^{23}Na NMR investigation of human lenses from patients with cataracts

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1. INTRODUCTION

Since the pioneering work of Huggert and Odleblad [1] a large number of investigations have been performed in vitro on intact ocular lenses by means of NMR spectroscopy to delineate and monitor specific changes in the lens that are related to the development of cataract. In particular, the state of water (^{1}H NMR), the polyol pathway (^{13}C NMR) and organophosphate metabolites (^{31}P NMR) have been extensively investigated in the ocular lens [2–10]. One aspect of research into the lens in which NMR can give important information is the study of electrolyte imbalance involved in the development of certain types of cataracts [12]. The electrolyte and water balance of the crystalline lens is largely determined by an active sodium pump, and by the passive permeability of the lens cell membrane. Although the characteristics of the cation pump and the permeability of the lens membranes have been largely elucidated for the normal mammalian lens, the course of events that lead to the derangement of electrolyte balance in the lens in many human cataracts is not understood. Moreover, a direct evaluation of membrane permeability properties in whole lenses is very difficult with traditional methods of investigation, and has led to conflicting reports [13–15].

Therefore, we decided to use ^{23}Na NMR spectroscopy and to investigate the intralenticular sodium resonance and the transmembrane fluxes in intact crystalline lenses incubated in TC-Earle culture medium.

Here we report the preliminary results of a ^{23}Na NMR study on human lenses taken from patients with cataract. The paramagnetic shift reagent $Dy(PPP)_2^{7-}$ was used to distinguish the resonances from intra- and extracellular $^{23}Na^{+}$.

2. MATERIALS AND METHODS

2.1. *Lenses from patients with cataract*

These lenses were obtained at surgery immediately after intra-capsular extraction. The lenses had been previously examined in situ with the slit lamp and classified according to the Chylack method [11]. Lenses were immediately placed in sterile vials containing TC-Earle medium (Difco, Detroit, MI), and were checked by biomicroscopy to exclude those with signs of capsule damage. Lenses were equilibrated for 2 h in TC-Earle medium at 37°C prior to the initiation of NMR analysis. Only one lens was used for each analysis.

2.2. *NMR measurements*

^{23}Na spectra were recorded on a Varian FT-80A spectrometer, utilizing a broad-band probe at 21.04 MHz, at 32°C, using 10-mm sample tubes. The spectrometer conditions used in the analysis were as follows: pulse width, 13 μs (90° flip angle); acquisition time, 0.5 s; sweep width, 4000 Hz; number of scans, 600. The relaxation times T_1 were

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obtained by the inversion recovery sequence and were determined by a non-linear three-parameter regression with percentage standard errors never greater than 10%. Sodium chloride in TC-Earle medium was used as a reference standard.

2.3. *Buffer*

The TC-Earle medium (116.4 mM NaCl, 5.6 mM dextrose, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.4 mM $MgSO_4$, 0.9 mM NaH_2PO_4, 26.4 mM $NaHCO_3$, with an osmolarity of 295 mosM, 37°C, pH 7.4) was used as the isotonic buffer.

2.4. *Shift reagent*

The shift reagent used was 0.1 M $DyCl_3$ and 0.1 M pentasodium tripolyphosphate (PPP) in aqueous solution, with a ratio of Dy/PPP = 1/2. The complex $Dy(PPP)_2^{7-}$ was then added to Earle medium to give a final concentration of 9 mM, as in [9].

3. RESULTS AND DISCUSSION

^{23}Na NMR spectra of TC-Earle culture medium and of an incubated human lens obtained from a patient with cataract are shown in fig.1a and b, respectively. It is clear that the ^{23}Na resonances in the two samples have similar chemical shifts, and this fact makes it very difficult to study the intralenticular $^{23}Na^+$. However, this objective may be reached by using $Dy(PPP)_2^{7-}$, a paramagnetic shift reagent that produces a higher field shift of the ^{23}Na signals; e.g., we show in fig.1c the ^{23}Na spectrum of TC-Earle medium with $Dy(PPP)_2^{7-}$ (9 mM), which has induced a paramagnetic shift (15 ppm) of the ^{23}Na spectrum with respect to the TC-Earle medium.

