CHANGES IN N.M.R. RELAXATION TIMES OF IRON OVERLOADED MOUSE TISSUE

O. Baffa[1], A. Tannus[2], M.A.Zago[3], M.S. Figueiredo[3], and H.C. Panepucci[2]

[1]*Dept. of Physics, University of São Paulo, 14.049 Ribeirão Preto;*
[2]*Magnetic Resonance Laboratory, IFQSC-USP, 13.560 São Carlos;*
[3]*Dept. of Clinical Medicine, School of Medicine,*
14.049 Ribeirão Preto, Brazil.

I. INTRODUCTION

The values of proton relaxation times (T_1 and T_2) of human or animal tissues play an important role in NMR imaging since the image contrast is mainly determined by these parameters. Based on T_1 and T_2 measurements of healthy and abnormal tissues pulse sequences can be proposed to improve the diagnosis of a specific disease. Many factors can effect relaxation rates such as the rate of water exchanges between compartments with different relaxation times, temperature and the presence of paramagnetic impurities in tissues.

Several human diseases are accompanied by excessive iron accumulation, either spontaneous, as in idiopathic hemochromatosis, or transfusion induced, as in thalassemias or sickle cell anemia. In normal individuals, iron is stored mainly in reticuloendothelial (RE) cells; however, excessive iron can be located either in RE or parenchymal cells of several tissues, including the liver. RE iron overload may have toxic effects. Within the cells, iron is stored primarily inside ferritin molecules as hydrous ferric oxide-phosphate, a paramagnetic complex. Hemosiderin is the other paramagnetic iron storage molecule lacking the protein coat that involves iron in ferritin (1).

Recently patients with hepatic iron overload have been studied by NMR imaging, thus demonstrating the ability of this technique to image and detect these anomalies (2,3,4). The question remains of the quantification of the iron concentration in the organs by NMR. The availability of a non-invasive technique of body iron measurement would be particularly useful to decide on the use and to monitor the effects of iron chelating agents to reduce the body iron excess.

In this work we report an *in vitro* study of the relaxation times of liver tissue of iron overloaded mice. The relaxation times were compared to the liver tissue iron concentration chemically measured and the relationship between these results and the theoretical models of relaxation process is discussed.

II. MATERIALS AND METHODS

Iron overload was induced in 28 adult female Swiss mice by repeated weekly intramuscular injections of a colloidal iron hydroxide complex. The livers were excised after ether anesthesia and put into sealed plastic tubes with physiological solution and maintained on ice for a time not longer than 6 hours prior to NMR measurements. Before NMR measurements all liquid was drained from the tubes and the samples were allowed to stand at room temperature for at least 20 minutes.

Except for a small piece of liver tissue which was cut for chemical and histological analyses, the whole organ was used in the NMR measurements. The material for histological analysis was fixed in 10% formalin and included in parafin for histological sections. Perl's Prussian blue reaction was used for the histochemical staining of

trivalent iron (5). The tissue iron concentration in the liver was measured by the chemical method of Bothwell et al (6).

III. NMR MEASUREMENTS

NMR measurements were made using a home made Fourier Transform Pulsed NMR spectrometer operating at 25 MHz described elsewhere (7). The data for the determination of the relaxation time T_1 were obtained by the inversion recovery technique (180-τ-90). Data for T_2 evaluation were obtained by the two pulse (90-τ-180) spin echo technique. All measurements were carried out at room temperature (25 °C).

IV. RESULTS AND DISCUSSION

The spin lattice relaxation time (T_1) was calculated by fitting of the experimental points by a non linear least squares fitting program designed to adjust two exponentials to the free induction decay (FID) amplitudes. The T_1 values were obtained for 28 livers with different iron concentrations, and at least 12 points were collected for each fitting. A single exponential was found for the T_1 fitting for liver iron concentration in the range 0.10-4.17 mg Fe(3+)/g liver wet weight. A linear relationship was found between the iron concentration and relaxation rate $1/T_1$ with a correlation coeficient of 0.86 (figure 1).

The slope of the semi-log plot of the exponential data obtained in the two-pulse spin-echo (Carr-Purcell) experiments increases with the increase of iron concentration. Only one exponential curve can be fitted to the experimental data for iron concentrations up to 3.0 mg/g. Above this value, a two component or biphasic curve was obtained, as shown in figure 2. The fast decay of these biphasic curves follows the same linear equation of $1/T_2$ of the single component curves.

The relaxation rate $1/T_2$ against liver iron concentration is plotted in figure 3. The correlation coeficient found is 0.96 and the equation relating the relaxation rate to iron concentration is shown. Above 3.0 mg/g liver wet weight the relaxation rate of the slow component of the biphasic curve is also presented.

