were determined by averaging over 20–80 runs. The measured data were collected in a HP storage oscilloscope. The maximum echo amplitudes were determined by an MS Excel 4.0 macro. The relaxation processes were analysed using MicroCal Origin 4.1 program on a PC.

As there are no generally accepted rules of how to determine how many decay terms are needed in describing the relaxation processes, as a rough rule of thumb, we use the following simple procedure. In all cases, the procedure starts with a single decay term fit with baseline correction and in the next steps a new decay term is added. The new decay term is accepted, whenever the drop in the sum of square of residuals is at least 20%. Otherwise, the new decay term is rejected and the procedure is halted.

3. Results

In total 48 lenses or lens nuclei were investigated: 3 normal human lenses, 25 cataractous human lens nuclei, 8 normal turkey, 4 chicken and 8 carp lenses. The results, the relative amplitudes and spin–spin relaxation times from all the CPMG measurements on the lenses are given in Table I. The mean values of the characteristic parameters and their standard errors for each lens/lens nucleus type are summarized in Table II.

The decay of CPMG echoes in the case of a normal turkey lens, together with the one and three exponential fits are shown in Figs 1 and 2. It is worth mentioning that the sums residual squares are 1·82, 0·12 and 0·06 for the single, two and three exponential models, respectively. This clearly demonstrates that in turkey lenses, there are at least three decaying components.

TABLE II

*Spin–spin relaxation times and their weights in different eye lenses (*ECCE)*

Species	A1 (%)	A2 (%)	A3 (%)	t1 (msec)	t2 (msec)	t3 (msec)
turkey	7·6 ± 0·4	56·4 ± 4·3	35·9 ± 4·5	0·6 ± 0·1	39·4 ± 1·1	93·4 ± 5·0
chicken	5·7 ± 0·5	37·6 ± 3·2	56·7 ± 3·5	1·35 ± 0·14	77·5 ± 7·3	255 ± 15·4
human normal (ExpDec3)	2·5 ± 0·3	87·1 ± 1·1	10·4 ± 1·2	2·1 ± 0·4	24·4 ± 0·2	62·9 ± 4
human normal (ExpDec2)	—	85·9 ± 1·2	14·1 ± 1·2	—	23·6 ± 0·2	55·9 ± 2·3
human normal nucleus*	—	85·4 ± 0·8	14·6 ± 0·8	—	30·3 ± 0·2	82·4 ± 2·7
human cataractous nucleus*	0	75·1 ± 0·8	24·9 ± 0·8	—	24·8 ± 0·3	63·5 ± 2·0
carp lens	38·3 ± 1·1	43·7 ± 1·2	18·1 ± 0·6	6·4 ± 0·2	35·7 ± 1·5	154·6 ± 12·6

The CPMG amplitude, $I(t) = \Sigma_i A_i \mathrm{Exp}(-t/t_i)$.

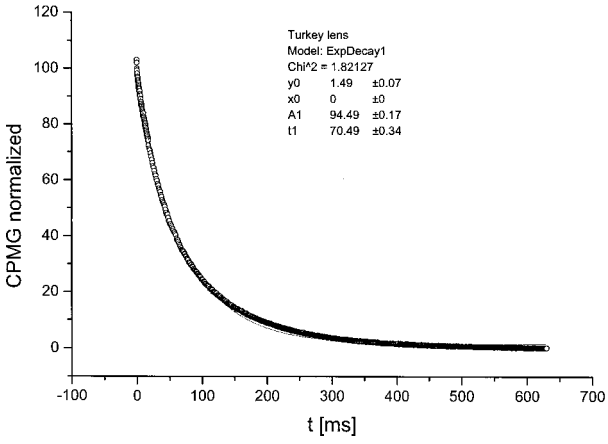

FIG. 1. The spin–spin relaxation in a turkey lens: single exponential fit with baseline correction. Measured CPMG amplitudes (○).

