RADIOLYSIS OF DNA AND PROTEINS BY HEAVY PARTICLES GENERATED BY THE NUCLEAR REACTION $^{10}B(n, a)^{7}Li$. A STUDY AT THE MOLECULAR LEVEL OF BORON NEUTRON CAPTURE ENHANCEMENT OF FAST NEUTRON RADIOTHERAPY.

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The implementation of boron neutron capture therapy (BNCT) presents some difficulties: the targeting of the boron-bearing drug and the delivery of a substantial flux of thermal neutrons in tumour are problems not yet solved. That is the reason why, some radiotherapists are moving towards a new technique, less satisfying from the theoretical point of view, but more easy to realise: the boron neutron capture enhancement of fast neutron radiotherapy.

During fast-neutron radiotherapy, a fraction of neutrons are thermalised in the irradiated volume by slowing down, as well as scattering by protons of the medium (70-80% water). Thus thermal neutrons are produced inside the tumour, solving by this way the problem of penetration. Targeting of boron can be considered as satisfactory by combining a concentration gradient of boron between tumour and safe tissues, with a very good spatial definition of the irradiation beam (collimator). Computations have shown that, in realistic cases, the dose due to the boron neutron capture in the irradiated volume could be sufficient to sterilise some tumours with sombre prognostic, resistant to γ rays, and difficult to treat using pure fast neutrons, like glioblastoma.

The biological consequences of ionising radiations occur mainly through two pathways. First, the direct effects result from the ionisation of the biological material itself. Second, the indirect effects are due to the chemical attack of the biological material, by the reactive species issued from water radiolysis. These indirect effects are by far the most abundant.

The radiobiological properties of such heavy particles α and Li^{3+} are very different from those of β or γ rays. Their linear energy transfer (LET = dE/dx) in water, that characterises the density of interacting events along the trajectory, is very high: 185 keV / µm in average, compared with less than 1 keV / µm for ^{60}Co γ rays, in fact not for γ rays, but for Compton electrons issued from γ rays interaction with water. (Remind that the mean energy required for water ionisation is 30 eV). The result is that the spatial distribution of the radiolytic events is completely different for γ rays and for both α and Li particles. For the same energy deposition by mass unit of irradiated medium (the dose, in gray (Gy) = 1 J kg^{-1}), the number of particle tracks is considerably smaller in the case of boron neutron capture, but the linear density of events along the trajectory of the particle is considerably greater. The biological implications of the physics of the tracks appear immediately. In the case of boron neutron capture, the mean distance between two radiolytic events (an order of magnitude of 1-2 Å instead of 0.1 µm for γ rays) is much smaller than the size of the target we are studying : DNA plasmid or protein (30-100Å).

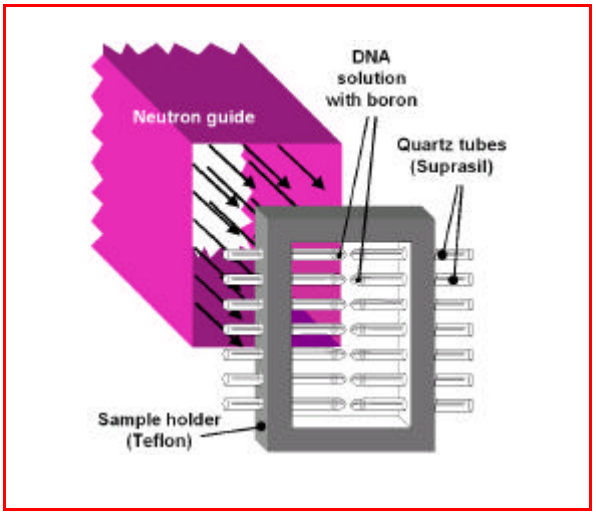
Our experiments at the LLB concern the induction of damages in DNA. Supercoiled DNA plasmids in aqueous solution are exposed to a flux of thermal neutrons (white beam on G5-4 line). The neutron fluence, 4.6 x 10^{8} n cm^{-2} s^{-1}, corresponds to a dose rate of 24 Gy min^{-1} for 1 M ^{10}B. The solution contains various concentrations of potassium borate, enriched at 99% in either ^{10}B or ^{11}B (used as reference to account for ? contamination of the beam). The induced DNA chain breaks (SSB as single strand breaks and DSB as double strand breaks) are assayed by relaxation / linearisation of the plasmid. The induction of damages to the bases of DNA is assayed by measuring the excess of strand breaks after enzymatic treatment by endonucleases which are specific of damaged purines or damaged pyrimidines.

The samples (typically 80 µl of DNA solution) are contained in 4 mm diameter thin quartz tubes (NMR type). A special device has been drawn and built to ensure a reproducible centring of the samples in the beam.

Our experiments started on 10th February 2003, and will finish on 31st March. So it is not yet possible to summarise and discuss here the results.

However, a test experiment was made in June 2002. We have shown that DNA single- and double-strand breaks are induced upon thermal neutron irradiation in the presence of ^{10}B, whereas the effects are very

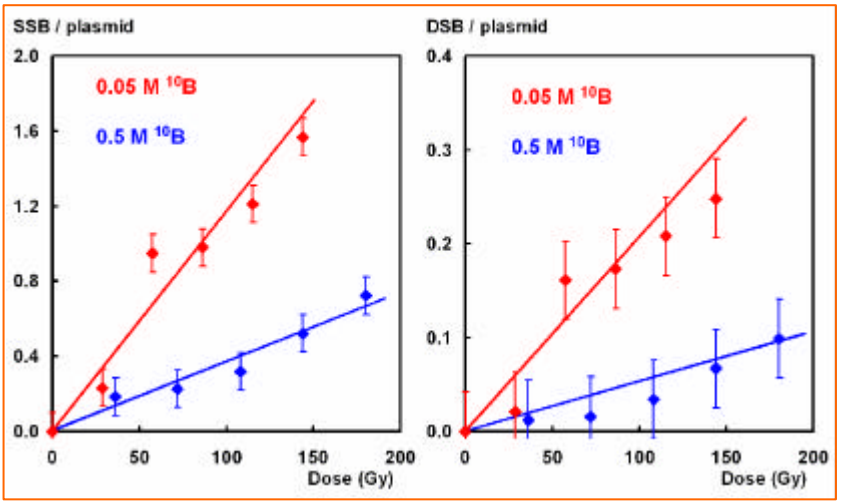
small in the presence of ^{11}B, may be due to the γ contamination of the neutron beam.



We can make the following remarks :

1 - The dose-response curve depends on the boron concentration. This fact can be explained by the previously observed presence of an impurity in the borate solution, that scavenges with a good efficiency the radicals produced in water. From this point of view, experiments at low boron concentrations are more accurate.

2 - The slope of the dose-response curve for small boron concentrations is smaller than those observed at GANIL with a $^{36}A^{18+}$ ion beam, whose LET is of the same order of magnitude (200 keV / µm). This could be due to a likely overestimation of the dose at the LLB. We do not take into account the scattering of neutrons by protons of water in the samples. Experiments with D_2O could help us to remove this difficulty.



3 - The ratio DSB / SSB is about 0.2 whatever the boron concentration. This very high value (0.02 for γ rays, 0.05 for fast neutrons) is close from that measured for high LET particles. As SSB are easily repaired by repair enzymes in the cells, whereas DSB are considered as major DNA damages, this observation is of real importance for biological and medical applications.