Polarized into the Bloodstream –
Spin-off from Polarized Solid Targets

Christian Heß
Ruhr-Universität Bochum, Institut für Experimentalphysik I, Universitätsstraße 150,
44801 Bochum, Germany
E-mail: hess@ep1.rub.de

Abstract. Magnetic Resonance Imaging (MRI) is one of the most powerful non-invasive
techniques in medical diagnostics that provides spatial and functional information. The
sensitivity of NMR, imaging or spectroscopy, is strongly limited by the low Boltzmann
polarization that can be reached at room temperature and any achievable field strength.
A way out is the use of the Dynamic Nuclear Polarization (DNP) scheme, used for the operation
of polarized solid targets in numerous nuclear and particle physics experiments since the early
1960s. Several imaging applications will benefit from the strong DNP-signal, e.g., available
from hyperpolarized $^{13}$C-labeled contrast agents. The input and present status of DNP-
hyperpolarization in this new emerging field is presented.

1. Introduction
Since the first Nuclear Magnetic Resonance experiments (NMR) by Bloch and Purcell in 1946
[1, 2, 3], the NMR technique has developed to an important analytical method used in physics,
biochemistry, materials sciences and medicine: In the early 1960s Cohn used NMR techniques
to investigate metabolic processes at the molecular level [4]. In the mid 1960s Ernst developed
the Fourier Transform NMR technique which offered a much better signal to noise ratio due
to faster measurements compared to the primary continuous wave NMR technique [5]. In the
1970s Lauterbur and Mansfield developed the technique of Magnetic Resonance Imaging (MRI)
[6, 7], today’s most powerful and versatile imaging method in medical diagnostics.

The major drawback of these NMR based methods is their low sensitivity caused by the
small magnetic moment of the nuclear spins, which results in a small Zeeman splitting of the
nuclear spin energy levels, and therefore leads to only a small Boltzmann polarization at room
temperatures.

Beside new hardware developments, such as magnets operating at very high fields and low
noise detectors, various strategies have been designed to overcome the sensitivity limitation
by increasing the nuclear polarization. All these techniques are based on the coupling of the
nuclear Zeeman reservoir, though not directly, to a highly polarized quantum system. The
nobel gases $^3$He and $^{129}$Xe can be polarized by the angular momentum transfer of photons
from circularly polarized laser light [8]. Similarly, triplet spin polarization of photoexcited
triplet states is transferred to the nuclear system by means of a microwave field [9]. The use of
parahydrogen in molecular addition reactions can form highly polarized nuclear spin systems
[10]. Another method, known as the Haupt effect, involves the conversion of rotational energy
bound in molecular rotors into nuclear polarization during fast temperature jumps [11].
But all these techniques are limited to a comparatively small class of molecules in each case. Unlike in the case of DNP, which has a large field of application both in solids and liquids, solely requiring a certain amount of paramagnetic centers in the sample. In the next section, the DNP technique will be introduced in more detail, including a short historical retrospective.

2. Dynamic Nuclear Polarization and EPR linewidth

In the thermal equilibrium (TE) the population of a particle’s Zeeman states is characterized by the Boltzmann distribution. The polarization is given by the Brillouin function

$$P_0 = B_I \left( \frac{I \gamma B}{kT} \right),$$

(1)
depending on the particle’s spin $I$, its gyromagnetic ratio $\gamma$, the magnetic field strength $B$ and the temperature $T$. For spin 1/2 particles this equation simplifies to

$$P_0 = \tanh \left( \frac{\gamma B}{2kT} \right).$$

(2)

Free electrons are much easier to polarize thermally than any nuclei, due to their more than 600 times larger gyromagnetic ratio (compared to protons). At moderate temperature and field conditions like 1 K and 2.5 T, electrons are polarized to more than 90% while the proton polarization is about 0.25%. Low gamma nuclei like deuterons or $^{13}$C are polarized to less than 0.07% at the same conditions.

Even in the early days of NMR, the idea came up to use the high electron polarization and transfer it to the nuclear system. When Overhauser [12] predicted the enhancement of the nuclear polarization in metals by electronic cross relaxation at a conference of the American Physical Society in 1953, leading NMR experts received this proposal with deep skepticism. But shortly afterwards, Carver and Slichter could experimentally verify Overhauser’s predictions in metallic lithium [13, 14]. Four years after Overhauser, Jeffries proposed a way to polarize nuclear spins in non-metals by saturation of certain so-called forbidden transitions by microwave irradiation [15]. He also established the term “Dynamic Nuclear Polarization”. In the following year, Abragam and Proctor could demonstrate this effect for the first time experimentally in LiF [16] and called it the “solid effect”. Within the next ten years, a lot of theoretical work in the field of DNP was done by Abragam, Goldman, Provoterov and Borghini. So the application of the Redfields spin temperature concept led to a fundamental understanding of the DNP process (see below). Since the early 1960s DNP is used for the operation of polarized targets in particle physic experiments. First for scattering experiments on the proton with hydrogen compounds, later for those on the neutron with deuterated compounds. A comprehensive overview about the achievements made in this field up to the late 1980s can be found in [17]. More recent overviews are given in [18, 19].