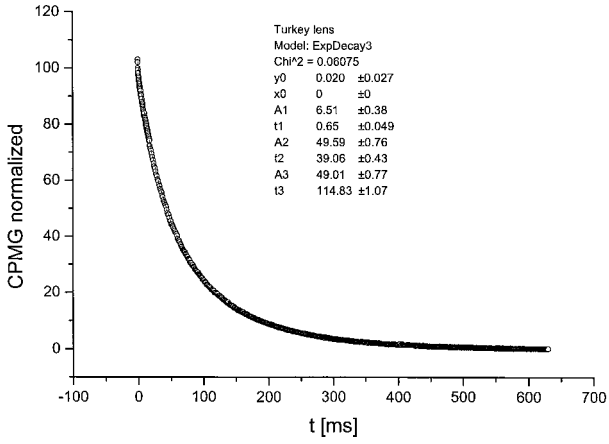

FIG. 2. The spin–spin relaxation in a turkey lens, triple exponential fit with baseline correction.

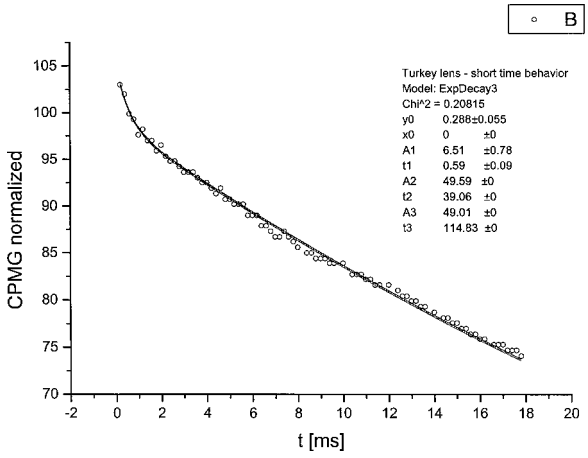

FIG. 3. The short time behavior of the CPMG echo train of a turkey lens.

In Fig. 3 the initial part of the CPMG echo train is plotted. It proves the existence of a fast relaxation component. This fast component is probably not due to the water protons, but coming from the protons in the lens proteins.

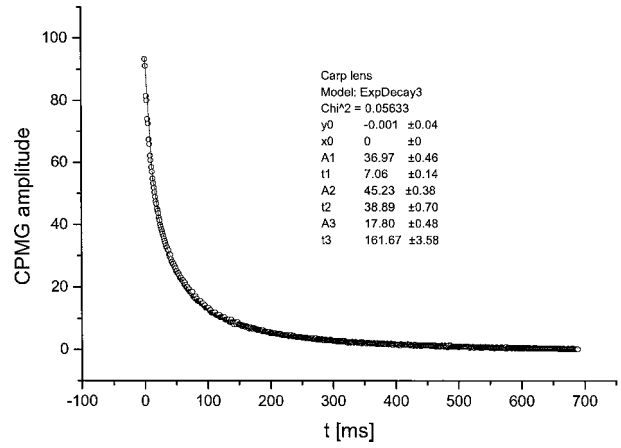

FIG. 4. The spin–spin relaxation in a carp lens: triple exponential fit with baseline correction.

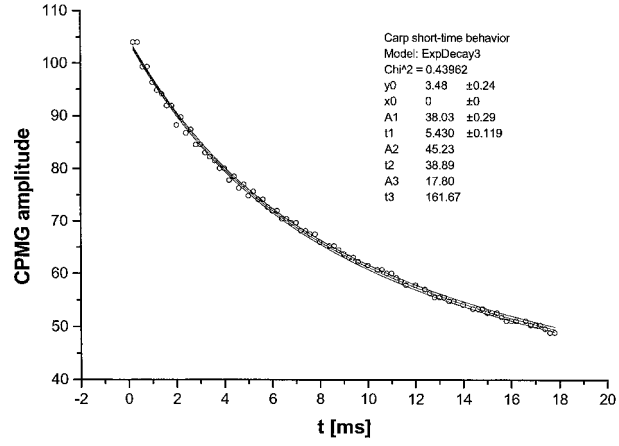

FIG. 5. The short time behavior of the CPMG echo train of a carp lens. The full lines represent the fitted curve and the 0·95 confidence band curves.

