DYNAMICS OF WATER IN DEOXYRIBONUCLEIC ACID STUDIED BY NEUTRON INELASTIC SCATTERING

V. S. Walder, L. A. Vinhas and R. Fulfaro

PUBLICAÇÃO IPEN 15 IPEN - Pub - 15	JUNHO/1980

CONSELHO DELIBERATIVO

MEMBROS

Dr. Luiz Cintra do Prado – Presidente
Dr. Edgardo Azevedo Soares Júnior – Vice-Presidente

CONSELHEIROS

Dr. Hélcio Modesto da Costa
Dr. Ivano Humbert Marchesi
Dr. Admar Cervellini
Dr. Waldyr Muniz Oliva

REPRESENTANTES

Dr. Jacob Charcot Pereira Rios
Dr. Paolo Enrico Maria Zaghen

SUPERINTENDENTE

Hernani Augusto Lopes de Amorim

PUBLICAÇÃO IPEN 15
IPEN - Pub - 15

JUNHO/1980

DYNAMICS OF WATER IN DEOXYRIBONUCLEIC ACID STUDIED BY NEUTRON INELASTIC SCATTERING

V. S. Walder, L. A. Vinhas and R. Fulfaro

CENTRO DE OPERAÇÃO E UTILIZAÇÃO DO REATOR DE PESQUISAS
COURP - AFN 074

INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
SÃO PAULO - BRASIL

Série PUBLICAÇÃO IPEN

INIS Categories and Descriptors

B12
A13

DNA: Nucleic acids
MOLECULAR BIOLOGY: Molecules
INELASTIC SCATTERING: Scattering
SLOW NEUTRONS: Neutrons
FREQUENCY MEASUREMENT: Spectra

DYNAMICS OF WATER IN DEOXYRIBONUCLEIC ACID STUDIED BY NEUTRON INELASTIC SCATTERING

V. S. Walder*, L. A. Vinhas and R. Fulfaro

ABSTRACT

In order to study the dynamics of water present in biological molecules neutron inelastic scattering measurements were performed on natural and dehydrated DNA samples, using a cold neutron time-of-flight spectrometer. The water frequency spectrum obtained by difference from the natural and the dehydrated DNA spectra, indicates that water molecule motions in the helicoidal DNA structure are quite similar to those in liquid water.

I – INTRODUCTION

It is known the interest in the state and role of water in the structure and functions of biological macromolecules[4]. Neutron inelastic scattering may be a valuable tool for further elucidating the controversy about bound water in hydrated biopolymers[4,17].

In biological matter such as living cells, it is found, to a large extent, a kind of bound water which does not freeze out on cooling and does not melt on subsequent heating of the solution, although it can be removed by conventional dehydration technique[4]. Nuclear magnetic resonance results and other studies have demonstrated that the mobility of these H_2O molecules, or of a certain fraction of them, is less pronounced than the mobility in the liquid state. This suggests some form of ice-like coordination of the H_2O close to the surface of the biological molecules[4,17]. Several hydration models were considered as applicable to deoxyribonucleic acid (DNA) as well, giving indication that the water hydrating DNA would have an "ice-like" structure[2,12,14]. Measurements in NaDNA using neutron inelastic scattering, which is the technique used in the present paper, also have given results similar to those metioned[4].

On the other hand, investigations on the motion of water molecules in samples of polyglutamic acid, also using neutron inelastic scattering, have given results providing evidence that the water molecule is tightly bound to the polypeptide with a behaviour similar to that of liquid water[18]. Results from infrared studies[7] and from measurements of neutron cross sections of H_2O present in DNA, performed in this Institute, have also given[8] indications that the water molecules hydrating DNA samples have the same behaviour.

So, in order to obtain additional informations on the behaviour of water molecules hydrating DNA samples, the present work was developed using the slow neutron scattering technique in dry and wet samples, which provides sensibly different results due to the water presence.

II – EXPERIMENTAL

Measurements were performed with a conventional cold neutron time-of-flight spectrometer[16] at the IEA-R1 2 Mw swimming pool research reactor. A polycrystalline beryllium filter cooled with

---

* Presently at the Instituto de Física – USP.

Aproved for publication in June 1980.