As mentioned above, the DNP process takes advantage of the large electron polarization and transfers it to the nuclear system. But to benefit from this electron polarization, free electrons must be present in the sample. In ordinary compounds, all electron spins are coupled anti-parallelly to spin zero. Therefore paramagnetic centers have to be embedded into the diamagnetic target material. Target materials which are liquid at room temperature can be doped by solving chemical radicals in it, before the solution is dropped into liquid nitrogen to form frozen beads. If solving chemical radicals is not possible due to some reasons, paramagnetic centers can be generated by low temperature irradiation, e.g., with an intensive electron beam.

There are basically two models that describe the polarization process of DNP. In case of the solid effect the microwave field drives the so-called forbidden transition generating simultaneous spin flips of electron and nucleon spin. In combination with the fast electron relaxation this leads
to an effective polarization of the nuclear spin system. Depending on the microwave frequency being the sum or difference of electron and nuclear Larmor frequency, the nuclei are polarized negatively or positively. Based on simple rate equations for this process, the maximum reachable nuclear polarization can be estimated by

$$|P_I|_{\text{max}} = |P_e| \frac{1}{1 + f_\alpha}$$

with

$$f_\alpha = n_I T_{1e} n_S T_{1n}'.$$  \hspace{1cm} (3)

where $P_e$ is the electron polarization, $n_I$, $n_S$ the nuclear and electron spin density, $T_{1e}$ the electron spin lattice relaxation time and $T_{1n}'$ the nuclear leak relaxation time. But the solid effect can only be applied to some of the proton target materials, where the nuclear (proton) Larmor frequency exceeds the EPR (electron paramagnetic resonance) linewidth significantly – for example, LMN (Lanthanum magnesium nitrate, one of the first used polarized solid target materials in the early 1960s [20]).

For other materials, especially those containing deuterons or other low gamma nuclei, the DNP is described by the spin temperature theory, developed by Abragam [21] and Goldman [22], based on the Provotorov theory [23]. The spin temperature theory treats the different spin systems as thermodynamical ensembles. Each occurring interaction is represented by an energy reservoir with a corresponding temperature. In this scheme, the DNP can be described as follows: By applying a saturating microwave field close to the electron Larmor frequency, the electronic dipolar reservoir can be cooled or heated effectively. The transfer of the high electron polarization to the nucleons then is realized by equalizing the nuclear Zeeman temperature to the electronic spin-spin temperature in terms of thermal contact (e.g., due to hyperfine interaction).

For a most effective polarization transfer, the thermal contact of the nucleons has to be optimized by matching the corresponding heat capacities, that is, of the energy width of the electronic dipolar reservoir $D$ to the nuclear Zeeman energy. That means practically that the EPR linewidth of the paramagnetic centers should be approximately equal to the NMR frequency of the nuclei to be polarized.

The quantitative theoretical treatment of this picture, including some additional assumptions, provides an upper limit for the nuclear polarization,

$$|P_I|_{\text{max}} = B_1 \left( I_1 J_\omega \frac{\omega_f}{2D} \sqrt{\frac{1}{\eta(1+f)}} \right),$$

\hspace{1cm} (4)
Figure 2. $^{13}$C spectrum of urea (natural abundance $^{13}$C, 60 mM aqueous solution) at 9.4 T. 

A) hyperpolarized to 20% by dissolution DNP. Single excitation, SNR = 4592. B) Thermal equilibrium spectrum of the same sample at room temperature (averaged during 65 h with 232128 excitations, SNR = 7). Figure taken from Ref. [26].

depending on the nuclear spin $I$, the inverse lattice temperature $\beta_L = \hbar/kT$, the electronic and nuclear Larmor frequencies $\omega_e$ and $\omega_I$, the EPR linewidth $D$, the ratio of the electronic Zeeman and dipolar relaxation times $\eta = t_Z/t_D$, and the "leakage factor" $f$ containing all nuclear relaxation processes, which do not process via the electronic dipolar reservoir. A more detailed description of these processes is given in [24]. In Fig. 1, this dependency is shown by a representative set of deuteron polarization values as a function of the EPR linewidth. Except for the remarkably high polarization values of EDBA\textsuperscript{1} doped d-butanol, these values show a clear correlation with the EPR linewidth: the narrower the EPR linewidth is, the higher is the maximum deuteron polarization. Furthermore, the correlation qualitatively shows the functional behavior given by the theory, as indicated by the dashed curve calculated from Eq. (4) with an arbitrarily chosen factor $\sqrt{\eta(f + 1)} = 3.5$, see [25].