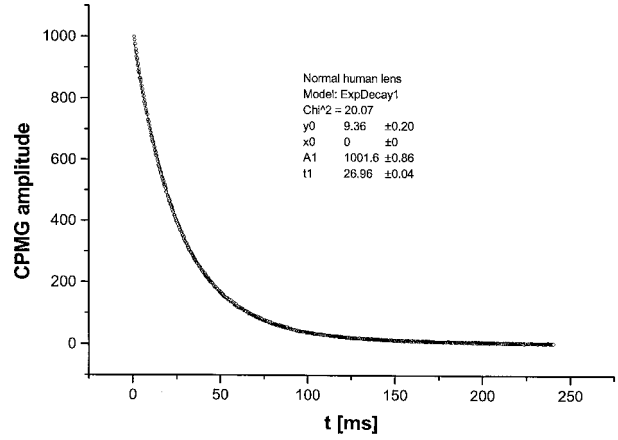

FIG. 6. The spin–spin relaxation in a normal human lens: single exponential fit with baseline correction. Lens code is 'humnor21'.

In Figs 4 and 5, the behavior of carp lenses is illustrated. In this case, the sums of squares of residuals are 4·78, 0·31 and 0·06 for one, two and three components decay, respectively. This suggests that

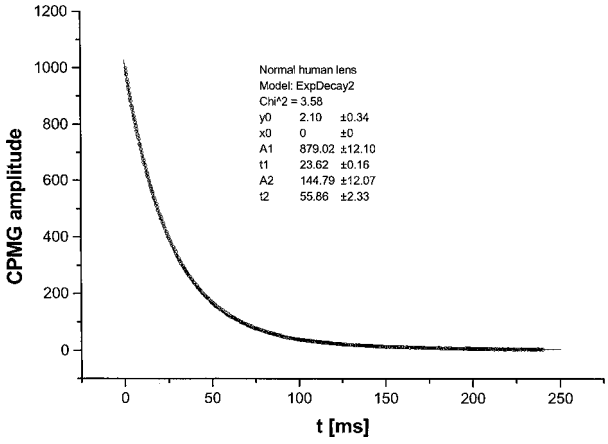

FIG. 7. The spin–spin relaxation in a normal human lens: double exponential fit with baseline correction. Lens code is 'humnor21'.

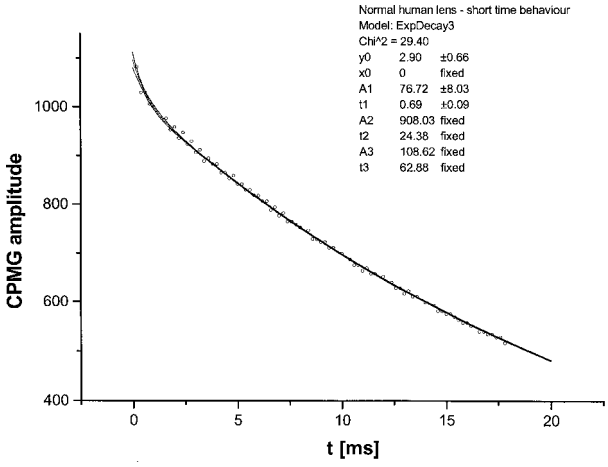

FIG. 8. The short time behavior of the CPMG echo train of a normal human lens. Lens code is 'humnor21'. The full lines represent the fitted curve and the 0·95 confidence band curves.

there are also three decaying components in the carp lenses. The short time behavior of the CPMG train, shown in Fig. 5, shows no sign of the fast component observed in turkey lenses.