Writing, orthography, concepts and final revision are of exclusive responsibility of the Authors.

liquid nitrogen transmits a neutron spectrum with a sharp cutoff at 3.95 Å (5.2 meV) and with a mean energy of 3.5 meV and a width of 2 meV; a Fb monocrystal filter is used to reduce γ-ray background. Neutrons scattered by the sample at angle Ω are pulsed by a curved slit slow neutron chopper operated usually at 13000 rpm and are detected by a bank of ^{3}He detectors after an evacuated flight path of 3.15 m. Scattered neutron spectra are recorded with a multichannel time-of-flight analyser and the time-of-flight resolution is 1.7% for 4 Å neutrons and 6.4% for 1 Å neutrons.

The sample was a Deoxyribonucleic acid ex-thymus in powder form (from Koch Light Laboratories Ltd.) with a specified moisture of 6%. Using a conventional technique of dehydration one can remove the water present in DNA, including the bound water[4]. For the dry sample preparation, a certain amount of DNA was placed in a vacuum dissecator, containing calcium chloride, for two months; by this procedure it was possible to eliminate practically all the water (5.9%) present in DNA. Part of the same sample of powdered DNA was treated so as to become hydrated in order to obtain the wet sample; a quantity of DNA was exposed for fifteen days to a surrounding atmosphere whose relative humidity was approximately 85%; through successive weighing, a gradual increase of mass due to the water presence was observed, until saturation was attained with a final total mass that corresponds to 7.5% of water, which is equivalent to 5.6 water molecules per tetra nucleotide.

The scattering samples, dry and wet DNA, contained each one in slab type aluminum cell, have a thickness of 0.2 mm, ensuring a 90% transmission to avoid multiple scattering. The samples were placed at an angle of 45° to the incident beam.

III – RESULTS AND DISCUSSION

Energy gain spectra corresponding to the neutron inelastic scattering measurements in dry and wet DNA, were collected at the scattering angle $\Omega = 50°$ with the sample at temperature 298K. After subtraction of background and sample holder scattering, spectra were corrected for chopper transmission, air scattering and detector efficiency; all these corrections are energy dependent. By monitoring the incident beam the spectra normalization was performed.

From the corrected and normalized experimental scattered neutron spectra (Figure 1), the scattering laws were obtained. These data were used to calculate the generalized frequency spectra by means of the Egelstaff relation[5,6].

Figure 2 shows calculated generalized frequency spectra for natural (wet) and dehydrated (dry) DNA; in each spectrum the full line represents the sum of seven gaussians adjusted to the experimental points, after peak assignments, as the result of the best fit obtained. The fact that the spectra present a similar behaviour indicates that the drying process did not cause drastic alterations in the dynamics and structure of the macromolecules. On the other hand, the differences in intensity and shape of the peaks observed in the DNA spectra can be interpreted as the result of water presence in natural DNA, corresponding to 11 hydrogen atoms in addition to the 49 characteristic of the DNA tetranucleotide unit, in view of the large neutron scattering cross section of hydrogen atoms.

The difference curve corresponding to the bound water frequency spectrum, describing the water molecule motions in the helicoidal DNA structure, was obtained perfoming a subtraction between natural and dehydrated DNA spectra. An analysis of the difference spectrum, shown in Figure 3, reveals the existence of six peaks associated with the following frequencies: 43 cm^{-1}, 79 cm^{-1}, 143 cm^{-1}, 244 cm^{-1}, 381 cm^{-1} and 530 cm^{-1}. Table I shows the comparison of these values with an average of characteristic frequencies for liquid water and ice, appointed in the literature in the same frequency range[1,3,9,10,11,13,15].