3. Dissolution DNP

As already explained, the DNP process takes advantage of the high electron polarization at low temperatures. That is why it does not work at room temperatures. But to benefit from this powerful tool even in room temperature measurements, the following procedure is used: The desired probe is mixed with a suitable radical and frozen into droplets. In an $^4$He evaporation refrigerator with a temperature close to 1 K and a magnetic field of several Tesla, the probe is positively polarized by microwave irradiation like it is done with a polarized target. The current polarization is monitored by NMR. When sufficient polarization is obtained, the sample container is lifted upon the level of liquid helium and boiling water is injected rapidly into the sample container through a tube. The sample is dissolved by the hot water and displaced via a second tube to a receiving container outside the refrigerator [26]. This hyperpolarized liquid is directly transferred to a high resolution NMR spectrometer. In the top panel of Fig. 2, a $^{13}$C spectrum of the polarized liquid gained by a single excitation is shown. Below that, for comparison, a thermal equilibrium spectrum at room temperature from the same sample is shown, acquired by averaging 65 h with 232128 excitations – still, the signal-to-noise ratio (SNR) of the hyperpolarized spectrum is 656 times higher. This example illustrates in an impressive way the capability of the DNP technique for liquid state NMR applications.

But besides aspects related to spectroscopy, there is another, much more exciting application of these hyperpolarized liquids; the use as a contrast agent for MRI. This means concretely

1 perdeuterated Cr(V)-EHBA: sodium bis(2-ethyl-2-hydroxy-butyl)oxochromate(V)monohydrate-d_{22}
injecting a hyperpolarized liquid into a patient’s bloodstream while doing the MRI investigation. Before coming to that, two points have to be considered: Which substance should be used for that purpose and what kind of radical?

4. Trityl Radicals
For the use of contrast agents in MRI studies, organic metabolic immanent compounds with polarized carbon turned out to be most applicable. Because the 98.9% naturally occurring $^{12}$C has spin zero, special $^{13}$C labeled compounds are used instead. The gyromagnetic ratio of $^{13}$C is only a quarter of that of the proton, thus for a correct description of the $^{13}$C DNP process the spin temperature theory is the only valid model, and as explained in Sec. 2, the EPR linewidth is the key parameter that controls the efficiency of the polarization process. Experiments using the nitroxy radical TEMPO (2,2,6,6-Tetramethylpiperidine-1-oxyl) in $^{13}$C labeled pyruvate give polarization values of 16% at 5 T and 1.1 K [27]. That is due to the radical’s large EPR linewidth which is 150 MHz at 2.5 T [25] – thus about six times larger than the $^{13}$C’s Larmor frequency of 26.7 MHz at the same magnetic field. Different from that, trityl radicals feature linewidths of about 25 MHz and according to the spin temperature theory, this perfectly fits to the $^{13}$C frequency. Using the trityl radical Ox063Me [28], polarization values of 75% for $^{13}$C have been achieved in labeled pyruvic acid at 5 T and 0.9 K, as shown recently [29].

5. Pyruvate
Especially for the use in cancer research, the substance pyruvic acid (or rather its corresponding salt pyruvate) is the appropriate agent, due to its central role in the cellular metabolism, as illustrated in Fig. 3. Pyruvate is an intermediate product of the energy generation in the cells, produced by the glycolysis (the decomposition of glucose) which is an anaerobic process (not consuming oxygen). The pyruvate itself is converted in the decarboxylation reaction to acetyl coenzyme A, which is broken down in the Krebs-cycle afterwards. But this path works only under aerobic conditions – if sufficient oxygen is available in the cell. Under anaerobic conditions (which can be an indication for cancer cells), the pyruvate is decomposed in lactic acid fermentation to lactate. The pyruvate can also be converted to the amino acid alanine.

By now injecting $^{13}$C labeled and hyperpolarized pyruvate, one is able to observe its
Figure 4. A) Metabolic pathways of the $^{13}$C labeled pyruvate. Hyperpolarized $^{13}$C spectra (B) and peak height plots (C) show the time course for the $[^1-^{13}$C] pyruvate and its metabolic products following the injection of 350 µl of hyperpolarized pyruvate in a mouse. Figure taken from Ref. [31].

decomposition and find out if the cells' metabolism works normal or not. This is possible because the chemical shift of the $^{13}$C NMR signal depends on the molecule in which it is embedded. So by analyzing the NMR spectrum, one is able to determine the relative concentrations of the different substances containing $^{13}$C, especially pyruvate, lactate and alanine. Figure 4 shows the results of an experiment with a mouse: During the first 12 seconds the pyruvate is injected, every 3 seconds an NMR spectrum is acquired. The pyruvate concentration quickly reaches a maximum before being converted to lactate and alanine.