We demonstrate the data and fits of a normal human lens in Figs 6 and 7. The measurement was carried out on the normal human lens 'humnor21' 48 hr later than the reported one in Table I. The Chi-square values are 20·1, 3·6 and 3·1 for the one, two and three components decay models, respectively. This indicates that in normal human lenses, the spin–spin relaxation process can be well described by two decaying components. The short time behavior of CPMG train in normal human lenses is illustrated in Fig. 8, a fast component shows up again. It should be emphasized that this fast component can be observed neither in cataractous lens nuclei nor in the carp lenses.

The typical relaxation curves and the results of two exponential fit on human cataractous lens nuclei are

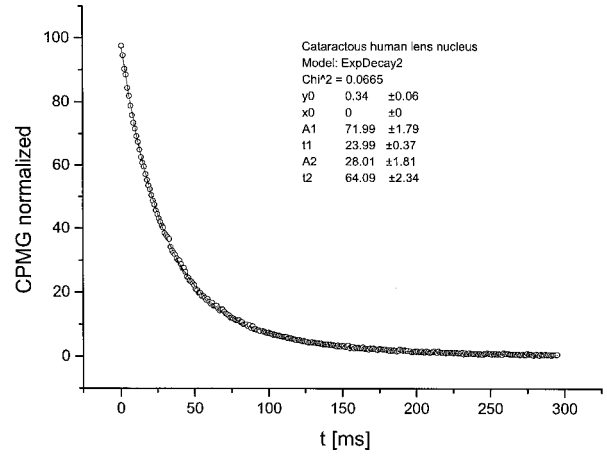

FIG. 9. The spin–spin relaxation in a cataractous human lens nucleus: double exponential fit with baseline correction.

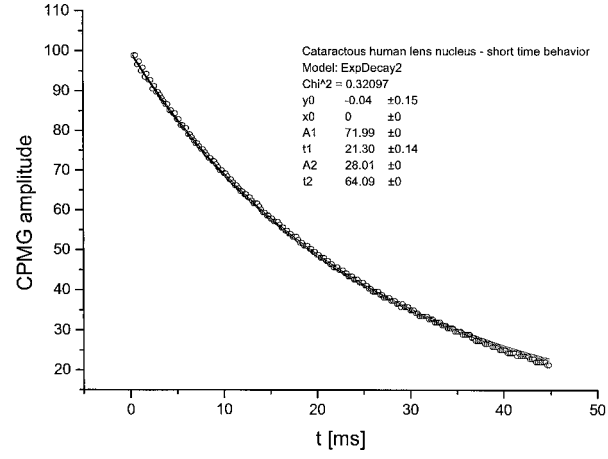

FIG. 10. The short time behavior of the CPMG echo train of a cataractous human lens nucleus. The full lines represent the fitted curve and the 0·95 confidence band curves.

shown in Figs 9 and 10. It is worth mentioning the similarity to the normal human lenses, the human cataractous lens nuclei can be well described by two exponential terms. In the cataractous lens nuclei, the fast decaying component is completely missing (Fig. 10).

4. Discussion

For all indications (Kumaraswamy et al., 1996; Hepburne-Scott, 1997) as to their physical properties, all the lens nuclei investigated in this study can be regarded as a high concentration aqueous solution of the lens proteins, crystallins. Israelachvili and Wennerström (1996) in general, treat the role of hydration in biological and colloidal interaction. In this NMR investigation, the resonant nuclei are protons. The protons in lenses can therefore be classified into three groups: (a) protons in lens protein; (b) water protons in the hydration layer around the crystallin molecules; and (c) protons in 'free' water. The measured spin–spin relaxation times for (a), (b)