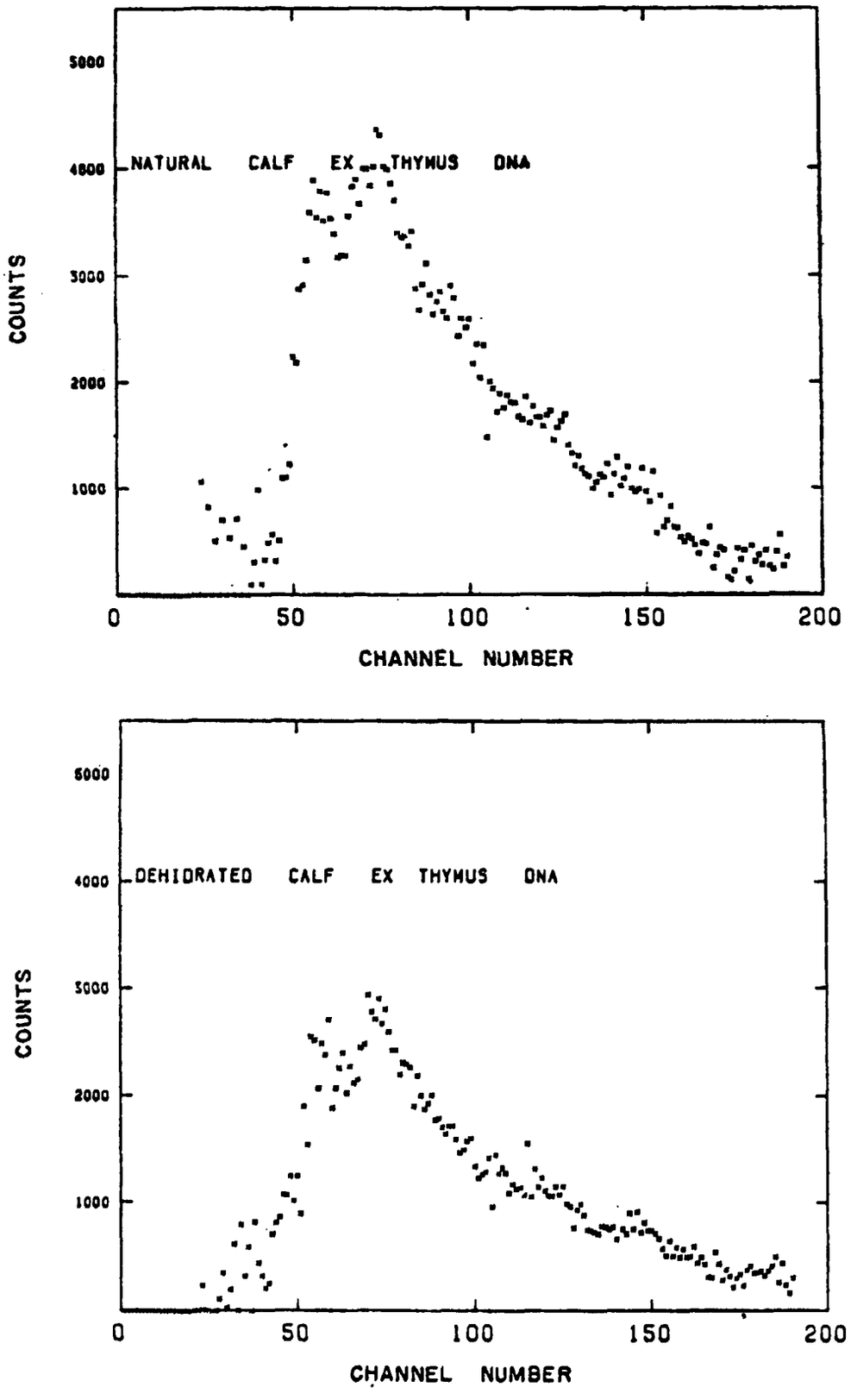

Figure 1 – Corrected time-of-flight spectra of scattered neutrons at an angle of 50° by natural and dehydrated DNA as a function of number of 16 μsec channels