When $Dy(PPP)_2^{7-}$ was added to the medium containing a human lens obtained from a patient with cataract, two well separated resonances of ^{23}Na were observed in the spectrum (fig.1d): a signal at higher field coincident with the signal of the sodium present in the medium (fig.1c), which can be attributed to the extralenticular sodium; and a second resonance, unaffected by $Dy(PPP)_2^{7-}$, which is attributable to the sodium present inside the lens. These findings, in accordance with previous investigations performed on cellular samples [16,17], indicate that $Dy(PPP)_2^{7-}$ distinguishes intralenticular from extralenticular

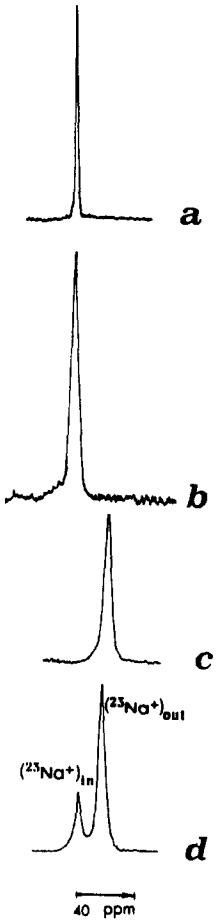

Fig.1. ^{23}Na NMR spectra (21.04 MHz, 32°C) of samples containing: (a) TC-Earle medium (1.5 ml) and D_2O (0.5 ml); (b) TC-Earle medium (1.5 ml), D_2O (0.5 ml) and a human lens obtained from a patient with cataract; (c) same as in (a), plus $Dy(PPP)_2^{7-}$ (9 mM); (d) same as in (b), plus $Dy(PPP)_2^{7-}$ (9 mM).

$^{23}Na^+$ due to its inability to cross the lens capsule. Since the chemical shifts of these resonances were practically unmodified even after 12 h, it may be suggested that this paramagnetic shift reagent is unable to cross the lens membrane, at least over this time-span. It is therefore suitable for long-time NMR analysis.

In fig.2a, the ^{23}Na NMR spectrum of a lens obtained from a patient with cataract is reported. It is noteworthy that the resonance relative to intralenticular $^{23}Na^+$ is composed of two partially superimposed signals: a less broad one, α, and a broader one, β. Confirmation of the existence of

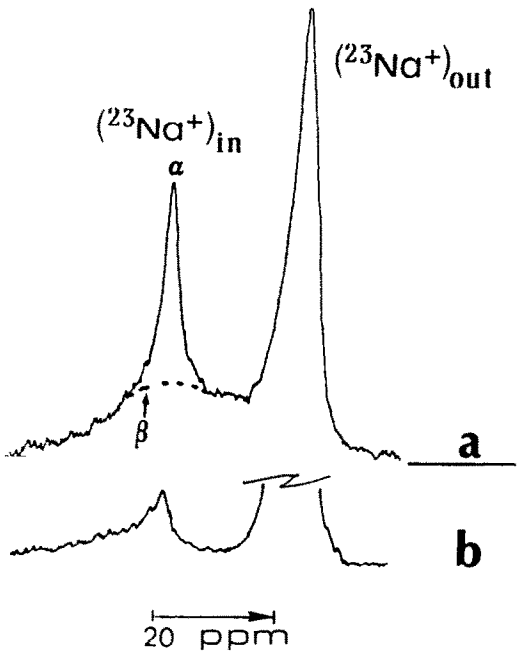

Fig.2. ^{23}Na NMR spectra (21.04 MHz, 32°C) of samples containing a human lens obtained from a patient with cataract in TC-Earle medium (1.5 ml), D_2O (0.5 ml) and $Dy(PPP)_2^{7-}$ (9 mM). (a) Whole lens, (b) lens nucleus.

the β-resonance was obtained by analysis of a dissected lens nucleus that, in the medium with $Dy(PPP)_2^{7-}$, exhibits a single, clearcut β-signal (fig.2b). Conversely, when the lens cortex alone was analyzed, the α-signal was shifted highfield as well as the extralenticular $^{23}Na^+$.