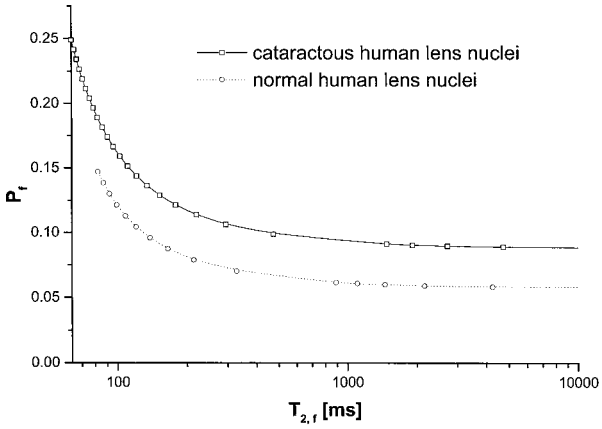

FIG. 11. The free water fraction, P_f vs the spin–spin relaxation time, $T_{2,f}$ of the free water protons in the normal and cataractous human lens nuclei. The open symbols are representing the solutions of the simultaneous equations (A2).

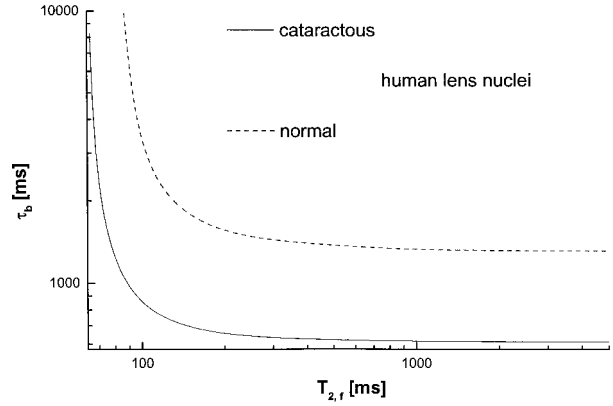

FIG. 12. The average residence time of the water protons in the hydration layer, τ_b vs $T_{2,f}$ in normal and cataractous human lens nuclei.

and (c) groups of protons show at least a two order magnitude rise from the covalently bound protons, group (a), to free water protons, group (c). The possible exchange between the different groups of protons and their true relaxation times and true weights in the CPMG signal should be accessed.

First we will discuss the normal and cataractous human lenses, as the spin–spin relaxation can be described as two exponential decay processes. The groups of water protons with the longer/shorter relaxation time will be regarded as the free/bound water protons. The relaxation environments of the free and the bound nuclei are different. Let $T_{2,f}$ be equal to the proper spin–spin relaxation time of the free water protons, P_f be their population fraction and τ_f the average residence time of a proton in the free environment before it is transferred to the bound environment. Similarly, let $T_{2,b}$ equal the proper spin–spin relaxation time of the bound water protons, P_b be their population fraction and τ_b the average residence time of a proton in the bound environment before it is transferred to the free environment. $P_f + P_b = 1$.

The very existence of two distinct decay processes indicates that the exchange between them will not be strong, i.e. rates of exchange, $1/\tau_f$ and $1/\tau_b$ should be small (Zimmerman, Holmes and Lasater, 1956; Zimmerman and Brittin, 1957; Hazelwood et al, 1974). Naturally, the effects of the exchange processes should not, however, be disregarded.

If $P_{eff,f}$ and $P_{eff,b}$ are the measured fraction of the longer and the short spin–spin relaxation time, $T_{2,feff}$ and $T_{2,beff}$ components, respectively:

$$P_{eff,f} + P_{eff,b} = 1.$$

We therefore have the following problem: three independent parameters from the measurements ($P_{eff,b}$, $T_{2,feff}$ and $T_{2,beff}$) and searching for five other parameters characterizing the two environments (the proper bound fraction P_b, the proper spin–spin relaxation times, $T_{2,f}$, $T_{2,b}$ and the average residence times, τ_f and τ_b). There are only four independent equations for our five unknown parameters: equations 6, 7 and 8, in Hazelwood et al. (1974) and the constraint by detailed balance, $P_f/\tau_f = P_b/\tau_b$ (equation 9 in Hazelwood et al., 1974).