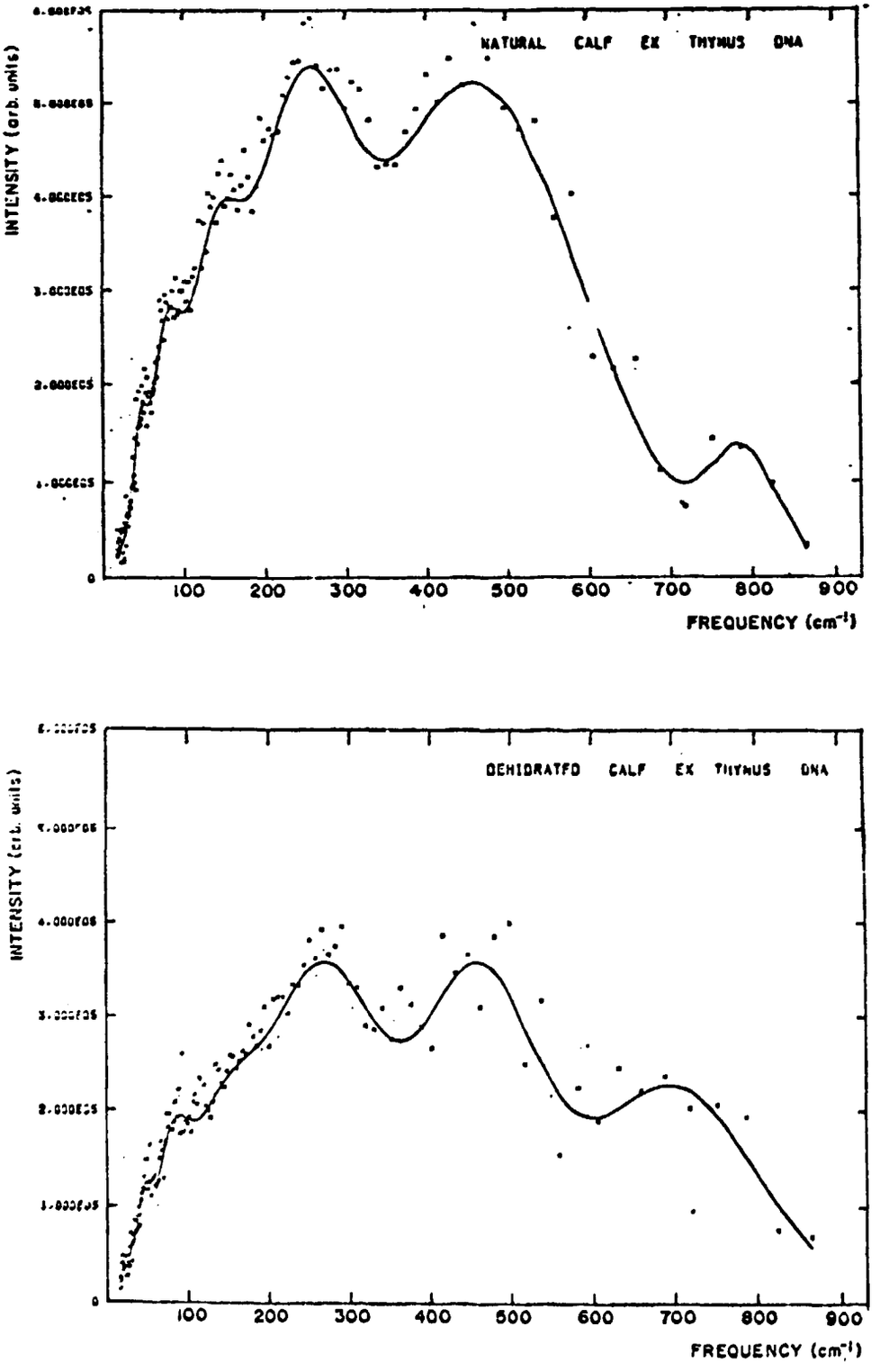

Figure 2 — Generalized frequency spectra of natural and dehydrated DNA. The full line represents the sum of seven Gaussian functions fitted to experimental points

