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Physics III Committee

PROPOSAL:

MUONIUM CHEMISTRY IN CONDENSED MATTER: SEARCH FOR TRANSIENT RADICALS IN D.N.A.

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1) Introduction

During the last decade, the well known asymmetry of the μ^+ decay into positron and neutrons has been exploited in the investigation of the properties of condensed matter. If a polarized μ^+ beam is made to stop in a gaseous, liquid or solid target, the detection of the emitted positrons enhables one to monitorize the μ^+ spin-direction at the instant of the decay. Since the μ^+ slowing down processes do not affect the spin direction, any change in such quantity occurs during the μ^+ life-time at rest in the target. Changes in the spin direction can be caused by the presence of externally applied magnetic fields, local magnetic fields, interaction with the electron of the target-system. The time-hystogram of the emitted positron in a given direction relative to the μ^+ initial spin polarization yields, in addition to the μ^+ life time exponential decay, the time dependence of the μ^+ spin as well. This last information is the basis for a studying the properties of the target system and has been proved very successfull in many gases, liquids and solids.

In this proposal we are particularly interested in the muonium atom formed by the capture of an electron by the μ^+ in its final part of the range in the target.
It has been already shown that muonium is a very gentle and rich source of information due to its properties of (i) being a light isotope of the hydrogen atom, (ii) being a highly reactive radical, (iii) having a large variety of interaction in different media (iiii) manifesting clearly its physico-chemical status via sharp changes in its hyperfine frequencies (1).

We suggest than the study of transient radicals formation in substances of biological interest such as D.N.A.. The formation of radicals in such systems is believed to be the first chemical step in the transformation of the macromolecular structure with genetical implications and its investigation is of great interest not only for a basic understanding of DNA trans-

formations but also in view of the protection and repair effects by external specific mechanisms.

2) Purpose of the experiments: Transient Radicals in D.N.A.

The most recent experimental investigations of the biophysical effects of radiation have indicated more and more clearly the lack of information on the events taking place in a molecule during short intervals of time of the order of 10^{-6} sec after the primary interaction with the radiation.

During the primary stage, or "physical "stage, of the order of 10^{-13} sec, the energy of radiation is transferred to the system leaving thus a state of ionizations and excitations. Subsequently, in a "physico-chemical" stage of approximately 10^{-10} sec, intermolecular energy transfers take place in the molecules. At this point one deals with molecular free radical or small diffusing radicals whose interaction with the "umperturbed" system is of "chemical" nature. In this stage, the system is composed by highly reactive species which give place, via a series of reactions, to stable final damages, i.e. to chemically modified molecules. This last chemical stage is the most unknown of all and is expected to last 10^{-6} sec. Traditional experimental techniques that have been used in the past in the attempt of studying the chemical stage of transient radical (pulse radiolysis, Electron Spin Resonance) suffer in fact from severe limitations, the most obvious of them being the impossibility of detecting changes in the system over a short time scale (for EPR) and the impossibility of optical observations of all radical species for pulsed radiolysis. To overcome this limitation, ESR measurements of transient free radicals have been done, in many cases of biological

material, in the solid state and at low temperature where the "chemical" life time of radical species can become very long.

In particular for DNA, there seems to be evidence from E.S.R. measurements (2) for radical species localized on the pyrimidine bases and similarly for the many components of the DNA, such as basis, nucleosides and nucleotides, also in the solid state, reliable evidence has been collected for the formation of hydrogen adduct radicals (3) only in tymine and cytosine derivatives. For the cases of purine compounds there is'nt yet a conclusive evidence for such radicals, even in the solid state (4). The possibility of using Muonium as a probe and simultaneously primary agent of the involved radical species (Muonium replaces hydrogen atoms in this role) seems to be, now-a-days, the only break through toward the understanding of the role of the hydrogen adduct radicals not in the solid state, but in the natural biological surrounding, i.e. in proper solutions similar to the situations"in vivo". Muonium's lifetime is of the useful order of magnitude for detecting the chemical changes in the radical species containing it. The μ^+ spin hyperfine frequencies in Muonium are sharply affected by the changes in localization of its electron.The "single-event" detection technique makes possible experiments with a total number of μ^+ "damaging" the sample still much smaller than it would require, for instance, ESR or other techniques.