Table 1

^{23}Na NMR parameters of lenses obtained from patients with cataract and from TC-Earle medium

Sample	Chemical shift[a] (ppm)	T_1 (ms)	T_2 (ms)
TC-Earle medium	0	60	55
Normal saline[b]	0	60	60
TC-Earle medium + $Dy(PPP)_2^{7-}$ (9 mM)	−15	8.8	3.6
$(^{23}Na^+)_{in}$ α	−0.2	10.7	5
$(^{23}Na^+)_{in}$ β	+0.2	≪10	<1

[a] Downfield shifts are indicated by a positive sign
[b] Reference values

Spin-lattice relaxation time (T_1) parameters and spin-spin relaxation time (T_2) parameters estimated by line-width measurements ($T_2 = 1/\pi\nu_{1/2}$) of the α- and β-signals were also analyzed, and their values are shown in table 1.

These parameters, exhibiting values significantly decreased with respect to those obtained from TC-Earle medium and from normal saline, indicate that the physical characteristics of intralenticular Na^+ are different from those of Na^+ present in the medium. Moreover, β-Na^+ gives relaxation time values decreased with respect to α-Na^+.

Therefore, we assume as a first approximation that the decrease in T_1 and T_2 is induced mainly by a lengthening of the ^{23}Na re-orientation correlation times, τ_c [17]. These findings may be interpreted in terms of an increased immobilization of intralenticular ^{23}Na with respect to ^{23}Na present in the

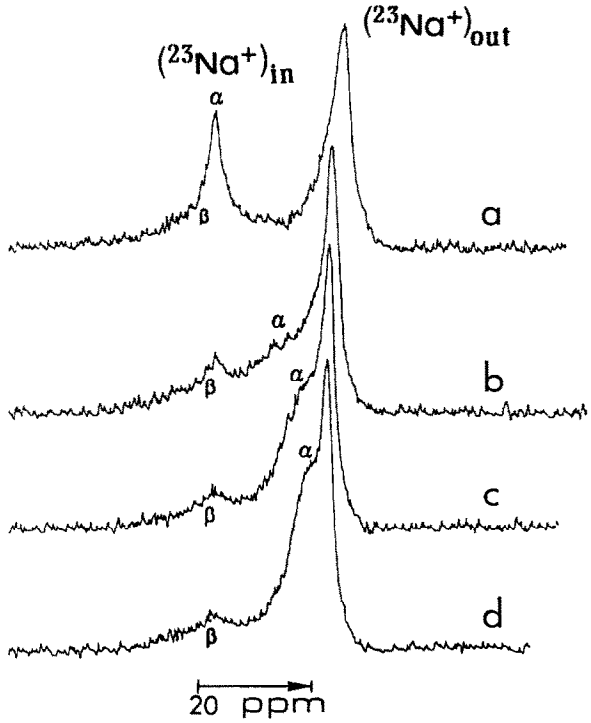

Fig.3. ^{23}Na NMR spectra (21.04 MHz, 32°C) of a human lens obtained from a patient with cataract immersed in aqueous solution (1.5 ml H_2O + 0.5 ml D_2O + $Dy(PPP)_2^{7-}$ 9 mM of KCl (0.9%) as a function of time: (a) 10 min after the preparation of the samples; (b–d) spectra registered 30, 60 and 90 min after the preparation of the samples.

medium, the β-Na^+ being the most strongly immobilized. To obtain more information on the characteristics of intralenticular sodium, and in particular on its mobility properties, we studied lenses, obtained from patients with cataract, maintained in an aqueous solution of 0.9% KCl.

Fig.3 reports the results of this experiment. Only the intralenticular α-signal is progressively shifted highfield whilst the spectral parameters of β-Na^+ resonance remain unmodified even after 12 h. These findings indicate that the efflux of ^{23}Na from the lens obtained from patients with cataract has at least two components: a faster component, of cortical and capsular origin (α), and a slower component, of nuclear origin (β). A similar result was found by Duncan [18] in the amphibian lens when, by ^{22}Na autoradiographic techniques, he showed the nuclear sodium to be more slowly exchangeable than elsewhere.

It appears therefore that ^{23}Na NMR represents a rapid method of investigation of these phenomena, and can be expected to furnish important data on their functional significance, at present still unknown.

The overall information obtained with this non-invasive technique, although at present only qualitative, suggests that ^{23}Na NMR may contribute to studies on the lens such as:

(1) Monitoring sodium levels in normal transparent lenses and in lenses affected by idiopathic or symptomatic opacities;
(2) The dynamic properties of Na^+ inside the lens;
(3) The evaluation of the efficacy of drugs to prevent or inhibit cataracts related to electrolyte imbalance.

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