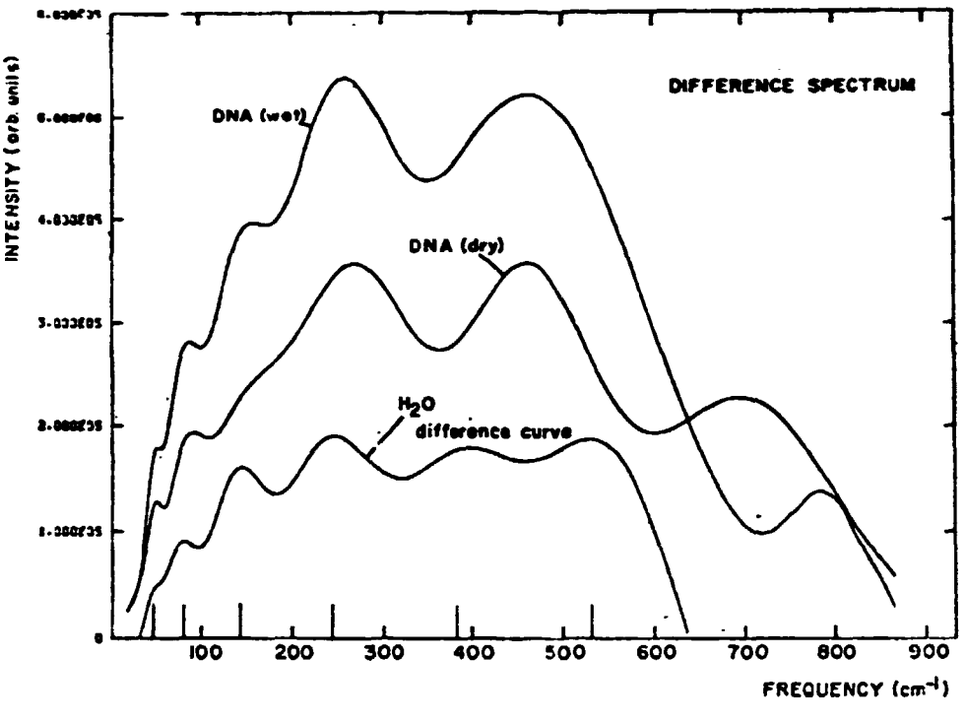

Figure 3 – Frequency spectra of "wet" and "dry" DNA. The difference spectrum is due to water in DNA. The assigned frequencies are indicated by the vertical lines in the lower part of the figure

Table I

Frequencies for water in DNA determined in the present work and literature data for water and ice (frequencies in cm^{-1})

Difference Spectrum	Water	Ice
530	547	600
381	–	–
244	229	280
143	136	132
79	81	90
43	43	52

Except for the frequency at 381 cm^{-1}, all the frequencies assigned in the difference curve are in agreement with those for liquid water; small differences between frequency spectra can be attributed to some factors that introduce changes in the experimental curves, such as the resolution that varies with $E^{3/2}$ and the fitting process that smooths the frequency spectra.

Since the frequency at 381 cm^{-1} is absent in the list of liquid water and ice characteristic frequencies, the observed peak in the difference curve is probably related to a frequency corresponding to a kind of motion resulting from the influence of the dynamics of DNA bases (adenine, guanine, cytosine and thymine) on the H_2O molecule. The same frequency was observed in a neutron scattering study[18] of the dynamics of water present in a sodium salt of polyglutamic acid, Na-PGA, with a helicoidal structure very similar to that of DNA.

Results of the present work indicate that water molecule dynamics in the host DNA molecule is more similar to that of liquid water than of ice, mainly with respect to the frequency at 530 cm^{-1} that corresponds to the literature frequency 547 cm^{-1}. This frequency is related to the fact that the usual accepted model for liquid water considers that the molecule executes torsional motion around the oxigen atoms, bending the two hydrogen bonds (each hydrogen atom in one molecule bonded to other molecule). Thus, through the correspondence between both mentioned frequencies, one may suspect that most atoms of the individual water molecules present on wet DNA tend to be tightly bound and that the molecule executes hindered rotations (torsional motion) much the same as in liquid water. This conclusion corroborates the results from infrared studies[7] and is in agreement with the results obtained in a previous work[8] made at this Institute, using neutron transmission technique through dry and wet DNA samples.

RESUMO

Com o objetivo de estudar a dinâmica da água presente em moléculas biológicas, foram realizadas medidas de espalhamento inelástico de nêutrons em amostras de DNA natural e desidratado, usando um espectrômetro de tempo de vôo para nêutrons lentos. O espectro de freqüência da água obtido por subtração do espectro do DNA desidratado daquele natural, indica que os movimentos das moléculas de água no DNA tem comportamento análogo aos das moléculas de água no estado líquido.

ACKNOWLEDGEMENTS

The authors wish to thank the "Comissão Nacional de Energia Nuclear" of Brazil and International Atomic Energy Agency for partial financing of this work.