The experiment is expected to proceed in three steps:

1) hydrogen adduct radicals in benzene, cycloexene and in solutions of tymine and cytosine (for which evidence already exists (2)).
2) hydrogen adduct radicals in all basis, nucleosides, nucleotides and DNA.

3) correlation between the radical's existence and chemistry in solutions and in the solid state.

3) Experimental Requirements and set up.

The basic features of the proposed experiment are determined by the range of muonium hyperfine frequencies on one side and by slower change of the spectrum into the free μ^+ Larmor frequency as transient radicals react and a diamagnetic surrounding of the μ^+ results. Thus:

1) The time resolution of the electronics should be at least of 1-2 nsec so as to assure a reasonably good detection of the main transitions between hyperfine levels.

2) The rate of stopping μ^+ must be 10^5 sec^{-1} since the time evolution of muonium or Mu-radical mode's amplitude so well as free μ^+ frequency must be followed up to times of the order of 10 μsec.

3) Since one single measurement is expected to be much shorter than the available beam-time in one shift, and since the target system needs to be changed (temperature, solvent concentration, ecc.) according to the indication of previous measurements, it is necessary to accumulate and analyze data-simultaneously.

Other characteristics of he experimental set up will account for the necessity of a small but homogeneous magnetic field that can be put either longitudinal or transverse to the polarization of the incoming muons.

More than one set of positron detectors will be programmed in order to allow a good determination of the residual polarization and initial phase in cases in which the Mu or Mu-radical chemical life is too short to make a direct detection of hyper-

fine frequencies feasible.

An automatic control of the target system, when it consists of solution of DNA as well as of its molecular components, will be built in order to allow the remote control of the experiment.

Finally it must be emphasized that since the entire set up is planned under the assumption of having a small computer on line with the experiment, all the other experimental parameters such as magnetic field, temperature etc. will be automatized by the computer.

4. Equipment and Beam-Time

According to the previous recommendations of the physics Committee III, our Group will coordinate its efforts in setting the experimental equipment up with the Uppsala Group (exp. code SC.65). This is made possible by the fact that the general requirements of the two experiments are quite similar. The only main differences are in the targets, targets thermostats or cryostats, and in the final part of the data analysis.

The beam-time that we plan to use will have a common part with the Uppsala Group in the testing of the μ^+ beam and of the experimental set up. Our experiment will need approximately 5 shifts for the preliminary measurements on benzene and cycloexene and 15 shifts for the measurements on DNA and DNA components in solution. This part of the work is expected to be carried out during the first year.

The essential parts of the equipment are:

1) Detection system: one multiple coincidence and anticoincidence system for stopping μ^+ and two positron "telescopes";

2) Counter-timer system with high linearity and long-time stability;
3) On-line computer and relative interfacing for direct and fast accumulation of data (data rate: 10^5 sec^{-1}; data format: integers between 1 and 15.000) in the form of a time-elapsed hystogram and for its analysis;
4) Target system: thermostatic container of proper solutions of macromolecules located either in the gap-space of an electromagnet or between two purposely built Helmoltz coils.

We are planning to ask CERN for (A) contributions in terms of laboratory space and facilities for the accomplishment of point 1) and 2); (B) making available, one a time-sharing basis, a small computer capable of satisfying the requirements of point 3) and equipped with monitor, magnetic tape, teletype. (C) making available some computer time for subsequent elaboration and analysis of data.

The funds for the fast electronic components, for the detection system, for the counter-timer system and for the specific requirements of the target system have been asked for the Italian Research Council.
As for the long term evolution of the research described here, we intend to present future specific proposals.

In the interest of a more profiteable coordination of the european efforts in this new field of research, we would like to emphasize our availability to interact and collaborate with other groups willing to start μ^+SR at CERN, along the line of the beginning collaboration with the Uppsala Group.

References

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