6. DNP in cancer diagnostics
In many cancer cells, the metabolism is different from that in normal cells. Due to their higher energy consumption and limited oxygen supply, cancer cells are forced to run more lactic acid fermentation, producing lactate – known as “Warburg effect”. And in fact, studies show [31] that the lactate level significantly increases with the cancer development: In Fig. 5 H&E-stained\(^2\) sections from different histologically defined groups of murine prostate cancer are shown together with their associated hyperpolarized $^{13}$C spectra. The spectrum from the normal prostate exhibited the lowest lactate level out of all the representative spectra, whereas pyruvate produced the largest peak in the normal spectrum. The carbon spectra showed significantly higher lactate levels in the primary tumors, with the high-grade tumors having the highest lactate levels. Pyruvate levels in the primary tumors were similar and were comparable with the normal prostate and lymph node metastases. Lactate level in the lymph node metastases fell between those of the primary tumors.

And finally, using the capability of spatial resolution of magnetic resonance imaging, one is able to locate a tumor by watching the lactate distribution. In Fig. 6, the concentrations of hyperpolarized $^{13}$C metabolic compounds calculated from MRI examination of a rat with an

\(^2\) hematoxylin and eosin stain: a popular staining method in histology, e.g., in the case of a suspected cancer
Figure 5. Representative H&E-stained sections (5 μm thick, ×40 magnification) and hyperpolarized $^{13}$C spectra for different histologically defined groups of prostate cancer using the transgenic adenocarcinoma of mouse prostate (TRAMP) model. The normalized spectra illustrate the strong correlation that exists between the amount of $^{13}$C lactate and the progression of the normal prostates to the low-grade and high-grade primary tumors. Figure taken from Ref. [31].

implanted P22 tumor are shown. The anesthetized rats were positioned in a clinical MRI scanner while the hyperpolarized sodium $^{13}$C$_1$-pyruvate with a polarization of $\sim 20\%$ was infused during 14 seconds. Thirty seconds after the start of the infusion, a chemical shift imaging sequence (CSI) was started. The metabolic images were calculated from the CSI data set estimating the peak amplitudes for alanine, lactate, and pyruvate at fixed frequencies. The highest pyruvate signal originates from the well-perfused tumor and aorta. Pyruvate has been partly metabolized into alanine and lactate. From the lactate distribution in Fig. 6, it can be seen that the tumor area contains the highest concentration of lactate [32].

Pyruvate transformation into lactate seems to occur abundantly in cancer cells, and it is, thus, envisaged that injection of hyperpolarized $^{13}$C labeled pyruvate can be used for early visualization and diagnosis of cancer tissue.

7. Conclusion

The ability to hyperpolarize $^{13}$C labeled molecules using DNP in the solid state and maintain the polarization following dissolution into the liquid state has opened up new pathways for biomedical applications. The large increase in SNR has made it possible to image the spatial in vivo distribution of labeled molecules following their intravenous injection and even to observe their metabolism in real-time.

Hyperpolarized $^{13}$C labeled pyruvate is abundantly decomposed into lactate in cancer cells. Therefore, lactate is a non-invasive biomarker for the presence of cancer and histological grade that could be used in future three-dimensional $^{13}$C spectroscopic imaging studies of cancer patients.
connected via a T-tube to a pressure recorder and a pump delivering saline (0.15 L min⁻¹) to prevent catheter clotting. ... Imaging by Hyperpolarized¹³C MRI

Figure 3. Erlangen, Germany) using a ¹H-¹³C T x/Rx birdcage coil (diameter = 8.3 cm, pressure were continuously recorded during the experiments. Based on the proton frequency found by the MR system, the MR frequency for the location of the tumor. All automatic inline adjustments of the MR scanner were disabled. This was done to avoid unwanted radiofrequency pulses, which would destroy the hyperpolarization signal. A 90-degree reference radio-frequency pulse was calibrated using the natural abundance¹³C-lipid signal. The chemical shift imaging (CSI) was nonlocalized MR spectroscopy sequence was run for setting of the¹³C-MR difference image, clearly showing the position of the tumor. All automatic inline adjustments of the MR scanner were disabled. This was done to avoid unwanted radiofrequency pulses, which would destroy the hyperpolarization signal. A 90-degree reference radiofrequency pulse was calibrated using the natural abundance¹³C-lipid signal. The chemical shift imaging (CSI) was nonlocalized MR spectroscopy sequence was run for setting of the¹³C-MR

Figure 4. MRI slice image of a rat: transversal ¹H-NMR image and color coded distribution plots for hyperpolarized ¹³C labeled pyruvate, alanine and lactate, projected on the anatomical ¹H image. Alanine is most prominent in the skeletal muscle around the spinal cord, whereas the P22 tumor tissue is indicated by the highest signal of lactate. Figure taken from Ref. [32].

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