REFERENCES*

1. BAJOREK, A.; JANIK, J. A.; JANIK, J. M.; NATKANIEC, I.; PARLINSKI, K.; POKOTILOVSKY, Y. N.; SUDNIK-HRYNKIEWICZ, M.; KOMAROV, V. E.; OZEROV, R. P. Investigation on the dynamics of water molecules in crystallo-hydrates by neutron inelastic scattering. In: INTERNATIONAL ATOMIC ENERGY AGENCY. *Neutron inelastic scattering: proceedings of a symposium on . . . held in Copenhagen, Denmark, 20-25 May, 1968, v.2.* Vienna, 1968 p.143-158 (Proceedings series).

2. BERENDSEN, H. J. C. Nuclear magnetic resonance study of collagen hydration. *J. chem. Phys.*, 36:3297-305, 1962.

(*) Bibliografic references related to documents belonging to IPEN Library were revised according with NB-66 of ABNT.

3. BURGMAN, J. O.; SCIESINSKI, J.; SKÖLD, K. Proton dynamics in water and ice studied by inelastic scattering of slow neutrons. *Phys. Rev.*, 170:808-15, 1968.

4. DAHLBORG, U. & RUPPRECHT, A. Hydration of DNA: a neutron scattering study of oriented NaDNA. *Biopolymers*, 10:849-63, 1971.

5. EGELSTAFF, P. A. The scattering of thermal neutrons by moderators. *Nucl. Sci. Engng*, 12:250-9, 1962.

6. EGELSTAFF, P. A. & SCHOFIELD, P. On the evaluation of the thermal neutron scattering law. *Nucl. Sci. Engng*, 12:260-70, 1962.

7. FALK, M.; HARTMAN JR., K. A.; LORD, R. C. Hydration of deoxyribonucleic acid II. An infrared study. *J. Am. chem. Soc.*, 85:387-91, 1963.

8. FULFARO, R.; WALDER, V. S.; VINHAS, L. A.; AMARAL, L. Q. *Slow neutron scattering cross section of deoxyribonucleic acid, ex-Thymus*. São Paulo, Instituto de Energia Atômica, 1974 (IEA-342).

9. HARLING, O. K. Slow neutron inelastic scattering study of light water and ice. *J. chem. Phys.*, 50:5279-96, 1969.

10. HAYWOOD, B. C. The spectral density of hydrogen in water. *J. nucl. Energy*, 21:249-62, 1967.

11. HUGHES, D. J.; PALEVSKY, H.; KLEY, W.; TUNKELO, E. Atomic motions in water by scattering of cold neutrons. *Phys. Rev.*, 119:872-81, 1960.

12. JACOBSON, B. Hydration sctructure of deoxyribonucleic acid and its physico-chemical properties. *Nature (London)*, 172:666-8, 1953.

13. LARSSON, K. E. & DAHLBORG, U. Proton motion in some hydrogenous liquids studied by cold neutron scattering. *Physica*, 30:1561-99, 1964.

14. MIGCHELSEN, C.; BERENDSEN, H. J. C.; RUPPRECHT, A. Hydration of DNA. Comparison of nuclear magnetic resonance results for oriented DNA in the A, B and C form. *J. Mol. Bio.*, 37:235-37, 1968.

15. PRASK, H.; BOUTIN, H.; YIP, S. Frequency spectrum of hydrogenous molecular solids by inelastic neutron scattering. Hexagonal H_2O ice. *J. chem. Phys.*, 48:3367-76, 1968.

16. RODRIGUEZ, C.; AMARAL, L. Q.; VINHAS, L. A.; HERDADE, S. B. Proton motions in methanol by cold neutron scattering. *J. chem. Phys.*, 56:3118-21, 1972.

17. SPRINGER, T. Quasielastic neutron scattering for investigation of diffusive motions in solids and liquids. *Springer Tracts Modern Physics*, 64:1-100, 1972.

18. WHITTEMORE, W. L. An investigation of polypeptide poly-L-glumatic acid, using neutron inelastic scattering. In: INTERNATIONAL ATOMIC ENERGY AGENCY. *Neutron inelastic scattering: proceedings of a symposium on . . . held in Copenhagen, Denmark, 20-25 May, 1968, v.2*. Vienna, 1968, p.175-184. (Proceedings series).

INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
Caixa Postal, 11 049 – Pinheiros
CEP 05508
01000 – São Paulo – SP

Telefone: 211-6011
Endereço Telegráfico – IPENUCLEAR
Telex – (011) 23592 - IPEN - BR