Using the averaged measured relaxation times and effective fractions for the normal and cataractous human lenses in Table II, the system of four non-linear equations has been solved for the characteristic times or their reciprocal values, i.e. the characteristic rates with the assumptions that $P_b = (1+\epsilon)P_{eff,b}$ and all the rates are non-negative.

There are physically acceptable, non-negative solutions only if $\epsilon \geqslant 0$ indicating the true bound fraction must not be smaller than the measured $P_{eff,b}$. For $\epsilon = 0$, both residence times, $\tau_f = \tau_b = \infty$, there is no exchange between the bound and free water protons, $P_f = P_{eff,f}$ and $P_b = P_{eff,b,}$ respectively. This is the trivial, no-exchange limit. According to our numerical calculations, there is, however, another limit, in that case $T_{2,f}$ goes to infinity, and meanwhile P_f remains a non-vanishing finite value. This second limit is the far end of the medium exchange range consistent with the two exponential decay mode. Meanwhile, the true spin–spin relaxation time, $T_{2,b}$, remains almost independent of the exchange processes in both cases of the normal and cataractous human lens nuclei.

As in the cases of both the normal and cataractous human lenses, $T_{2,f}$ defines the boundary of medium exchange range compatible with the existence of the two exponential decay mode, in Figs 11 and 12 P_f and τ_b are plotted against $T_{2,f}$ on logarithmic scale.

If the proton exchange is completely neglected, T_{2f}-s are exactly the measured values, in the cataractous human lens nuclei P_f is around 5/3 times higher than in the normal case. To see what happens when the proton exchange is switched on, we assume that the true $T_{2,f}$ values are equal both in the cataractous and normal lenses. Under this assumption, the ratio $P_{f,cata}/P_{f,normal}$ is between 1·29–1·54. It is very probable

therefore, that in the cataractous human lens nuclei there is 29–54% more free water than in the normal ones.

Similarly, on the basis of our common $T_{2,f}$ hypothesis, the average residence time of the water protons in the hydration layer, τ_b, is always at least a factor of 2·5 higher in normal lenses than in the cataractous lenses (Fig. 12). In the free water phase, τ_f is always higher in the normal lenses than in the cataractous ones, but the differences are not as marked.

Considering these results, 90–95% of water protons are bound on the surface of crystallin in normal human lenses, therefore the hydration layer must be thick. Also, the water protons in the normal hydration layer are rather reluctant to exchange with protons in the free water phase. During the development of the cataract, the normal hydration layer is degraded and some part of the bound water from the hydration layer of the crystallin is squeezed out to become free water. Thus, the cataract formation and the syneresis are simultaneous processes.

Syneresis is a process in which bound water from the hydration layer of macromolecules is released and becomes free water. This process has been implicated as one of the major contributors to cataract formation. Syneresis in the lens participates is not only in the pathological and aging process but is also involved in relieving small stresses that may result from hydrostatic pressure. Such hydrostatic pressure creates osmotic imbalance that can be relieved by changing the activity of water, i.e. changing the free/bound water ratios (Bettelheim, 1979, 1999).

Having concluded from the measured values, we can presume that in the course of the human cataractous process, some part of the originally bound water in the lens nucleus becomes mobile water, demonstrating the mentioned pathological changes in the molecular organization of the lens proteins.

It is worth mentioning that we have observed a fast decay component with relaxation times around 1 msec in all the normal lenses, except the case of carp lenses. This fast component shows as a sharp up-turn in the short time behavior of the CPMG train. The fastest decay component is probably due to the protons in the crystallin. This fast decay is certainly not the consequence of diffusive motion, but it is very probably the high exchange rate between the covalently bound protons in crystallin and the water protons in the first hydration layer around the crystallin surface. During the cataract formation, the syneresis depletes the water protons in the first hydration layer, thus drastically reducing the exchange rate and raising the spin–spin relaxation time of the protons in the crystallin. In the case of the carp, the same behavior is the consequence of the extremely rapid drying of the carp lens.