The brightness (I) of the NMR image is a multiparametric function given by

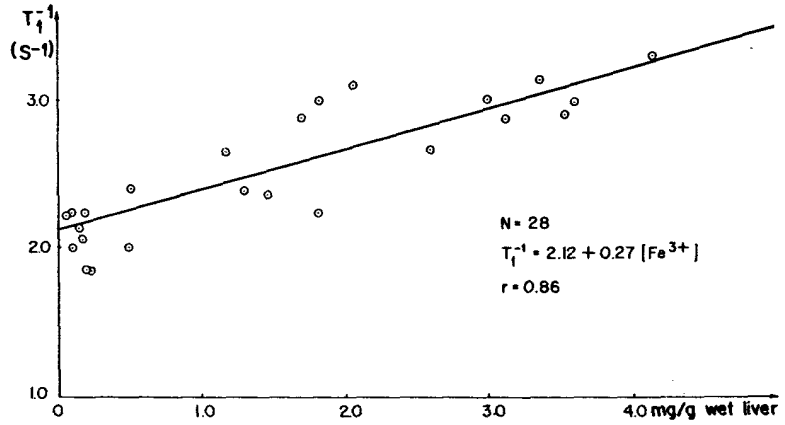

Figure 1. Increase of the relaxation rate $1/T_1$ with increasing tissue iron concentration. Not all the data for normal livers are displayed since overlap occurs near ordinate, smudging the picture. The equation found by linear regression of all 28 experimental points and the correlation coeficient (r) are also shown.

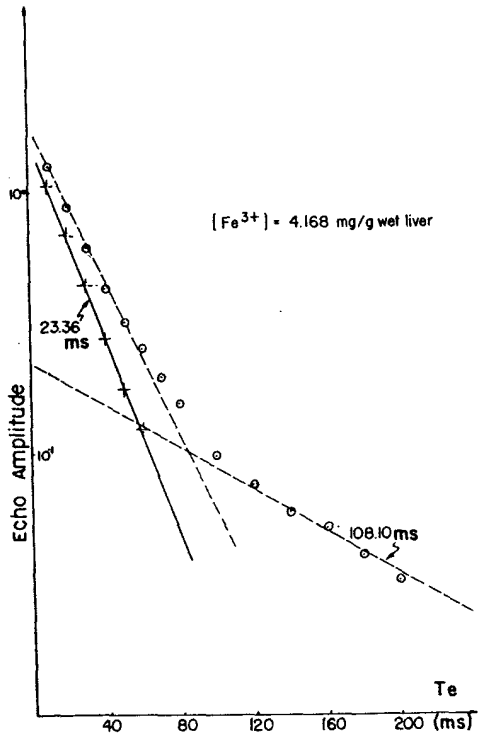

Figure 2. Graph of the echo amplitude versus time to echo (Te) obtained in a Carr-Purcell spin echo experiment. A two component decay is observed with a different spin-spin relaxation time T_2.

the following equation (1):

$$I = n(H)f(V)\exp(-TE/T_2)[1-\exp(-TR/T_1)]$$

where n(H) is the hydrogen density, f(V) is a function of hydrogen motion, TR is the repetition rate of the pulse sequence and TE is the echo delay.

Proton densities n(H) of normal and of iron overloaded rat livers are not significantly different (4). It is also supposed that proton motion is not affected by iron excess so that f(V) is the same for normal and iron overloaded livers. Thus, the main intrinsic parameters affecting the intensity of the image are T_1 and T_2. The plot of T_2 against T_1 (figure 4) shows the possibility of discriminating between normal and iron overloaded livers, since both parameters decrease as iron accumulates. The normal livers are indicated by points in the upper right corner and the iron overloaded by points at botton left. The correlation found between T_2 and T_1 (r=0.87) suggests a common mecha nism for the decrease of the two relaxa tion times.

The histological specimens of the iron overloaded mouse livers show a homogeneous distribution of iron within the parenchymal cells in contrast with the specimens of normal livers where practically no iron is detected by the routine histological methods. Furthermore as the iron concentration increases some regions with dark granules in the histological sections exhibit a great accumulation of iron.

Our in vitro results shown that the relaxation rate $1/T_2$ fits well with the chemically measured iron content of the mouse liver. This suggests that $1/T_2$ could be useful to quantify the iron concentration of liver by a non invasive method. At present there are technical problems with this measurement. At high iron concentration the images have an intensity as low as the background leading to a poor signal to noise (S/N) ratio which prevents an acurate T_2 measurement. To overcome this problem by enhancing the S/N ratio, special surface coils could be developed to measure this parameter acurately even in iron overloaded patients.

Another crucial problem is that some routine procedures determine T_2 by fitting an exponential curve on the basis of only two experimental points. With this crude approximation base line is not corrected, and multicomponent exponential decays like the biphasic curves that we have obtained for iron concentrations above 3.0 mgFe/g tissue are mistaken for single exponentials leading to incorrect T_2 determination. Thus, another point to be improved is data acquisition for T_2 measurement.