By using this analysis it may be possible to monitor the other syneretic processes in eye lenses suggested by Bettelheim (1999).

Furthermore, we think the coming regular application of NMR imaging (MRI) on the eye can have advantages incorporating the use of more than one (two or three) spin–spin relaxation time values and data processing for elucidating the changes in the state of lens water in vivo in a cataractous eye.

Conclusions

Regarding the measurement method, it is important to consider at how many points the measurement is done, what succession of measuring time is chosen and finally what is the accuracy of the individual measurements.

None of the lenses investigated have shown single exponential relaxation, i.e. none of them can be characterized by a single spin–spin relaxation time. The multiple nature of relaxation processes is the consequence of the internal molecular structure and interactions in the lenses.

Having taken into account the proton exchange between the hydration layer around the lens protein and the free water, it is found that: first, in normal human lenses 90–95% of the protons are in the hydration layer around the crystallin molecules; and second, the free water fraction in the cataractous human lenses is 29–54% higher than in normal human lenses.

During cataract formation some of the originally bound water in the lens nucleus is released from the hydration layer around the crystallin, significantly increasing the mobile water fraction. Thus, giving experimental evidence to the statement that the cataract formation and the syneresis are simultaneous processes.

The spin–spin relaxation time of the fast component is increased in the cases of the fish lenses and the human cataractous lens nucleus. Indicating that the exchange between the protons in lens protein and the water protons in the hydration layer is very much reduced in these cases. The appearance of the fast component in the bird eye lens and in the human normal lens nucleus does prove the existence of the very strong exchange between the two groups of protons.

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Appendix

The input parameters are

$P_{eff,b}, P_{eff,f} = 1 - P_{eff,b}$,
$R_{eff,b} = 1/T_{2,beff}$, $R_{eff,free} = 1/T_{2,feff}$,
$C_1 = (R_{eff,b} + R_{eff,f})/2, C_2 = (R_{eff,b} - R_{eff,f})/2$.
Let $R_b = 1/T_{2,b}, R_f = 1/T_{2,f}, \rho_f = 1/\tau_f, \rho_b = 1/\tau_b$

The original system of equations,

$$\left.\begin{aligned} &P_{eff,b} = 0{\cdot}5 - 0{\cdot}25((P_b - P_f)(R_f - R_b) + \rho_f + \rho_b)/C_2,\\ &C_1 = 0{\cdot}5(R_f + R_b + \rho_f + \rho_b),\\ &C_2 = 0{\cdot}5[(R_b - R_f + \rho_b - \rho_f)^2 + 4\rho_f\rho_b]^{1/2},\\ &P_f\rho_f = P_b\rho_b,\\ &(P_{eff,f}R_{eff,f} + P_{eff,b}R_{eff,f}) = (P_fR_f + P_bR_b),\\ &P_f + P_b = 1, \end{aligned}\right\} \quad \text{(A1)}$$

has no solution, probably because of the fact that the fifth relation can be derived from the other five equations, or it contradicts the other five.

Therefore, we have introduced a new parameter, ϵ, so that $P_b = (1+\epsilon)P_{eff,b}$ and the simultaneous equations are reduced to:

$$\left.\begin{aligned} &P_{eff,b} = 0{\cdot}5 - 0{\cdot}25((2(1+\epsilon)P_{eff,b} - 1)\\ &\times (R_f - R_b) + \rho_f + \rho_b)/C_2,\\ &C_1 = 0{\cdot}5(R_f + R_b + \rho_f + \rho_b),\\ &C_2 = 0{\cdot}5[(R_b - R_f + \rho_b - \rho_f)^2 + 4\rho_f\rho_b]^{1/2},\\ &(1 - (1+\epsilon)P_{eff,b})\rho_f = (1+\epsilon)P_{eff,b}\rho_b. \end{aligned}\right\} \quad \text{(A2)}$$

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