What are the biological implications of the biphasic curve obtained for T_2, if any? About 80% of the liver water is located within parenchymal cells - hepatocellular water, primarily cytosol

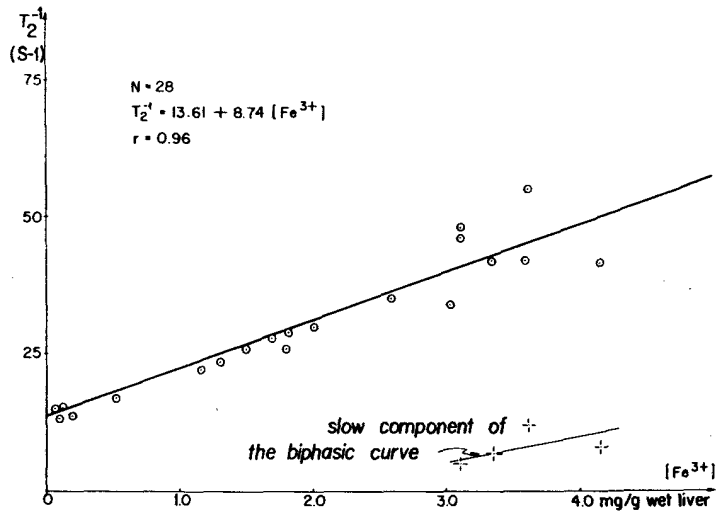

Figure 3. Graph of the relaxation rate T_2 versus iron concentration. Not all the data for normal livers are displayed since overlap occurs near the ordinate, but all data were taken into occount in the equation presented.

water - which accounts for the main relaxation features obtained for the liver tissue. However, reticuloendothelial water is about 10% and can also contribute to the relaxation properties of the tissue.

The two-exponential curves obtained for livers with iron concentrations above 3.0 mgFe/g allow the determination of the two amplitudes, two relaxation times and the base line. The amplitudes of the slow and fast component of the biphasic curve are proportional to the protons or water molecules in the slow and fast relaxing micro environment, respectively. The fraction of protons in the slow relaxing environment calculated from our data is 12 ± 3%, a value very close to that ascribed to the water within reticuloendothelial cells, suggesting that the biphasic behaviour of the exponential decay may be related to the heterogeous distribution of the iron overload within the liver. However this point needs further investigation, probably by using different methods of iron overload so that hepatocytes or Kupfer cells can selectively affected. Nevertheless at present magnetic resonance imaging appears to be a potential tool to distinguish between iron overload in these different sites.

At iron concentrations below 3.0 mg Fe/g liver wet weight, the T_2 relaxation time decreases continuosly as the iron concentration increases, in the same way as when the concentration of a paramagnetic impurity in water solution increases, suggesting a homogeneous distribution of this impurity (i.e., iron) in the tissue. However above that critical point, the curve has a biphasic shape with two relaxation times that can be the consequence of the protons at two states. One of these states would be the cytoplasmic water homogenously doped with paramagnetic iron whereas the other would be the water near the iron aggregates, which possibly create a magnetic field inhomogeneity, thus shortening the relaxation

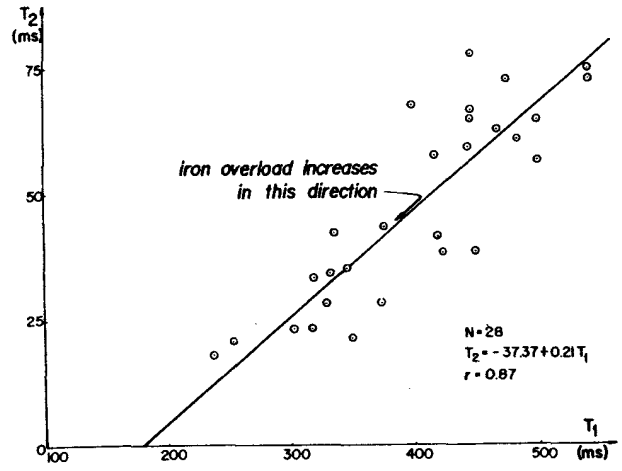

Figure 4. T_2 versus T_1 relaxation times for livers with different iron concentration.

time.

Thus, by the demonstration of changes of the two relaxation times which are dependent on the tissue iron concentration, our results indicate that NMR can be potentially useful for the non-invasive evaluation of excessive iron deposits in humans.

ACKNOWLEDGMENT

The authors are indebted to Mr. Adalberto V. Verceze and Ademir G. Jardim for technical help. Supported by FAPESP and CNPq.

REFERENCES

1) P.M. Harrison, *Sem. Hematol.* 14, 55 (1977).
2) V.M. Runge, J.A. Clanton, F.W. Smith, J. Hutchison, J. Mallard, C.L. Partain and A.E. James Jr., *Am. J. Radiol.* 141, 943 (1983).
3) R.C. Brasch, G.E. Wesbey, C.A. Gooding and M.A. Koerper, *Radiology* 150, 767 (1984).
4) D.D. Stark, M.E. Moseley, B.B. Bacon, A.A. Moss, H.I. Goldberg, N.M. Bass and T.L. James, *Radiology* 154, 137 (1985).
5) R. Willians, P.J. Scheur and S. Sherlock, *Q. J. Med.* 31, 249 (1962).
6) T.H. Bothwell, R.W. Charlton, J.D. Cook and C.A. Finch, *Iron Metabolism in Man.* Oxford-Blackwell (1979) pages 440-443.
7) A. Tannus, *PhD Thesis - University of São Paulo* (1986).